Upon petition, the State Medical Board of Ohio has the authority to approve and designate conditions or diseases as qualifying medical conditions for treatment with medical marijuana. For the calendar year of 2024, the board will accept petitions for consideration between **November 1, 2024** and **December 31, 2024**.

Covered Existing Qualifying Conditions

The following conditions are already part of the program: AIDS, amyotrophic lateral sclerosis, Alzheimer's disease, cachexia, cancer, chronic traumatic encephalopathy, Crohn's disease, epilepsy or another seizure disorder, fibromyalgia, glaucoma, hepatitis C, Huntington's disease, inflammatory bowel disease, irritable bowel syndrome, multiple sclerosis, pain that is either chronic and severe or intractable, Parkinson's disease, positive status for HIV, post-traumatic stress disorder, sickle cell anemia, spasticity, spinal cord disease or injury, terminal illness, Tourette syndrome, traumatic brain injury, and ulcerative colitis.

The board's Medical Marijuana Committee determined that the following are considered to be covered by an existing qualifying condition:

- Arthritis (determined to be covered by pain that is either chronic or intractable, February 2021)
- Chronic Migraines (determined to be covered by pain that is either chronic or intractable, February 2021)
- Complex Regional Pain Syndrome (determined to be covered by pain that is either chronic or intractable, February 2021)
- Degenerative Disc Disease (determined to be covered by pain that is either chronic or intractable, February 2022)
- Lupus where pain is present (determined to be covered by pain that is either chronic or intractable, February 2022)

You do not need to submit a petition for any of these conditions. Read the board's Position Statement (PDF).

Petition Consideration Denials

The petition will not be considered if:

- Received after **December 31, 2024**
- It seeks to add a broad category of diseases or conditions
- The condition that has been previously reviewed by the board and rejected unless new scientific research that supports the request is offered

Previously Considered Condition

If you are petitioning for a previously considered condition:

- Do not resubmit documents which have already been reviewed by the board
- Only new scientific research should be submitted for previously rejected petitions
- View a catalogue of submitted research and documents.

Public Record

Most information submitted as part of a petition is public record and may be posted on the Medical Board's website at med.ohio.gov. This includes the submitter's name provided contact information, and responses.

Instructions

- All sections below are required to be completed per Ohio Administrative Code 4731-32. All text boxes are required. Applicants may type "see attached" or "previously submitted" in the required fields.
- If you would like for the Medical Board to consider multiple conditions, please complete a separate submission for each one.
- Please refrain from providing personal medical information as all submissions are subject to public record requests.

First Name *	Last Name *	Email *
Douglas	Woo	dougthekinezo1@gmail.com
Address *	City *	State *
1513 Clover Court	Lancaster	OHIO
Zip Code *	County *	Specific Disease or Condition *
43130	FAIRFIELD	Refractory Autism Spectrum Disorder

Please do not include any links in the text fields. All materials submitted for review must be attached in the format of a Microsoft Word document or PDF.

NOTE: Links within submitted documents will not reviewed.

Specialized Experts

Information from experts who specialize in the disease or condition *

1. Bonni Goldstein MD is pediatric emergency room physician in California who has been treating both adult and pediatric patients with medical cannabis since 2008, including many with autism spectrum disorder. She has authored multiple books, co-authored multiple studies in peer-reviewed journals, and has spoken at numerous national and international scientific conferences.

2. Patricia Frye MD is a pediatrician in Maryland with subspecialized training in pain management and obesity medicine. She is also certified in Cannabis Science and Medicine from the University of Vermont School of Medicine and authored a curriculum on cannabis treatment in pediatric patients while on the faculty of the University of Maryland School for Pharmcy. She has treated 300 patients with autism spectrum disorder with medical cannabis over a career spanning 40 years.

3. Mohsin Maqbool MD is a child neurologist in Texas who is fellowship trained in sleep medicine and has multiple publications in peer-reviewed journals. He has been treating multiple patients with autism spectrum disorder (ASD) since 2019 and recently completed an open-label trial of cannabis in treating 120 patients with sleep disorders related to ASD, as well as a separate trial in 80 patients with ASD that evaluated effect of cannabis on sleep, behavior, and quality of life.

File Name	Size *
Dr. Bonni Goldstein (signed).pdf	1.88 MB
Dr. Patricia Frye (signed).pdf	94.65 kB
Dr. Mohsin Maqbool (signed).pdf	172.84 kB
CNS Poster 2023 Final (Maqbool).pdf	251.01 kB
Abstract_237 (Maqbool).pdf	27.28 kB

Medical or Scientific Evidence

Relevant medical or scientific evidence pertaining to the disease or condition *

Please see attached.

File Name	Size
Refractory ASD (Medical or Scientific Evidence).pdf	400.45 kB

Conventional Medical Therapies

Consideration of whether conventional medical therapies are insufficient to treat or alleviate the disease or condition *

Please see attached.

File Name	Size
Refractory ASD (Conventional Medical Therapies).pdf	175.85 kB

Supporting Evidence

Evidence supporting the use of medical marijuana to treat or alleviate the disease or condition, including journal articles, peer-reviewed studies, and other types of medical or scientific documentation *

Please see attached.

File Name	Size
Refractory ASD (Supporting Evidence).pdf	711.54 kB

Physician Letters of Support

Letters of support provided by physicians with knowledge of the disease or condition. This may include a letter provided by the physician treating the petitioner, if applicable. *

Please see attached.

File Name	Size
Dr. Bridget Williams (signed).pdf	152.86 kB
Dr. James Weeks (signed).pdf	312.26 kB

Autism, Sleep, and Medicinal Cannabis: Evaluating 18-Month Efficacy and Safety Outcomes

M. Maqbool[1]
[1]University of Texas Dallas, Neuroscience, Richardson, United States

Type

Presentation type: Oral presentation

General data

Topic: Pediatric
Secondary topic: Neurological Disorders Affecting Sleep

Abstract text

Introduction: Sleep disturbances are common in children with Autism Spectrum Disorder (ASD), affecting up to 80% of this population. Poor sleep onset, frequent awakenings, and reduced sleep quality contribute to behavioral challenges, impaired cognition, and increased caregiver stress. Standard treatments like melatonin or sedatives offer limited relief, making medicinal cannabis a promising alternative. Cannabis-based therapies with cannabidiol (CBD) and delta-9-tetrahydrocannabinol (THC) in tailored ratios have shown encouraging short-term results for sleep improvement in ASD. This study evaluates the long-term efficacy and safety of medicinal cannabis for sleep management over 18 months, focusing on sustained outcomes and side effects.
Materials and methods: This longitudinal observational study enrolled 120 pediatric patients (ages 3-17 years) with a confirmed diagnosis of ASD who experienced chronic sleep disturbances. Patients were treated with medicinal cannabis formulations containing varying ratios of CBD and THC, customized based on individual tolerance and therapeutic response.
Baseline data were collected three months prior to initiating cannabis treatment, including metrics on sleep onset latency, sleep maintenance, and overall sleep quality, as reported by caregivers and validated sleep assessment tools. Follow-up assessments were conducted three months post-treatment initiation and at 18 months to evaluate long-term efficacy and safety. Primary endpoints included improvements in sleep parameters (sleep onset latency, sleep maintenance, and overall sleep quality) and caregiver-reported quality of life. Secondary endpoints included the identification of adverse effects and the development of treatment tolerance. Data were analyzed using paired t-tests and repeated measures ANOVA for longitudinal comparisons.
Results: Initial results demonstrated significant improvements in sleep onset latency, maintenance, and quality within the first three months of treatment, with over 80% of patients showing measurable benefits. At the 18-month follow-up, 68% of patients sustained their sleep improvements without requiring substantial dosage adjustments.
Adverse effects were minimal, with increased appetite and agitation reported in less than 10% of patients, primarily during the initial adjustment phase. No severe adverse events were recorded. A small subset of patients (12%) showed signs of developing tolerance, necessitating dosage increases to maintain efficacy. Overall, caregiver-reported quality of life improved in parallel with sleep improvements, highlighting the positive impact of treatment on both patients and their families.
Conclusions: This 18-month observational study highlights the long-term efficacy and safety of medicinal cannabis for managing sleep disturbances in ASD. Tailored treatments sustained sleep improvements with minimal side effects, making this a viable option for a challenging population. While tolerance development is a concern for some, the overall safety profile and lasting benefits support medicinal cannabis as part of a comprehensive sleep management strategy. Future studies should refine dosing regimens, including terpene and flavonoid profiles, to establish standardized therapeutic guidelines.
Acknowledgments: The authors acknowledge the patients and caregivers for their participation and trust throughout this study. This research was conducted independently without external funding. Data were derived from patients receiving care at a neurology sleep clinic, where progress was monitored as part of routine care. Gratitude is extended to the clinic staff for supporting data collection and patient management. Their dedication made this study possible.

General

1. I confirm that the abstract and that all information is correct: Yes
2. I confirm that the abstract constitutes consent to publication: Yes
3. I confirm that I submit this abstract on behalf of all authors.: Yes
I understand that the presenting author MUST register for the congress: Yes

The Effects of Medical Cannabis on Sleep, Behavior, and Quality of Life for Children with Autism Spectrum Disorder – An Open Label Case Series

Jyona Kate Nazareno[1], Samuel Poelker-Wells[1], Mohsin Maqbool, M.D.[1-2] | University of Texas, Dallas.[1] Texas Child Neurology. Plano, TX[2]

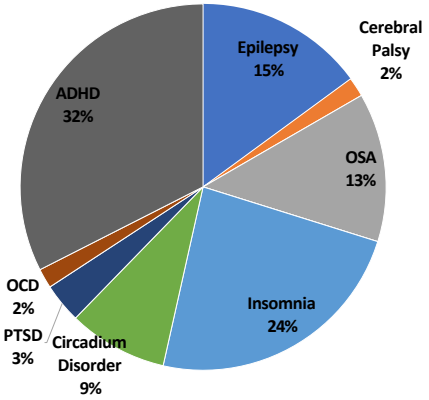
Purpose

To study the effects of medical cannabis on sleep, behavior, anxiety, focus/attention, sensory issues, and quality of life for children with Autism Spectrum Disorder (ASD).

Demographics

N = 80 (M:F 59:21)
Age: 3-17 y (mean: 9.2 years)

Comorbidities



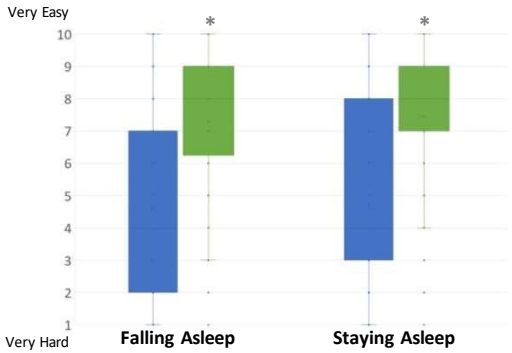
Methodology

- Parents of children diagnosed with ASD were given an option to be treated with medical cannabis (oral tincture or gummies).
- Varying ratios of CBD and THC were prescribed under Texas's Compassionate Use Program of 2019.
- A caregiver global impression of change (CGI-C) was obtained after a minimum of 3 months of treatment.
- The dose of THC ranged from 2.5 to 10 mg BID.
- Performed a two-tailed t-test for each domain ($p < 0.05$).

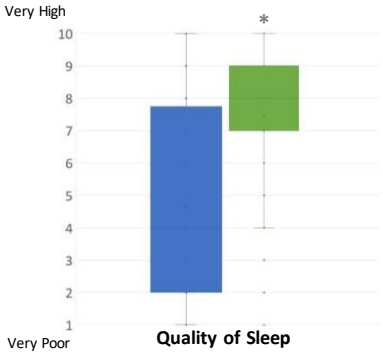
Conclusion

- Medical cannabis was well tolerated and had clinically significant effects on multiple domains of autism.
- Of the formulations, 1:1, 3:1 and 4:1 ratios of CBD:THC were the most effective compared to 20:1 or THC only.
- Further studies are needed to establish the long-term efficacy and safety of medical cannabis.

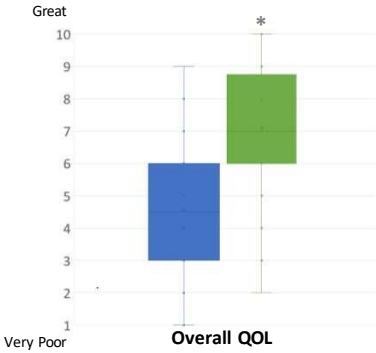
Sleep/Insomnia



Quality of Sleep

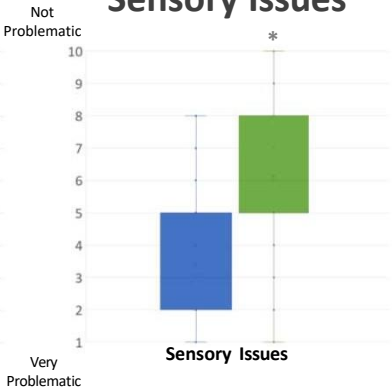


Quality of Life

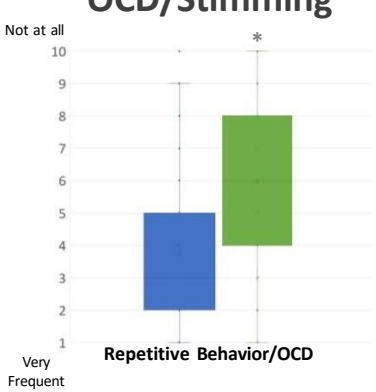


Focus/Attention

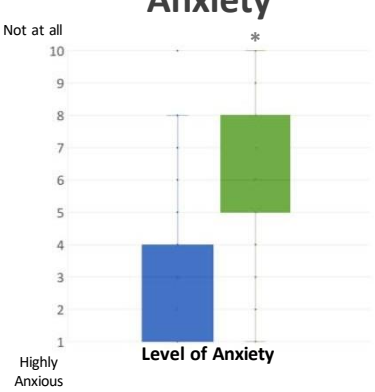
Sensory Issues



OCD/Stimming



Anxiety



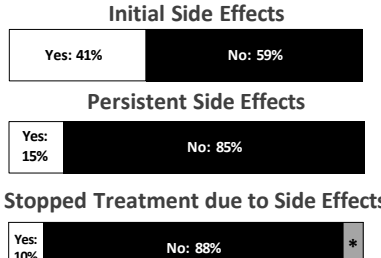
Effectiveness by Ratios of CBD:THC

CBD:THC Ratio	(1:1)	(3:1)	(4:1)	(20:1)	THC Only
n =	49	9	9	3	2
Falling Asleep	2.91E-07	0.007	0.021	0.423	0.17
Staying Asleep	1.94E-10	0.231	0.028	0.423	0.295
Overall Quality	3.23E-10	0.008	0.041	0.423	0.295
Sensory Issues	2.92E-12	0.051	0.025	0.58	0.295
Focus & Attention	3.76E-13	0.017	0.05	0.3	0.126
Anxiety	1.02E-11	0.034	0.02	0.346	0.09
Repetitive Behavior	1.04E-11	0.03	0.024	0.497	0.126
Quality of Life	1.50E-09	0.019	0.028	0.84	N/A

$p < 0.05$

Common Side Effects

Increased appetite, drowsiness and worse behavior



***Stopped due to tolerance** = <3% (2)

Before Treatment
After Treatment

Initial Side Effects

Increased appetite: 27%
Drowsiness: 20%
Worse behavior: 13%

Persistent Side Effects

Increased appetite: 10%
Drowsiness: 7%
Worse behavior: 5%

December 15, 2024

To Whom It May Concern:

I am certified by the American Board of Pediatrics and hold professional certification in Cannabis Science and Medicine from the University of Vermont School of Medicine. As board member and chair of the Education Committee for the Society of Cannabis Clinicians, I oversee medical cannabis continuing education for physicians. Having served on the faculty of the University of Maryland School of Pharmacy master's program in Medical Cannabis Science and Therapeutics, I authored the curriculum on cannabis use in the pediatric population. In 2018 I authored "The Medical Marijuana Guide: Cannabis and Your Health" published by Rowman & Littlefield, which is used as text and required reading for several university medical cannabis programs in the US.

I have been in practice for over forty years. In the past ten years of including cannabis medicine as a therapeutic modality in my practice, I have evaluated approximately 300 pediatric and young adult patients diagnosed with autistic spectrum disorder. Many of these patients are referred by their primary care pediatricians, behavioral pediatricians, or pediatric neurologists, while some parents come to me directly, seeking medical cannabis advice for their children.

Most families with a child with autism seek out cannabis medicine due to highly disruptive behaviors affecting the child, the family, and/or the school, or because the child has not improved or has experienced significant adverse effects from conventional therapies. Many of these families have reported benefits from medicinal cannabis. Co-occurring conditions such as anxiety, depression, OCD/repetitive behaviors, seizures, sleep disturbances, sensory sensitivities, and gastrointestinal issues have improved in some patients. Parents also report enhancements in their child's ability to expand a palate typically restricted to highly refined carbohydrates, to the extent that they are willing to try new foods like fruits and vegetables. The importance of improved nutrition cannot be overemphasized. Adverse effects are generally limited to increased anxiety, daytime sleepiness, or decreased appetite, all of which respond to adjustments in doses or discontinuing treatment.

While many patients respond positively to non-impairing cannabidiol (CBD), cannabigerol (CBG), and/or cannabidivarin (CBDV) containing less than 0.3% THC, some severely affected patients have not experienced significant improvement until the addition of small doses of Δ9-THC combined with CBD, usually at a CBD:THC ratio of about 10:1. When used together in proper ratios, CBD mitigates the impairing effects of THC, avoiding the adverse effects associated with high-dose THC, which are commonly seen in excessive use of flower and concentrates, as well as in Δ9-THC use disorder.

Even in relatively low doses, Δ9-THC can be particularly effective in reducing irritability, self-injurious behaviors, and destructive behaviors, without the adverse effects—such as obesity, metabolic syndrome, and steatohepatitis—that affect patients treated with atypical antipsychotics like risperidone and aripiprazole. Typical antipsychotics are also prescribed. While they are not associated with the excessive weight gain we see with the atypical antipsychotics, they too, are associated with significant adverse effects like tardive dyskinesia, that may not improve after the medication is discontinued. Δ9-THC has also been found to be more effective in treating tics, another common comorbidity, than CBD alone. When we are able to discontinue atypical antipsychotics, patients lose much, if not all, of the weight they gained, and can reverse iatrogenic pre-diabetes, dyslipidemia, and steatohepatitis.

The conventional treatment of co-occurring conditions often relies on the off-label pharmaceutical use of SSRIs, SNRIs, antihypertensives, psychostimulants, benzodiazepines, and nutritional supplements, with limited supporting evidence of efficacy. FDA-approved options are limited to melatonin for sleep, methylphenidate for ADHD, and atypical antipsychotics for irritability. However, these options can be only

marginally effective, if at all, for many patients. While single-molecule medications may target specific neurotransmitter systems such as serotonin, glutamate, or dopamine, none address the neuroinflammatory problems that contribute to symptoms like mood disorders that are associated with autism. Cannabis, on the other hand, modulates neurotransmitter systems that include serotonin, glutamate, GABA, dopamine, and adenosine (sleep), oxidative stress, inflammation, as well as chronic stress, anxiety, and gastrointestinal motility.

When starting patients on cannabinoid therapy, I advise parents not to inform teachers, therapists, and caregivers, so that I can obtain unbiased feedback. A common response from these ancillary care providers when asked how the patients are doing in school or therapy is, "I don't know what you're doing, but whatever it is, keep on doing it." Patients are generally described as more engaged, easier to transition, and exhibiting fewer and less severe problems with aggressive or self-injurious behaviors.

Currently, there are no FDA-approved medications to address the core symptoms of autism, which include impaired social interaction, expressive language deficits, and repetitive behaviors. Although not necessarily targeted symptoms, approximately 20-30% of the patients I see have displayed improvements in these core symptoms and are noted by both teachers and parents.

Furthermore, including autism as a qualifying condition for medicinal cannabis allows parents access to qualified medical care and guidance, as well as regulated medical cannabis products from licensed sources. When healthcare providers are unable to certify and advise these patients, many desperate parents turn to social media for advice, where well-meaning but untrained and unqualified individuals provide medical recommendations on products and dosing. Such advice frequently includes unsafe practices, such as vaping Δ8-THC concentrates, and promotes unvetted over-the-counter or underground products that may not be safe.

In summary, I strongly support the use of medical cannabis in patients with autism who have not benefited from conventional therapy or for whom conventional therapies have caused or have the potential to cause significant adverse effects.

If I can be of any further assistance, please do not hesitate to contact me.

Sincerely,

Patricia C Frye, MD FAAP

Bonni S. Goldstein, MD
46 Peninsula Center Drive
Rolling Hills, CA 90275

December 18, 2024

Dear Members of the Ohio State Medical Board,

I am writing to strongly urge the Ohio State Medical Board to add refractory autism spectrum disorder as a qualifying condition for medical cannabis. As a pediatric medical cannabis specialist with over 17 years of experience in this field, I have directly witnessed the transformative impact cannabinoid-based treatments have had on children with refractory autism and their families. Excluding children with this diagnosis from access to this safe and effective therapy would be a profound disservice to thousands of Ohio families seeking relief.

I am a California-licensed physician who transitioned to cannabinoid medicine in 2008 after 14 years practicing pediatric emergency medicine. I trained at Children's Hospital Los Angeles, where I served as Chief Resident, and have since become widely recognized as a leading expert in pediatric cannabis medicine. Over the past 17 years, I have treated over 2,400 children with medical cannabis, including many with refractory autism, intractable epilepsy, and other debilitating conditions.

I have authored two books on medical cannabis and co-authored three peer-reviewed studies documenting the impact of medical cannabis using salivary biomarkers in pediatric autism patients.[1-3] This research provided the first objective evidence that cannabinoid treatment shifts physiological pathways involved in inflammation, pain, and behavior toward neurotypical levels, correlating with significant symptom improvement reported on validated parent surveys.

There is growing scientific evidence that ASD is associated with a deficiency in endocannabinoids, particularly anandamide, a key compound that regulates the endocannabinoid system. Two studies, one from Stanford University and one from a large autism center in Israel, demonstrate that children with autism have significantly lower circulating levels of endocannabinoids compared to neurotypical peers.[4,5] Just as we would never withhold treatment for other medical deficiencies (such as insulin for diabetes or thyroid hormone for hypothyroidism), it is unacceptable to deny children with refractory autism access to a treatment that could address this critical imbalance.

A comprehensive review of 13 studies investigating the effects of cannabis and CBD on autism found that all studies reported substantial improvements in behavior and symptoms, with between 61% and 93% of subjects experiencing meaningful benefit.[6] Additionally, those studies evaluating concurrent psychotropic medication use, up to 80% of participants were able to reduce or discontinue other medications after starting cannabinoid therapy.[6]

Bonni S. Goldstein, MD
46 Peninsula Center Drive
Rolling Hills, CA 90275

The safety profile of cannabinoids in refractory autism far exceeds that of current pharmaceutical treatments. Many children with ASD are prescribed atypical antipsychotics (such as risperidone or aripiprazole) or other off-label psychotropic medications, which are associated with serious side effects, including substantial weight gain, metabolic dysfunction, sedation, and long-term risks to cardiovascular and neurological health.[7] In contrast, cannabinoids are well-tolerated, with minimal side effects when appropriately administered under medical supervision.[8] I take my oath to do no harm very seriously, and medical cannabis allows me to uphold this oath, as it is extremely well tolerated with an acceptable safety profile. This is documented in every clinical trial that has evaluated the use of cannabis in children with autism.

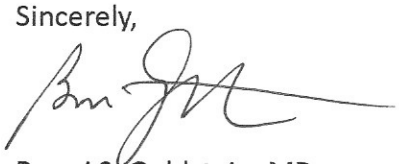
As a physician who has spent decades caring for pediatric patients, I fully support expanding access to treatments that are safe, effective, and life-changing for children with refractory autism. Medical cannabis profoundly improves quality of life, reduces aggressive and self-injurious behaviors, and brings much-needed relief to families navigating this challenging condition. Those of us on the front lines working with these families see these positive results every day.

The evidence is clear: medical cannabis is a safe and effective option for pediatric autism patients, addressing underlying physiological deficiencies while offering meaningful clinical outcomes. Denying access would ignore both the science and the lived experiences of families and clinicians who have seen its impact firsthand.

I respectfully urge the Ohio State Medical Board to recognize autism spectrum disorder as a qualifying condition for medical cannabis. I am confident this decision will bring relief and hope to many families in need.

Please do not hesitate to reach out if I can provide additional information or answer any questions you may have. I would be more than happy to assist in any way that I can as you consider this important decision.

Sincerely,



Bonni S. Goldstein, MD
Medical Director, Canna-Centers
Email: bgoldsteinmd@canna-centers.com

Bonni S. Goldstein, MD
46 Peninsula Center Drive
Rolling Hills, CA 90275

Sources:

[1]Siani-Rose, Michael, et al. "Cannabis-responsive biomarkers: A pharmacometabolomics-based application to evaluate the impact of medical cannabis treatment on children with autism spectrum disorder." *Cannabis and Cannabinoid Research* 8.1 (2023): 126-137.

[2]Siani-Rose, Michael, et al. "The potential of salivary lipid-based Cannabis-responsive biomarkers to evaluate medical cannabis treatment in children with autism spectrum disorder." *Cannabis and Cannabinoid Research* 8.4 (2023): 642-656.

[3]Quillet, Jean-Christophe, et al. "A machine learning approach for understanding the metabolomics response of children with autism spectrum disorder to medical cannabis treatment." *Scientific Reports* 13.1 (2023): 13022.

[4]Karhson, Debra S., et al. "Plasma anandamide concentrations are lower in children with autism spectrum disorder." *Molecular autism* 9 (2018): 1-6.

[5]Aran, Adi, et al. "Lower circulating endocannabinoid levels in children with autism spectrum disorder." *Molecular autism* 10 (2019): 1-11.

[6]Fletcher, Sarah, et al. "Medicinal cannabis in children and adolescents with autism spectrum disorder: A scoping review." *Child: Care, Health and Development* 48.1 (2022): 33-44.

[7]Aishworiya, Ramkumar, et al. "An update on psychopharmacological treatment of autism spectrum disorder." *Neurotherapeutics* 19.1 (2023): 248-262.

[8]Goldstein, Bonni S. "Medical Cannabis for Children and Adolescents." *Understanding Medical Cannabis* (2020): 134-161.

Robert S. Chudnow, MD, PA
Gerald M. So, MD, PA
Lina Shah, MD, PA
Mohsin Maqbool, MD, PA
Patricia S. Mireles, MD
Daniel Gossett, MD, PA
Safiullah Shareef, MD, PA
Andrew Hurd, MD, PA
Cerin Jacobs, MD

Letter of Support

Monday, December 23rd, 2024

Ohio State Medical Board

To Whom It May Concern,

I am writing to express my strong support for the petition to include autism spectrum disorder (ASD), with medically refractory symptoms, as a qualifying condition for medical marijuana in the state of Ohio. As a pediatric neurologist and sleep specialist practicing in Texas, I have firsthand experience in utilizing medical cannabis as part of a comprehensive treatment plan for the medically refractory symptoms in children with ASD under Texas' Compassionate Use Program, which has been in place since 2019.

Through my clinical practice, I have observed significant improvements in patients with ASD, with medically refractory symptoms, who were treated with medical cannabis. These improvements include:

- Sleep: Helped with the ability to fall asleep and stay asleep, enhanced sleep duration and quality, reducing sleep disruptions. With secondary effects of improved focus, attention, and anxiety, enhanced sleep has been a pivotal factor in the therapeutic success of medicinal cannabis.

- Anxiety: Noticeable reductions in anxiety levels, enabling better social interactions, learning potential, and emotional regulation.

- Self-Stimulatory Behaviors (Stimming): Decreased frequency and intensity of stimming behaviors, contributing to improved focus and adaptability.

- Focus and Attention: Enhanced ability to sustain attention and participate in educational and therapeutic activities.

- Quality of Life: Overall improvements in daily functioning, social engagement, and family dynamics.

These outcomes align with the findings presented in my poster on the effects of cannabis on children with autism, which I am attaching for your review.

Texas Child Neurology, PLLC
4032 McDermott Road, Suite 100, Plano, TX 75024
www.texaschildneurology.com
Telephone: (972) 769-9000 Facsimile: (972) 769-0035
Subsidiary of Child Neurology Clinics of Texas

Robert S. Chudnow, MD, PA
Gerald M. So, MD, PA
Lina Shah, MD, PA
Mohsin Maqbool, MD, PA
Patricia S. Mireles, MD
Daniel Gossett, MD, PA
Safiullah Shareef, MD, PA
Andrew Hurd, MD, PA
Cerin Jacobs, MD

Medical cannabis, when administered under proper medical guidance and dosage, has demonstrated a favorable safety profile in my practice. The low incidence of adverse effects, coupled with the significant benefits, underscores its potential as a therapeutic option for ASD.

Patients and their families frequently report improved well-being and reduced dependence on traditional pharmacological interventions that often carry substantial side effects.

Given my clinical experience and the growing body of evidence supporting the use of medical cannabis for medically refractory symptoms of ASD, I strongly advocate for its inclusion as a qualifying condition in Ohio. This decision would provide families and physicians with a much-needed therapeutic option to address the complex needs of individuals with ASD who fail to benefit from traditional pharmaceutical interventions.

Thank you for considering this vital qualifying condition. Please do not hesitate to contact me for further information or clarification.

Sincerely,

Mohsin Maqbool, MD.
Board Certified American Board of Psychiatry and Neurology/Child Neurology
Board Certified Sleep Medicine
Consultant: Texas Child Neurology, LLC.
Neuroscience Faculty: University of Texas, Dallas.
Office: (972) 769-9000 x2156
Fax: (972) 769-0035
Cell: (313) 289-1179
mmaqbool@texaschildneurology.com
Mohsin.Maqbool@UTDallas.edu

Section 2: Medical or Scientific Evidence (Relevant medical or scientific evidence pertaining to the disease or condition)

Autism spectrum disorders (ASD) are a group of complex neurodevelopment disorders involving challenges with social communication and interaction along with characteristic patterns of repetitive behavior that result in heterogeneous levels of disability. The diagnostic criteria involve the following[1]:

A. Persistent deficits in social communication and social interaction across multiple contexts that can include deficits in the following:

1. Social-emotional reciprocity
   - failure of normal back-and-forth conversation
   - reduced sharing of interests, emotions, or affect
   - failure to initiate or respond to social interactions

2. Nonverbal communicative behaviors used for social interaction
   - poorly integrated verbal and nonverbal communication
   - poor eye contact and body language or deficits in understanding and use of gestures
   - lack of facial expressions and nonverbal communication

3. Developing, maintaining, and understanding relationships
   - difficulties adjusting behavior to suit various social contexts
   - problems with sharing imaginative play or in making friends
   -absence of interest in peers.

B. Restricted, repetitive patterns of behavior, interests, or activities that can include the following:

1. Stereotyped or repetitive motor movements, use of objects, or speech.
   - simple motor stereotypies
   - lining up toys or flipping objects
   - echolalia or idiosyncratic phrases

2. Insistence on sameness, inflexible adherence to routines, or ritualized patterns or verbal nonverbal behavior.
   - extreme distress at small changes
   - difficulties with transitions
   - rigid thinking patterns or greeting rituals
   - need to take same route or eat food every day

3. Highly restricted, fixated interests that are abnormal in intensity or focus.
   - strong attachment to or preoccupation with unusual objects
   - excessively circumscribed or perseverative interest

4. Exaggerated or diminished reactions to sensory input or unusual interests in sensory aspects of the environment.
   - apparent indifference to pain/temperature
   - adverse response to specific sounds or textures
   - excessive smelling or touching of objects
   - visual fascination with lights or movement

The diagnostic criteria specify that the symptoms must be present in the early developmental period but may not become fully manifest until adulthood when social demands exceed limited capacities or may be masked by learned strategies in later life, and that the symptoms cause clinically significant impairment in social, occupational, or other important areas of current functioning.

Refractory ASD has been defined as involving behavioral symptoms requiring medication adjustment despite previous trials of risperidone and aripiprazole or previous trials of three psychotropic drugs targeting the symptom cluster, one of which was risperidone or aripiprazole[2]. These disruptive symptoms particularly include aggression, self-injurious behavior, and severe tantrums. Refractory ASD can involve as much as 40% of those with ASD, particularly those with intellectual disability and who are at least 12 yrs old.

The pathophysiology of ASD is complex with multiple different genetic and environmental risk factors leading to abnormal neuron development, changes in brain structure, and alterations in brain function; these are summarized in the following figure[3]:

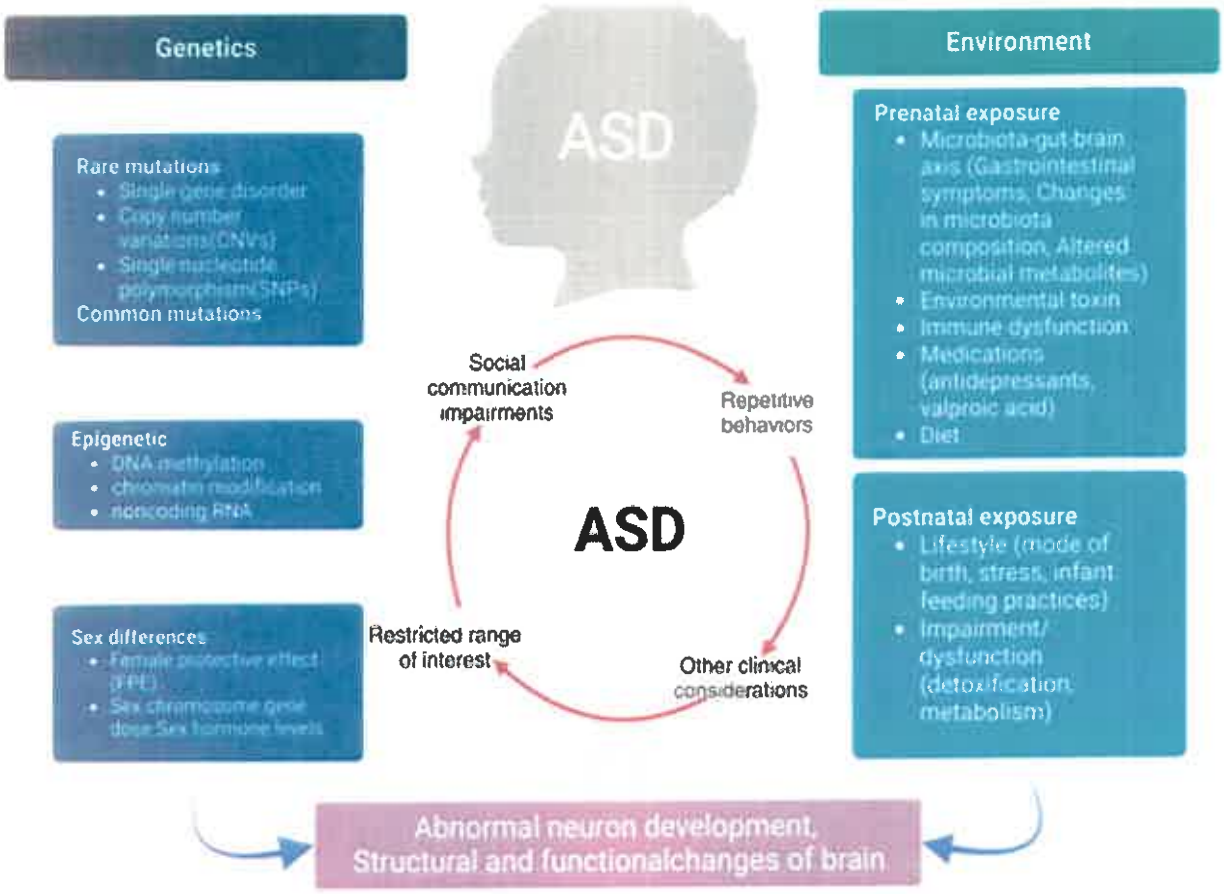


FIGURE 1 Potential influences of autism spectrum disorder (ASD). ASD is a heterogenous group of neurodevelopmental disorders characterized by social communication impairments, repetitive behaviors, restricted range of interest, and other clinical considerations. ASD is a multifactorial disease that involves the interactions of genetic and environmental factors. The genetic factors include genetics (single gene disorder, copy number variations and single-nucleotide polymorphism), epigenetic (DNA methylation, chromatin modification and noncoding RNA), and sex differences factors (female protective effect and sex chromosome gene dose sex hormone levels). In contrast, the environmental factors comprise prenatal exposure (microbiota–gut–brain axis, environmental toxin, immune dysfunction, medications, and diet) and postnatal exposure (lifestyle and impairment/dysfunction). These factors lead to abnormal neuron development, changes in the structure and function of the brain, resulting in ASD.

MedComm. 2024;5:e497.

In the U.S., the prevalence of ASD amongst children ages 3 to 17 yrs old increased by almost 300% between 1997 and 2008[4]. In subsequent years, the disorder rose further in prevalence from 1.1% of children in 2008 up to 2.7% in 2020[5]. The estimated prevalence in the American adult population is 2.2%, which is equivalent to 5.5 million individuals[6].

People diagnosed with ASD experience significant disruptions to their health and function. Compared to people without ASD, they have higher rates of depression (20% vs 7%), anxiety (11% vs 5%), sleep difficulties (13% vs 5%), and epilepsy (21% vs 0.8%)[7]. Largely due to their difficulties with communication and social interaction, children with ASD are bullied by peers at a rate 3-4x that of non-disabled peers, with a concomitant 3x increased rate of suicidal ideation and suicide attempts[8]. The following Adverse Childhood Events (ACEs) also occur more frequently:

- Income insufficiency (ASD 40%, control 23%)
- Parental divorce (ASD 28%, control 20%)
- Neighborhood violence (ASD 11%, control 8%)
- Household mental health (ASD 18%, control 7%)
- Household substance use problems (ASD 14%, control 10%)

In comparison to healthy control peers, children with ASD are twice as likely to have experienced four or more ACEs (10.2 versus 5.1%), which is unfortunately associated with a prolonged time to diagnosis of ASD and a 22-27% increase in the median age of entry into intervention services. Children with ASD experience much higher rates of emotional and behavioral problems (EBP) compared to those without ASD (Figure 1, below).

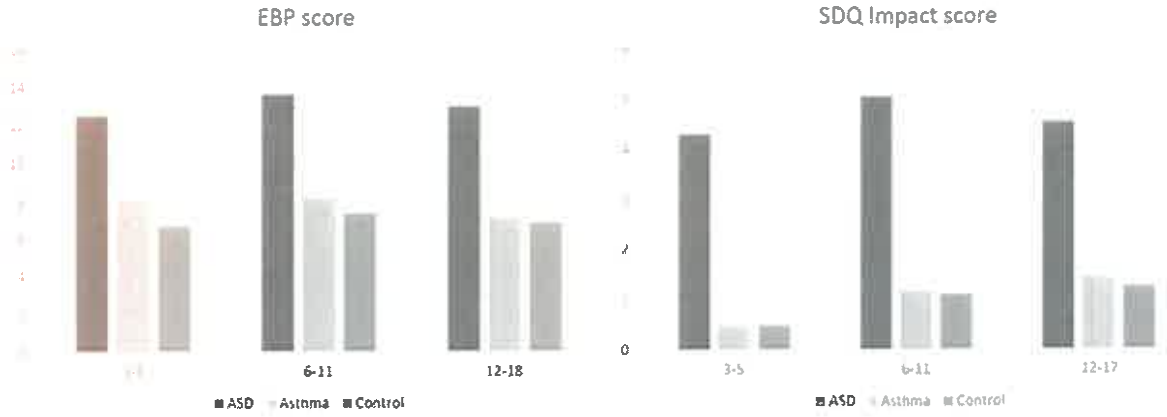


Figure 1: Emotional-Behavioral Problems (EBP) and impairment, by age and clinical group
Abbreviations: EBP: Emotional and Behavioral Problems; SDQ: Strengths and Difficulties Questionnaire; ASD: Autism Spectrum Disorder

J Dev Behav Pediatr. 2022 April 01; 43(3): 140–148.

There is also a highly significant correlation between EBP and impaired function (R =0.64), the impact of which was separate from the equally significant adverse effect of impaired social responsiveness (Table 3 below)[9].

Table 3.

Predictors of impairment in 440 children with ASD

Predictor	B (SE)	β	*p*	r_s	r_s^2	Pearson's r	Product measure	U	C	Total (U+C)	RW	GDW
Constant	−5.652 (.627)											
SRS t-score	.102 (.010)	.437	<.001	.901	.812	.653	.285	.113	.313	.426	51.7	51.5
EBP score	.200 (.024)	.358	<.001	.880	.774	.637	.228	.076	.329	.406	45.9	46.0
Age 6-11	.516 (.203)	.086	.017	.141	.020	.099	.009	.007	.002	.010	1.7	1.7
Parental education: High school or less	−.718 (.300)	−.081	.011	−.043	.002	−0.031	.003	.006	−.005	.001	0.7	0.8

Abbr.: SRS: Social Responsiveness Scale; EBP: Emotional Behavioral Problem score (see text)

Explanatory note: In this multiple regression analysis, the SDQ impact score that measures impairment in child's functioning was regressed on 10 pre-selected variables (8 socio-familial variables, the Emotional Behavioral Problem score (EBP), the autism symptomatology score (SRS)). Only 4 variables contribute significantly to the final model. Child age and parental education have negligible effects whereas emotional/behavioral problems and autistic symptoms strongly and independently predict child's impairment and at about the same level (see text for details).

Model summary: multiple R=.724; R^2=.525; R^2_{adj}=.520:

Regression statistics: B (SE): regression weights (standard error); β: standardized regression weights; r_S: structure coefficient (r/R); r_S^2: squared structure coefficient; r: zero-order correlation between predictor and impairment variable; Product measure: predictor contribution to R^2 (product of r and β)

Commonality analysis: U: % of impairment variance explained uniquely by predictor; C: % of impairment variance explained by the predictor that is shared with one or more other predictors; Total: % of impairment variance (unique and shared) explained by the predictor; See Table S2 for detailed results on commonality coefficients and relative weights

Relative importance analysis: RW: Relative Weights; GDW: General Dominance Weights. Raw RW and GDW sum up to R^2 (see Table S2). In the Table, RW and GDW have been rescaled to 100% and values represent the proportion of R^2 explained by the predictor.

J Dev Behav Pediatr. Author manuscript; available in PMC 2023 April 01.

Despite determined worldwide efforts, no treatments have yet been found to reverse the effects of ASD. As a consequence, people with the disorder continue to face life-long challenges that extend past childhood into adulthood, particularly pertaining to employment. Those who particularly suffer impairments in conversation and social skills from ASD have 50% less odds of employment following high school graduation[10]. People with ASD also have more difficulty earning a living wage and finding a suitable job for their skill set, especially amongst younger individuals and those with a lower level of academic achievement[11].

The ramifications of ASD extend far beyond the affected individual, with caregivers experiencing the following multitude of related difficulties:

a. Significant impairment in physical activity, social relationships, perceived Quality of Life, and health compared to caregivers of those with cerebral palsy or cognitive impairment without ASD[12].

b. Increased symptoms of parenting stress (negative parental self-views, lower satisfaction with parent–child bond, difficult child behaviors) and depression[13], with higher prevalence of depression and anxiety occurring in those caring for children with challenging behavioral problems[14].

c. A disproportionate impact on women as they are the primary caregivers in up to 80% of those with ASD, and who consequently experience restrictions on activities and reduced feelings of personal wellbeing[15].

d. Increased odds by 14x of being unemployed[16].

e. Nearly double the divorce rate compared to parents of children without ASD (23.5% vs 13.8%), with divorces continuing to occur up even up until the child reaches 30 years of age (Figure 1 below)[17].

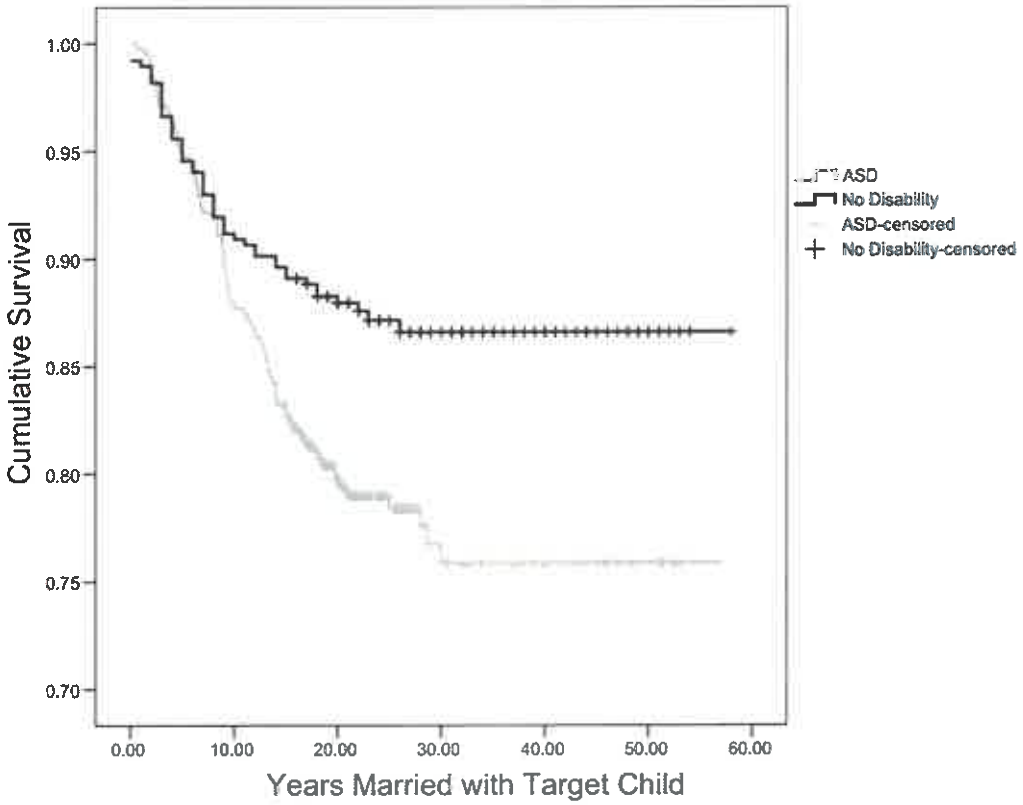


Figure 1. Survival plot for divorce in parents of children with an autism spectrum disorder (ASD) and parents of children without a disability.

Journal of Family Psychology
2010, Vol. 24, No. 4, 449–457

There has been exhaustive research into the financial impacts of autism, which extend far beyond childhood into adulthood and include several key findings[18, 19, 20]:

a. Average expenditure 10x that of children without ASD, due to increased requirement for inpatient psychiatric care.

b. A greater than 50% increase in length-of-stay for hospitalizations compared to those without ASD.

c. Compared to those without ASD, an increase in annual expenditure for outpatient office visit by 5x, for emergency room care by 6x, and for prescription drug use by 2x; these are primarily due to psychiatric co-morbidity.

d. Estimated annual loss in 2019 of $38,000 work productivity by each person with ASD, $31,000 in employment by each caregiver, and $56,000 in contribution to household services by caregivers.

e. Estimated lifetime societal cost in 2003 of $3.16 million, with over $2 million accruing after 18 years of age (Table 1 below).

Table 1. Age-Specific and Lifetime per Capita Incremental Societal Costs of Autism*

Age Group, y	Average Per Capita Cost per Age Group			Total Per Capita Cost
	Direct Medical	Direct Nonmedical	Indirect	
3-7	35 370	10 805	43 066	446 203
8-12	5013	15 709	41 138	314 297
13-17	5014	13 560	38 453	285 082
18-22	2879	10 720	36 090	248 446
23-27	1574	27 539	51 740	404 260
28-32	1454	23 755	35 757	304 828
33-37	1389	20 492	30 852	263 662
38-42	1283	17 676	29 132	240 457
43-47	1440	15 248	26 600	216 439
48-52	1447	13 152	24 331	195 650
53-57	1290	11 292	17 776	151 790
58-62	1218	9489	0	53 535
63-66	1027	7908	0	35 738
Total lifetime costs	305 956	978 761	1 875 667	3 160 384

*Costs presented in 2003 dollars. Costs for age 4 years and older are discounted to 2003 dollars using a discount rate of 3%. Life expectancy for men is age 66 years and for women, age 65 years.

Arch Pediatr Adolesc Med. 2007;161:343-349

With regard to the ASD population specifically residing in the state of Ohio, the following have been reported[19]:

a. A greater than 75% increase in the prevalence of ASD from 2008 to 2015.

b. Increase in the estimated number of new ASD diagnoses from 12,000 in the decade of 1990-1999 to 37,000 in the decade of 2010-2019.

c. A projected increase in new ASD cases for the decade of 2020-2029 of at least 36,000 and ranging up to 68,000.

d. Estimated expenditure in 2019 for Home and Community Based Services (HCBS) for each enrolled individual with ASD of $47,000 to $52,000, with cumulative lifetime per capita cost of $2.3 million.

e. Lifetime cost of the autism population in the following decades, in 2019 dollars:

1990-1999 = $42.8 billion

2000-2009 = $74.4 billion

2010-2019 = $130.8 billion

2020-2029 = $127.1 billion to $240.9 billion

In addition to the direct healthcare costs, there are substantial expenses for intensive therapy and specialized schooling, in-home support services, residential facilities for the care of individuals whose families cannot manage them at home, vocational training, and work-related accommodations.

In summary, refractory autism spectrum disorder that does not improve despite treatment clearly incurs tremendous difficulties on the individual and devastating effects on caregivers, with exponentially growing costs to society. The burgeoning prevalence of the disorder in the past three decades is without foreseeable limit and is conferring a logarithmic growth in the burden of the disorder. Treatments that would improve cognitive function and reduce psychiatric symptoms by a meaningful degree would have wide-ranging and consequential benefits.

References:

1. American Psychiatric Association. (2022). Neurodevelopmental Disorders. In *Diagnostic and statistical manual of mental disorders* (5th ed., text rev.). https://doi.org/10.1176/appi.books.9780890425787.x01 Neurodevelopmental Disorders

2. Adler BA, Wink LK, Early M, Shaffer R, Minshawi N, McDougle CJ, Erickson CA. Drug-refractory aggression, self-injurious behavior, and severe tantrums in autism spectrum disorders: a chart review study. Autism. 2015 Jan;19(1):102-6. doi: 10.1177/1362361314524641. Epub 2014 Feb 26. PMID: 24571823.

3. Zhuang H, Liang Z, Ma G, Qureshi A, Ran X, Feng C, Liu X, Yan X, Shen L. Autism spectrum disorder: pathogenesis, biomarker, and intervention therapy. MedComm (2020). 2024 Mar 2;5(3):e497. doi: 10.1002/mco2.497. PMID: 38434761; PMCID: PMC10908366.

4. Boyle CA, Boulet S, Schieve LA, Cohen RA, Blumberg SJ, Yeargin-Allsopp M, Visser S, Kogan MD. Trends in the prevalence of developmental disabilities in US children, 1997-2008. Pediatrics. 2011 Jun;127(6):1034-42. doi: 10.1542/peds.2010-2989. Epub 2011 May 23. PMID: 21606152.

5. Maenner MJ, Warren Z, Williams AR, et al. Prevalence and Characteristics of Autism Spectrum Disorder Among Children Aged 8 Years - Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2020. MMWR Surveill Summ. 2023 Mar 24;72(2):1-14. doi: 10.15585/mmwr.ss7202a1. PMID: 36952288; PMCID: PMC10042614.

6. Dietz PM, Rose CE, McArthur D, et al. National and State Estimates of Adults with Autism Spectrum Disorder. J Autism Dev Disord. 2020 Dec;50(12):4258-4266. doi: 10.1007/s10803-020-04494-4. PMID: 32390121; PMCID: PMC9128411.

7. Hirota T, King BH. Autism Spectrum Disorder: A Review. JAMA. 2023 Jan 10;329(2):157-168. doi: 10.1001/jama.2022.23661. PMID: 36625807.

8. Hoover DW, Kaufman J. Adverse childhood experiences in children with autism spectrum disorder. Curr Opin Psychiatry. 2018 Mar;31(2):128-132. doi: 10.1097/YCO.0000000000000390. PMID: 29206686; PMCID: PMC6082373.

9. Fombonne E, Croen LA, Bulkley JE, Varga AM, Daida YG, Hatch BA, Dickerson JF, Lynch FL. Emotional and Behavioral Problems in Youth with Autism: High Prevalence and Impact on Functioning. J Dev Behav Pediatr. 2022 Apr 1;43(3):140-148. doi: 10.1097/DBP.0000000000001028. Epub 2021 Oct 21. PMID: 34693924; PMCID: PMC9021329.

10. Roux AM, Shattuck PT, Cooper BP, et al. Postsecondary employment experiences among young adults with an autism spectrum disorder. J Am Acad Child Adolesc Psychiatry. 2013 Sep;52(9):931-9. doi: 10.1016/j.jaac.2013.05.019. Epub 2013 Jul 31. PMID: 23972695; PMCID: PMC3753691.

11. Espelöer J, Proft J, Kemmer P, Falter-Wagner CM, Vogeley K. What is specific about employment status, workplace experiences and requirements in individuals with autism in Germany? Autism Res. 2023 Jul;16(7):1389-1402. doi: 10.1002/aur.2958. Epub 2023 May 23. PMID: 37218532.

12. Mugno D, Ruta L, D'Arrigo VG, Mazzone L. Impairment of quality of life in parents of children and adolescents with pervasive developmental disorder. Health Qual Life Outcomes. 2007 Apr 27; 5:22. doi: 10.1186/1477-7525-5-22. PMID: 17466072; PMCID: PMC1868708.

13. Lai WW, Goh TJ, Oei TP, Sung M. Coping and Well-Being in Parents of Children with Autism Spectrum Disorders (ASD). J Autism Dev Disord. 2015 Aug;45(8):2582-93. doi: 10.1007/s10803-015-2430-9. PMID: 25800867.

14. Sharpley, C. F., Bitsika, V., & Efremidis, B. (1997). Influence of gender, parental health, and perceived expertise of assistance upon stress, anxiety, and depression among parents of children with autism. *Journal of Intellectual & Developmental Disability*, *22*(1), 19–28. https://doi.org/10.1080/13668259700033261

15. Paruk, S., & Ramdhial, M. (2018). Prevalence of caregiver burden, depressive and anxiety symptoms in caregivers of children with psychiatric disorders in Durban, South Africa. *South African Journal of Psychiatry, 24*, 1 page. doi:**https://doi.org/10.4102/sajpsychiatry.v24i0.1314**

16. Lynch FL, Bulkley JE, Varga A, et al. The impact of autism spectrum disorder on parent employment: Results from the r-Kids study. Autism Res. 2023 Mar;16(3):642-652. doi: 10.1002/aur.2882. Epub 2022 Dec 22. PMID: 36546608.

17. Hartley SL, Barker ET, Seltzer MM, Floyd F, Greenberg J, Orsmond G, Bolt D. The relative risk and timing of divorce in families of children with an autism spectrum disorder. J Fam Psychol. 2010 Aug;24(4):449-57. doi: 10.1037/a0019847. PMID: 20731491; PMCID: PMC2928572.

18. Rogge N, Janssen J. The Economic Costs of Autism Spectrum Disorder: A Literature Review. J Autism Dev Disord. 2019 Jul;49(7):2873-2900. doi: 10.1007/s10803-019-04014-z. PMID: 30976961.

19. Cakir, J., Frye, R.E., & Walker, S.J. (2020). The lifetime social cost of autism: 1990–2029. *Research in Autism Spectrum Disorders, 72*, 101502. ISSN 1750-9467, https://doi.org/10.1016/j.rasd.2019.101502.

20. Ganz ML. The Lifetime Distribution of the Incremental Societal Costs of Autism. *Arch Pediatr Adolesc Med.* 2007;161(4):343–349. doi:10.1001/archpedi.161.4.343

SECTION 3: Conventional Medical Therapies (Consideration of whether conventional medical therapies are insufficient to treat or alleviate the disease or condition)

The current state of therapy for ASD is limited to attempts at controlling symptoms, particularly aggression and psychomotor agitation. There are no treatments proven to reverse the core ASD features of communication deficits and restricted/repetitive behaviors. In 2018, a panel of British experts released a summary review of the available pharmaceutical options for treating ASD, along with treatment guidelines[1]. Amongst the notable statements from this review are the following:

- "**Risperidone** is approved by the FDA for treatment of irritability in ASD (2006) with up to 57% reduction in irritability. It appears to be tolerated reasonably well but long-term (6 months) use **was associated with persistent side-effects**, including increased appetite, weight gain, mild sedation, hyper salivation and hyperprolactinemia."

- "**Aripiprazole** is also approved by the FDA for treatment of irritability in ASD (2010). A line-item analysis of the two randomized controlled trials revealed that aripiprazole had **no effect** on self-injurious behaviour. A large long term study of aripiprazole reported that the benefits of aripiprazole on irritability were maintained over the study period (Marcus et al., 2011). However, discontinuation due to side effects occurred in about 10%, with aggression and weight increase the most commonly reported. In conclusion, there is a reasonable body of evidence indicating that risperidone and aripiprazole are effective at treating irritability in ASD with moderate to large effect sizes. However, their potential benefits should be weighed against the risk of side effects . . . In view of the risk of persistent side-effects, **we also recommend periodic attempts to reduce the daily dosage and discontinue** to either confirm the necessity for on-going exposure, or establish that the need for the drug has resolved."

- "Taken together, the studies of dopamine receptor blockers provide evidence that these agents may be beneficial for the treatment of repetitive behaviours in ASD . . . in view of the potential risk of adverse effects, it is **not recommended** that they are routinely used to treat repetitive behaviors."

- "Overall, the evidence is currently **too limited** to support the routine use of any of the agents discussed above (**citalopram, fluoxetine, fluvoxamine, memantine, risperidone, aripiprazole, methylphenidate, oxytocin**) for the core symptoms of ASD (deficits in social communication and interaction, and restricted and repetitive interests or behaviours). Although risperidone and aripiprazole have both shown modest efficacy for the management of repetitive behaviours (evidence level IIa), these studies focussed on individuals with high levels of irritability and it is **unclear** whether the findings would generalize to the wider ASD population. Furthermore, side effects should be carefully considered."

- "**SSRIs** and other anti-depressants are widely prescribed for people with ASD. However, there have been **no rigorous studies** have investigated the role of SSRIs in treating mood disorders in children with ASD. Given the lack of direct evidence for SSRIs and ASD, the use of SSRIs to treat depression is therefore based on extrapolation of trials in patients without ASD."

- "Overall, there is **little or no evidence** for treating anxiety or OCD symptoms with **risperidone, clomipramine or an SSRI**. The studies of risperidone are limited to participants with high levels of irritability and did not select participants on the basis of clinically significant anxiety or OCD symptoms. Hence it is unclear whether any positive effects are clinically meaningful or pertain to those with co-occurring anxiety/OCD. Same applies to studies of SSRIs, although here the combined evidence failed to identify an effect on anxiety or OCD symptoms."

- "Overall, **minocycline**'s potential benefit for reducing irritability **needs additional study** before routine use can be recommended."

- "Amantadine is a non-competitive NMDA antagonist. Despite encouraging case reports and small open-label studies, a small controlled trial by King et al. (2001) reported **no effect of amantadine** on responder rate or irritability ratings (evidence Ib). Thus, current evidence does not support the use of amantadine for irritability."

- "In view of the limited data available for minocycline, randomized, double blind controlled studies are required before recommendations can be made. The current evidence **does not support the use of arbaclofen or amantadine** for irritability."

- "Methylphenidate . . . these findings suggest that, although effective, **methylphenidate may not be as effective in people with ASD** as in people with ADHD and that individuals with ASD are more likely to experience side-effects."

- "**Atomoxetine**, a non-stimulant drug for ADHD is an alternative to methylphenidate. Evidence from one small and one medium study **demonstrate improvement in symptoms of hyperactivity but not inattention** (Hedge's g=0.83, effect size d=0.90) (Harfterkamp et al., 2012; Arnold et al., 2006) (evidence level Ib). The most common side effects were nausea, fatigue and sleeping difficulties."

- "The evidence for treating mood disorders in adults with Autism Spectrum Disorder is very limited. Only one SSRI (**fluoxetine**) has been studied in adults with ASD, **with no change in depression** relative to placebo (Buchsbaum et al., 2001) (evidence level IIa)."

- "In summary, benefits have been reported in small studies using SSRIs (fluoxetine, fluvoxamine) as a treatment for anxiety disorders, predominantly OCD, in adults with ASD. Although **SSRIs are generally well tolerated, the beneficial effects are modest, and the evidence is limited. There is currently insufficient evidence to recommend risperidone**."

- "Treating irritability has been less well studied in adults than it has been in children with ASD.

- In summary, there **is limited evidence to guide the treatment of irritability in adults with ASD**. A dopamine blocker such as risperidone or SSRI could be tried cautiously and side-effects should be carefully monitored (including relevant medical assessments and lab tests)."

- "There have **not been any** randomized controlled trials that have investigated the role of stimulant or non-stimulant medications in treating ADHD in adults with ASD."

- "**No current studies** are available for treating tic disorders in children or adults with ASD specifically."

This same expert panel also reviewed the available evidence for non-pharmacological (i.e. psychological) approaches for treating the core symptoms of ASD in children and reported, "there was evidence of efficacy for some of these programmes over treatment as usual at reducing symptoms of social interaction impairment. . . However, these effects **were small and often not clinically meaningful**". In adults with ASD, the results of a randomized clinical trial with emotion recognition training were reviewed and found "**no positive treatment effect** . . . on general emotion recognition".

A more recently published review in 2021 reported that two double-blind, placebo-controlled studies on selective serotonin reuptake inhibitors (SSRIs) provided evidence **against** the use of SSRIs for repetitive behaviors in youth with ASD. In addition, multiple clinical trials of oxytocin, vasopressin, and memantine did not demonstrate any consistent benefit on social impairment in ASD[2].

Deep brain stimulation (DBS) has been evaluated in a very small number of patients and revealed mixed results.[3] Accelerated theta burst transcranial magnetic stimulation (aTBS) has been studied 10 subjects with refractory major depressive disorder in ASD, with full remission in 5 patients and partial remission in 3 patients, but this was an open-label trial without a placebo group [4]. Both DBS and aTBS are only available at highly specialized academic tertiary care centers at considerable expense, so their availability is extremely limited.

The consistent theme in these summary reviews is that utilization of the current armamentarium of pharmaceutical agents in treating people with ASD is based on an extraordinarily limited body of evidence. These standard therapies confer only modest and short-term efficacy on a limited number of symptoms, and do not improve the most disruptive

features of the condition. The two medications that are currently FDA approved for ASD (risperidone, aripiprazole) are only indicated for the treatment of irritability and not for core symptoms of ASD, with their long-term use restricted by the significant potential for permanent and disruptive adverse effects. Despite the lack of robust clinical evidence supporting any meaningful benefit to offset their considerable risk and cost, these pharmaceutical medications remain in widespread use for treatment of ASD. An alternative treatment is clearly indicated particularly for those with refractory ASD who do not respond to any interventions, as the currently available options do not address the root cause of the disorder and are not otherwise effective in any meaningful way.

References:

1. Howes OD, Rogdaki M, Findon JL, Wichers RH, Charman T, King BH, Loth E, McAlonan GM, McCracken JT, Parr JR, Povey C, Santosh P, Wallace S, Simonoff E, Murphy DG. Autism spectrum disorder: Consensus guidelines on assessment, treatment and research from the British Association for Psychopharmacology. J Psychopharmacol. 2018 Jan;32(1):3-29. doi: 10.1177/0269881117741766. Epub 2017 Dec 14. PMID: 29237331; PMCID: PMC5805024.

2. Thom RP, Pereira JA, Sipsock D, McDougle CJ. Recent Updates in Psychopharmacology for the Core and Associated Symptoms of Autism Spectrum Disorder. Curr Psychiatry Rep. 2021 Oct 13;23(12):79. doi: 10.1007/s11920-021-01292-2. PMID: 34643815.

3. Marini S, D'Agostino L, Ciamarra C, Gentile A. Deep brain stimulation for autism spectrum disorder. World J Psychiatry. 2023 May 19;13(5):174-181. doi: 10.5498/wjp.v13.i5.174. PMID: 37303931; PMCID: PMC10251363.

4. Blank E, Gilbert DL, Wu SW, Larsh T, Elmaghraby R, Liu R, Smith E, Westerkamp G, Liu Y, Horn PS, Greenstein E, Sweeney JA, Erickson CA, Pedapati EV. Accelerated Theta Burst Transcranial Magnetic Stimulation for Refractory Depression in Autism Spectrum Disorder. J Autism Dev Disord. 2024 May 15. doi: 10.1007/s10803-024-06244-2. Epub ahead of print. PMID: 38744742.

SECTION 4: Supporting Evidence (Evidence supporting the use of medical marijuana to treat or alleviate the disease or condition, including journal articles, peer-reviewed studies, and other types of medical or scientific documentation)

The basis of cannabis treatment is a concept called Clinical Endocannabinoid Deficiency, which arose following the discovery of cannabinoid receptors, initially with the description of CB1 in 1988 and followed by the elucidation of CB2 in 1991[1,2]. This led to the hypothesis that these receptors must be interacting with ligands produced within the human body itself, called endocannabinoids. This is analogous to how the presence of mu receptors implies the synthesis of endorphins (endogenous opioids). Cannabinoid receptors are not simply waiting in a dormant state until a person ingests cannabis, they are an active and ongoing participant in normal human physiology. The suspected presence of endocannabinoids was confirmed in 1992 by the discovery of N-arachidonoylethanolamine which was named "anandamide"[3], and the subsequent elucidation of a second compound called 2-arachidonoylglycerol (2-AG)[4,5]. Several other compounds have also been described to have varying interactions with cannabinoid receptors, but anandamide and 2-AG are considered to be the predominant endocannabinoids[6].

Cannabis contains over 120 phytocannabinoids which are separate compounds from endocannabinoids, but both types of cannabinoid molecules bind to cannabinoid receptors. Many different cell types of the human body produce endocannabinoids as part of a signaling pathway called the endocannabinoid system which plays a central role in maintaining homeostasis and recovery from pathological insults[7]. As an example, elevated levels of both endocannabinoids and cannabinoid receptors have been found in colonic polyps and colon cancer, suggesting that the human body itself is attempting to eradicate neoplastic tissue[8].

The concept of Clinical Endocannabinoid Deficiency postulates that intractable health conditions arise from either an innate or acquired deficiency of these endocannabinoids, where the body is unable to produce sufficient levels of these compounds[9]. This impairs the ability of the endocannabinoid system to maintain homeostasis and allows the emergence of disease. Evidence is mounting that this hypothesis is involved in a variety of human diseases and disorders. As an example, multiple studies have shown that CSF levels of endocannabinoids are significantly reduced in people with migraine, epilepsy, multiple sclerosis, and PTSD (figures below)[10,11,12,13].

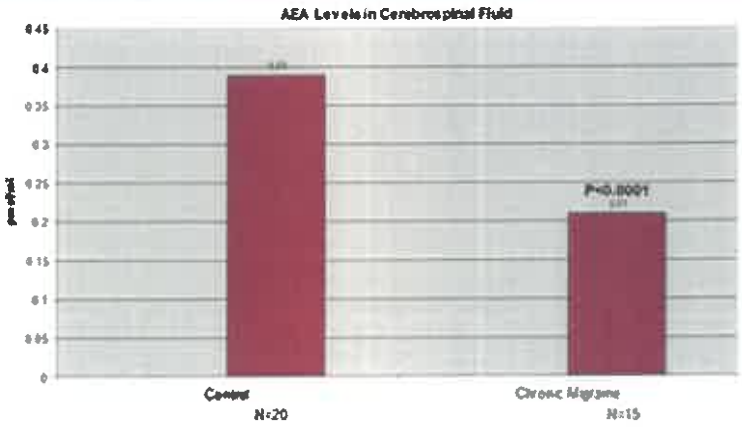


FIG. 2. Anandamide levels in cerebrospinal fluid of chronic migraine patients versus controls, adapted from data obtained from Sarchielli et al.[51]

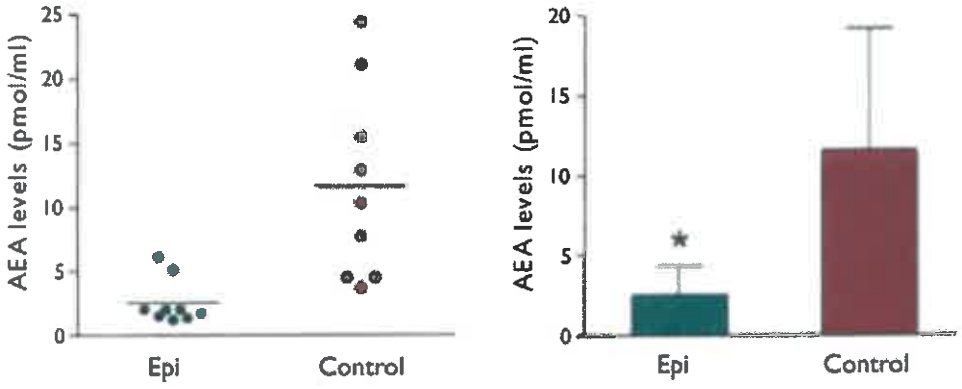


Figure 1.
AEA and 2-AG levels in cerebrospinal fluid (CSF) of epileptic and control patients. (**A**) The graphs show that AEA levels are significantly reduced in the CSF of epileptic versus control

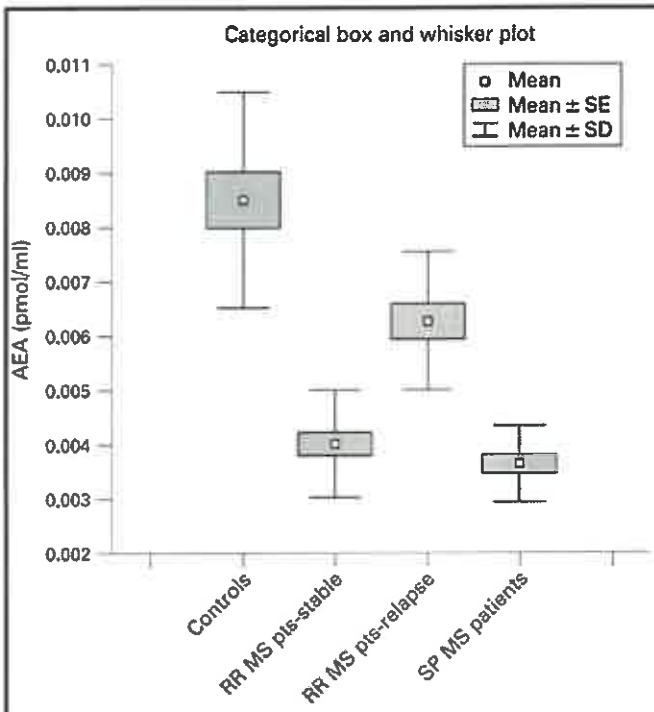


Figure 1 Plot of the levels of AEA, expressed as pmol/ml in the control subjects, in RR MS patients (both in a stable phase and during a relapse) and in SP MS patients. AEA, arachidonoyl-ethanolamine; RR MS, relapsing remitting multiple sclerosis; SE, standard error; SD, standard deviation; SP MS, secondary progressive multiple sclerosis.

J Neurol Neurosurg Psychiatry 2008;**79**:1224–1229. doi:10.1136/jnnp.2007.139071

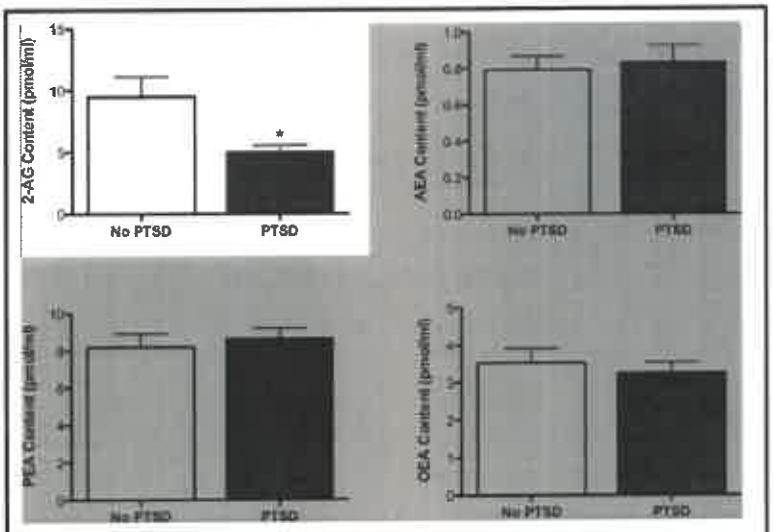
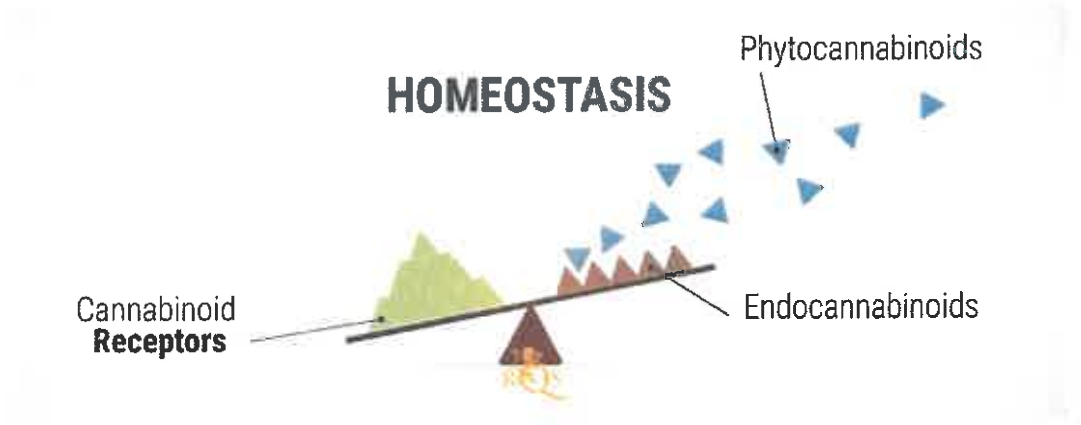


Figure 1.
Plasma 2-arachidonoylglycerol (2-AG), anandamide (AEA), palmitoylethanolamide (PEA) and oleoylethanolamide (OEA) concentrations in individuals who were diagnosed with post-traumatic stress disorder (PTSD) or healthy age and gender matched controls (No PTSD). Plasma levels of 2-AG, but not AEA, PEA or OEA, are found to be reduced in PTSD. Data are presented as mean +/− SEM. Significant differences ($p < 0.05$) are denoted by *.

Psychoneuroendocrinology. 2013 December ; 38(12)

The therapeutic benefit of medical cannabis arises from the ability of phytocannabinoids in cannabis to replace the deficient endocannabinoids and restore the ability of the endocannabinoid system to repair the human body.



This accounts for the potential of cannabis to treat conditions ranging from cancer to PTSD to multiple sclerosis, all of whom involve loss of physiological equilibrium. Cannabis is not merely a soporific "Band-Aid" to make people forget about their afflictions, it can potentially address the pathophysiologic linchpin underlying their disease.

The endocannabinoid system is essential for regulating synaptic function by judicious inhibition of neurotransmitter release from presynaptic neurons. Deficiency of endocannabinoids would disrupt this function and contribute to the impaired synaptic transmission and disrupted neural connectivity that leads to ASD[14].

Multiple studies corroborate the presence of endocannabinoid deficiency in people with ASD. Endocannabinoid levels are reduced in those with ASD compared to normal controls (Figures A, B)[15,16], while cannabinoid receptors are more highly expressed in people with ASD, likely as a compensatory mechanism for the loss of signaling by the diminished endocannabinoid levels (Figure C)[17]. This validates the rationale treating ASD with cannabis, as a means of correcting the endocannabinoid imbalance that is likely propagating the disorder.

Figure A

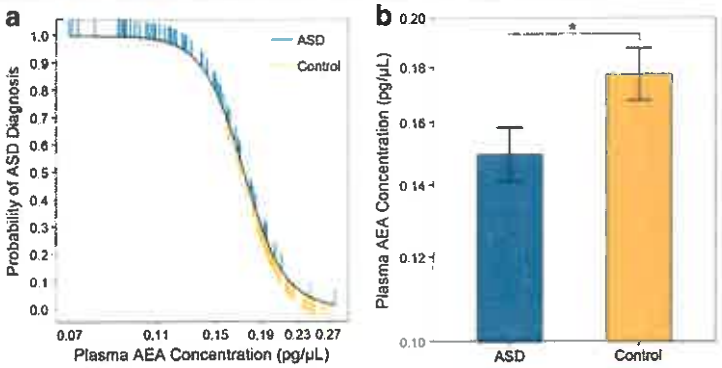


Fig. 1 Plasma AEA concentrations in children with ASD and neurotypical control children. a Plasma AEA concentration significantly differentiates cases from controls. Plasma AEA is plotted partialed (adjusted) for other variables in the statistical model. ASD individuals plotted above, and control individuals plotted below, the dashed lines are correctly classified. b Plasma AEA concentrations in children with and without ASD, back-transformed from logged values. Data are presented as LSM ± SEM. Children with ASD had lower plasma AEA concentrations (mean 0.149 pg/μl) compared to control children (mean 0.177 pg/μl)

Karhson *et al. Molecular Autism* (2018) 9:18

Figure B

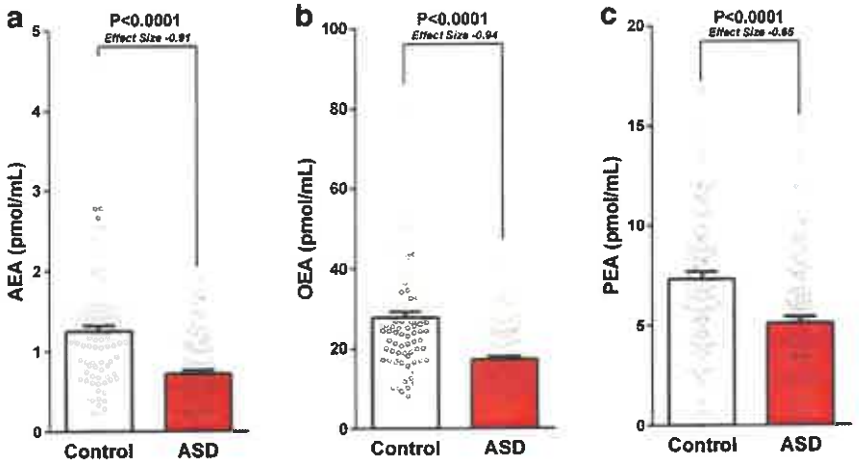


Fig. 1 Lower serum endocannabinoid levels in children with ASD. Legend: low endocannabinoid "tone" in serum samples of 93 children with ASD compared with 93 age- and gender-matched controls. Results of anandamide (AEA; panel a), oleoylethanolamine (OEA; panel b), and palmitoylethanolamide (PEA; panel c) are presented as mean, standard error, and distribution respectively

Aran *et al. Molecular Autism* (2019) 10:2

Figure C

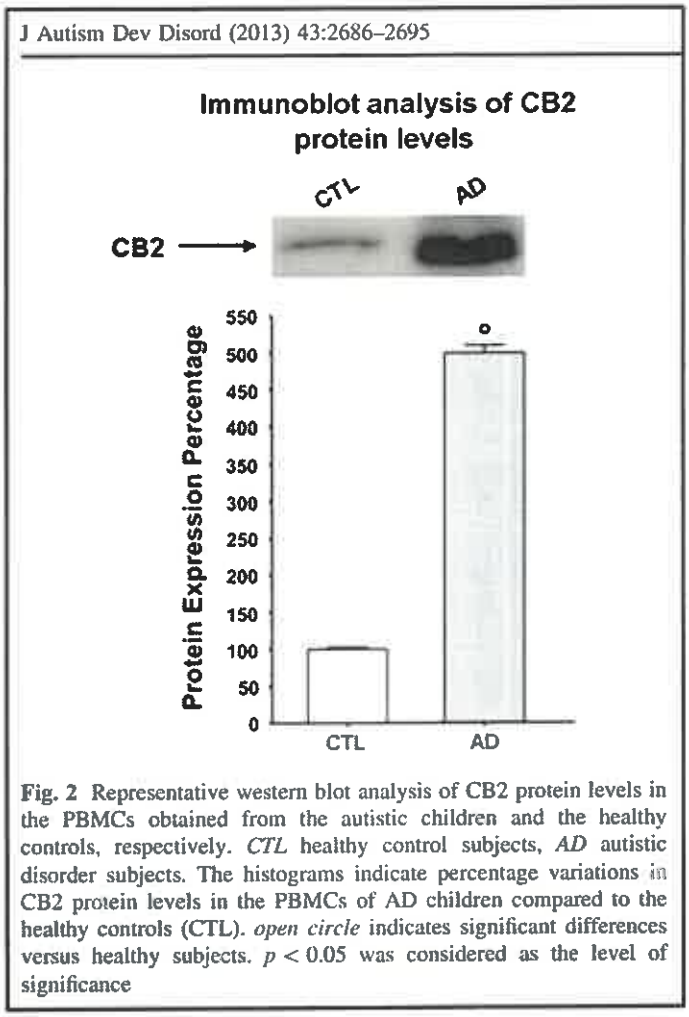
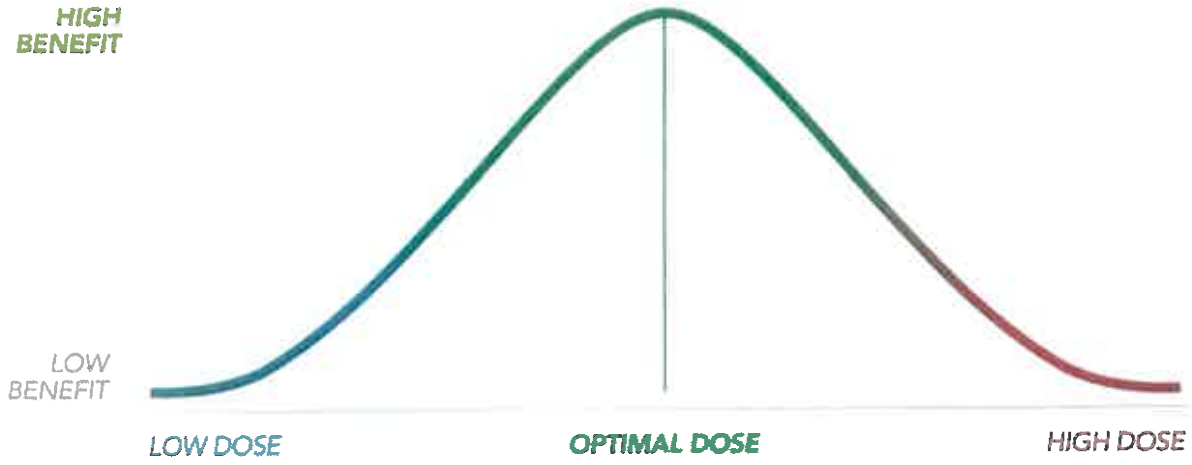


Fig. 2 Representative western blot analysis of CB2 protein levels in the PBMCs obtained from the autistic children and the healthy controls, respectively. *CTL* healthy control subjects, *AD* autistic disorder subjects. The histograms indicate percentage variations in CB2 protein levels in the PBMCs of AD children compared to the healthy controls (CTL). *open circle* indicates significant differences versus healthy subjects. $p < 0.05$ was considered as the level of significance

There are multiple potential mechanisms of action for cannabinoids in treating both the core and symptomatic features of ASD[18]. This includes modulating the elevated levels of neuroinflammation and microglial activation that contribute to dysregulated synaptic pruning, reducing neurotoxicity induced by beta-amyloid, regulating activation of the hippocampus to improve cognition and reduce anxiety, correcting circadian rhythms to improve restorative sleep, and inhibiting activity of the amygdala to improve socialization.

With respect to cannabis dosing, patients typically experience incremental improvements in their condition as they gradually increase the dose, with a diminution of benefit after a certain level. The dose at which the zenith of benefit occurs is designated the Optimal Dose, and

represents the quantity of phytocannabinoids that is sufficient to replete the deficient endocannabinoids and empower the endocannabinoid system to regain homeostasis[19].



This biphasic dose-response curve of cannabis is the same as that of the vast majority of pharmaceutical agents which are typically up-titrated until optimal effect is achieved, and decreased upon the advent of adverse effects and/or loss of benefit. Examples of this in conventional medicine include finding the precise dose of levothyroxine to achieve euthyroidism or the optimal insulin regimen to maintain euglycemia.

What is remarkable about cannabis is that the Optimal Dose for treating ASD is typically much lower than those of other conditions such as PTSD; this dosing strategy is referred to as micro dosing. One singular example of this is a case report in 2020 of a child with selective mutism, anxiety, and epilepsy related to ASD who was treated with a cannabis tincture containing a THC to CBD ratio of 1 to 20 (0.001% THC and 0.02% CBD)[20]. His symptoms became manifest at 4 yrs old and psychotherapy for mutism was started when he was 9 yrs old. ASD was formally diagnosed at 12 yrs of age, at which time he was reported to have no empathy, poor eye contact, and selective mutism with poor conversation; these symptoms had continued despite three years of ongoing intervention. With respect to seizure control, he had failed carbamazepine due to GI upset, clobazam due to suicidal ideation, and valproic acid due to weight gain of 13 kg. Cannabis treatment was initiated at 13 yrs of age with 0.1mL BID (four drops a day) and was increased after 3 months to 0.2mL BID (eight drops a day). This is

equivalent to a cannabinoid dose of only 0.4mg THC and 8mg CBD per day. This dose was maintained for the subsequent 3 years, after which he exhibited marked improvements in aggressiveness, irritability, concentration, sleep, talkativeness, and social interaction. No side effects were observed. By the end of the reporting period, he was able to initiate and reciprocate conversation with acquaintances (e.g. doctors) which he had previously been unable to do, as well as maintain both a vegetarian diet and exercise program with weight loss of 6.4 kg. These improvements culminated in his starting a part-time job involving customer service and maintaining a romantic relationship with a girlfriend (Figure 1 below).

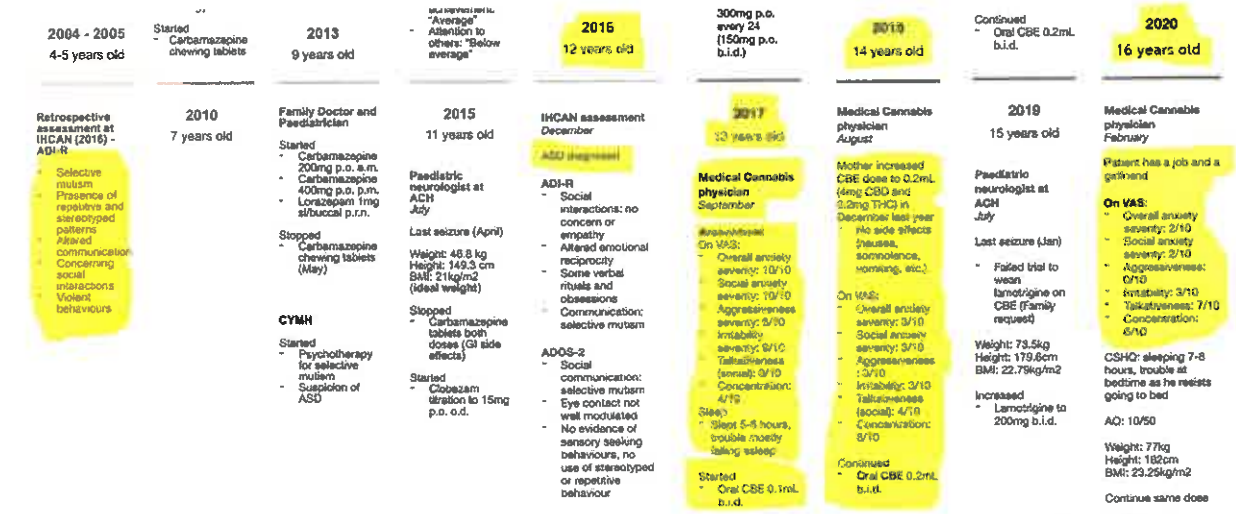


Fig. 1 Patient's timeline depicting important dates and events. *ACH* Alberta Children's Hospital, *ADI-R* Autism Diagnostic Interview – Revised, *ADOS-2* Autism Diagnostic Observation Schedule 2, *ASD* autism spectrum disorder, *AQ* Autism Spectrum Quotient (Adult), *BC* British Columbia, *BMI* body mass index (calculated by Du Bois method), *CBD* cannabidiol, *CBE* cannabidiol-based extract, CSHQ Children's Sleep Habits Questionnaire (Abbreviated), *CYMH* Child and Youth Mental Health, *IHCAN* Interior Health Children's Assessment Network, *OCD* obsessive-compulsive disorder, THC delta-9-tetrahydrocannabinol, *upset stomach* gastrointestinal side effects, *VAS* visual analog scale, *VPA* valproic acid. VAS severity for overall anxiety, social anxiety, aggressiveness and irritability, 0 = least severe, 10 = most severe. VAS for talkativeness, 0 = quiet, 10 = very talkative. VAS for focus, 0 = unfocused, 10 = focused

Ponton *et al. Journal of Medical Case Reports* (2020) 14:162
https://doi.org/10.1186/s13256-020-02478-7

This patient had a high premorbid likelihood of being unemployed and socially isolated but was instead was able to beat the odds with an extremely small amount of cannabis. These may seem like abstract words on a page, but one has to imagine the outcome on both the patient and his family if his severe social impairments had continued without change. In general, single case reports are not a high level of evidence but the remarkable degree of positive impact with the patient attaining normal employment and socialization status is noteworthy as, by

comparison, there are no published reports of similarly consequential improvement occurring with any of the conventional pharmaceutical treatments currently being employed.

There is understandable concern about the potential adverse effects of THC as this cannabinoid is implicated in causing most of the unwanted side effects of cannabis, particularly increase risk of schizophrenia[21]. The available literature regarding cannabis in ASD, however, highlights the role of CBD in providing most of the potential benefit in these patients[22]. This includes effects in pathways for generating empathy and responsiveness to social rewards (e.g. responding to happy faces), increased anxiolysis, normalization of circadian rhythm, and correction of neurodevelopment. This premise is exemplified by the afore-mentioned case report where the predominant cannabinoid was CBD and only a 5% fraction of the total cannabinoid dose was comprised of THC. As such, the optimum cannabis regimen for ASD would predominantly contain CBD and greatly minimize the risks associated with THC.

A large case series reported in 2018 described the experience of 188 people with ASD who received treatment with medical cannabis tinctures over a 2-year period[23]. The majority of tinctures contained 30% CBD and only 1.5% THC, with daily THC doses ranging from 3mg to 21mg. Over 80% of subjects were able to continue treatment for 6 months, by which time a striking number of participants reported improvement in multiple behavioral symptoms, sleep, cognition, and seizures (Table 2 below).

		Change at six months		
	Intake prevalence Total (188)	Symptom disappeared	Improvement	No change or deterioration
Restlessness, No. (%)	170 (90.4)	1 (1.2)	71 (89.8)	7 (8.8)
Rage attacks, No. (%)	150 (79.8)	1 (1.3)	65 (89.0)	7 (9.5)
Agitation, No. (%)	148 (78.7)	1 (1.4)	57 (83.8)	10 (14.7)
Sleep problems, No. (%)	113 (60.1)	9 (19.5)	27 (58.6)	10 (21.7)
Speech Impairment, No. (%)	113 (60.1)	—	15 (30)	35 (70)
Cognitive impairment, No. (%)	91 (48.4)	—	15 (27.2)	40 (72.7)
Anxiety, No. (%)	69 (36.7)	—	24 (88.8)	3 (11.1)
Incontinence, No. (%)	51 (27.1)	2 (9.0)	7 (31.8)	13 (59.0)
Seizures, No. (%)	23 (12.2)	2 (15.3)	11 (84.6)	—
Limited Mobility, No. (%)	17 (9.0)	2 (18.1)	—	9 (81.8)
Constipation, No. (%)	15 (8.0)	1 (12.5)	6 (62.5)	2 (25)
Tics, No. (%)	15 (8.0)	1 (20.0)	4 (80.0)	—
Digestion Problems, No. (%)	14 (7.4)	1 (12.5)	5 (62.5)	2 (25.0)
Increased Appetite, No. (%)	14 (7.4)	1 (33.3)	1 (33.3)	1 (33.3)
Lack of Appetite, No. (%)	14 (7.4)	2 (40.0)	1 (20.0)	2 (40.0)
Depression, No. (%)	10 (5.3)	—	5 (100.0)	—

Table 2. Symptom prevalence and change. Symptom prevalence at intake in 188 patients assessed at intake and change at six months in patients responding to the six-month questionnaire.

In terms of symptom improvement (quality of life, positive mood, dressing/showering independently, sleep, concentration), over 80% reported either significant or moderate improvement. Over 34% were able to **reduce** their consumption of pharmaceutical medication which included anti-psychotics, anti-epileptics, sedative hypnotics, and anti-depressants, with 20% of those taking anti-psychotics at the start of the trial being able to **discontinue** them altogether. The majority of those who discontinued treatment did so due to lack of therapeutic effect, with only 3% of the entire cohort discontinuing due to side effects (restlessness, sedation,

psychoactive effect, appetite change, digestion problems, dry mouth); these side effects resolved upon cannabis discontinuation. It is again notable that improvements of this magnitude with such sustained duration have never been reported with any conventional pharmaceutical agent. It is especially remarkable that this single therapeutic intervention was associated with benefits involving multiple symptom complexes, including cognition.

In 2022, a multi-center group in Brazil published the results of a **randomized double-blind placebo-controlled trial** of cannabis treatment in ASD[24]. A cohort of 64 children between the ages of 5-11 years were randomized to receive either a cannabis tincture or placebo for 12 weeks. The trial subjects were evaluated using a semi-structured interview answered by caregivers and quantified using the Autism Treatment Evaluation Checklist (ATEC) and Childhood Autism Rating Scale (CARS). The assessed outcome measures included aggressiveness, psychomotor agitation, concentration, number of meals per day, number of hours of sleep per day, social interaction with peers, verbal language, anxiety, and stereotypies. The cannabis product contained a THC:CBD ratio of 1:9 (i.e. CBD-rich) at a concentration of 0.5% (5mg/mL, 0.5mg THC/mL and 4.5mg CBD/mL). The placebo product had the same consistency, color, odor, and other organoleptic characteristics such that it was indistinguishable from the cannabis product. The study groups were instructed to start with 6 drops daily of the tincture (1 drop = ~0.05mL), which translated to a starting daily THC dose for the cannabis group of only 0.15mg. The daily dose was increased as needed by two drops at a time up to optimal clinical response as directed by the researchers, no more frequently than twice a week, and up to maximum daily dose of 70 drops (equivalent to 3.5mL of tincture and 1.75mg of THC). A total of four children dropped out of the study (1 cannabis, 3 placebo). At the end of the trial period, statistically significant improvements occurred in psychomotor agitation, meals eaten per day, social interaction, and anxiety (Table 3). Ability to concentrate also improved in the cannabis treatment group, although only in those subjects with mild ASD (Table 4).

Table 3 - Assessment of different variables in children with autism spectrum disorder (ASD) in the control group and the treatment group

Variable	Treatment group (n = 31)	Control group (n = 29)	p-value
Aggressiveness	0.81 (0.00) ± 1.05	1.39 (1.00) ± 1.36	0.2149
Psychomotor agitation	1.64 (2.00) ± 1.28	2.65 (3.00) ± 1.14	0.00295* ←
Concentration	1.71 (2.00) ± 1.07	2.96 (3.00) ± 0.86	0.269
Meals	1.32 (0.00) ± 1.90	0.38 (0.00) ± 0.82	0.045† ←
Sleep	0.77 (0.00) ± 1.61	0.28 (0.00) ± 0.59	0.0711
Social interaction	1.68 (2.00) ± 1.01	2.83 (3.00) ± 1.10	0.000268* ←
Speech	1.32 (1.00) ± 1.42	1.72 (1.00) ± 1.55	0.3918
Anxiety	1.84 (2.00) ± 1.39	2.90 (3.00) ± 1.23	0.0159* ←
Stereotypy	1.45 (1.00) ± 1.06	2.07 (2.00) ± 1.03	0.3853
ATEC L	12.16 (12.00) ± 7.49	13.14 (13.00) ± 8.18	0.254
ATEC S	13.64 (15.00) ± 6.31	17.83 (18.00) ± 9.83	0.113
ATEC P	13.68 (13.00) ± 7.77	16.86 (18.00) ± 8.53	0.212
ATEC SC	25.35 (25.00) ± 10.79	27.17 (25.00) ± 11.03	0.119
ATEC T	64.84 (63.00) ± 26.82	75.00 (78.00) ± 32.89	0.098
CARS	33.47 (31.00) ± 8.48	37.83 (39.00) ± 9.02	0.188

CARS = Childhood Autism Rating Scale.
Results are expressed as average (median) ± standard deviation.
All p-values were calculated for the treatment after versus the control after groups using the two-factor mixed analysis of variance (two-way ANOVA) test followed by Tukey and Wilcoxon.
The Autism Treatment Evaluation Checklist (ATEC) subscales are as follows: ATEC L, related to language; ATEC S, related to socialization; ATEC P, related to sensory and cognitive perception; and ATEC SC, related to health, physical aspect, and behavior, while ATEC T, is the total sum of the scale.
* $p < 0.01$; † $p < 0.05$; ‡ $p < 0.001$.

Table 4 - Mixed analysis of variance for two factors (R software version 4.0.2)

Variable	df	Sum Sq	Mean Sq	F value	Pr(>F)
Social interaction	1	17.63	17.633	14.133	0.000268*
Residuals	116	144.73	1.248		
Psychomotor agitation	1	14.70	14.700	9.225	0.00295†
Residuals	116	184.84	1.593		
Anxiety	1	10.21	10.208	5.989	0.0159‡
Residuals	116	197.73	1.705		
Number of meals per day	1	9.63	9.633	4.109	0.045§
Residuals	116	271.99	2.345		
Concentration (mild group)	1	5.56	5.558	6.747	0.0124‖
Residuals	48	39.54	0.824		

All p-values were calculated for the treatment after versus the control after groups using the two-factor mixed analysis of variance (Two-way ANOVA) test followed by Tukey and Wilcoxon.
* $p < 0.001$; † $p < 0.01$; ‡ $p < 0.05$.

The cannabis regimen utilized in the trial involved very small doses of THC that limited the risk for adverse effect. Only 10% of subjects in the cannabis group experienced adverse effects which included dizziness, insomnia, colic, and weight gain that were all reported to be mild. Symptom improvements were maintained even after 12 weeks of treatment in spite of these very small doses of the cannabis; this is consistent with the micro dosing that has been used in previous studies. Anecdotally, the clinical experience of myself and others has been that clinical improvements can continue to accrue even after 2 years of continuous treatment. By virtue of its randomized, double-blind, placebo-controlled design, this trial adds significant validation to the role of cannabis in treating both the core features and symptoms of ASD.

The science behind cannabinoid medicine in treating ASD is becoming more sophisticated and recently included the identification of 65 specific biomarkers whose physiological levels shifted following cannabis treatment from a profile seen in people with ASD to one seen in neuro-typical people[25]. These biomarkers were involved in pathways that included anti-inflammatory, bioenergy associated, neurotransmitters, amino acids, and endocannabinoids. Additional study of these biomarkers using machine learning has identified a specific cannabis-responsive biomarker called lysophosphatidylethanolamine that reliably distinguishes between ASD and neurotypical groups, novel phytochemicals that contribute to the therapeutic effect of medical cannabis by inhibiting acetylcholinesterase, and biomarkers that separated into distinct groups depending on strength of association with CBD versus THC[26]. These advances raise the potential for medical cannabis treatment to be tailored to the specific biomarker profile of an individual person with ASD by utilizing a formulation that contains a particular phytocannabinoid combination that more precisely addresses their endocannabinoid deficiency.

As a point of comparison, a study of cannabis treatment in 70 people with Tourette's syndrome demonstrated the following statistically significant improvements in the 57 participants who completed the trial: 67% noticed improvement in obsessive-compulsive disorders, 89% experienced reduction in anxiety comorbidities, and the average number of conventional psychotropic medications being taken was reduced by 75%. This led to 19% of participants gaining employment after starting medical cannabis treatment[27]. The potential exists to achieve similar advancements with medical cannabis treatment in people with ASD.

Many physicians who are currently proponents of medical cannabis were initially skeptics, as their formative years occurred during the Nixon administration's "War on Drugs" which created the presumption that cannabis must be harmful and without benefit because it was deemed illegal to possess and consume. A shared impulse in this group was reluctance to admit that they had committed a mistake in assuming the veracity of this presumption. The suggestion to "Be Curious, Not Judgmental" is apropos to the consideration of medical cannabis as a treatment. If a person can suspend their presumptuous judgement that cannabis is inherently detrimental and engage their curiosity, they could then appreciate the science that is steadily revealing the true nature of the plant.

Multiple academic institutions in other states have developed cannabis research centers specifically devoted to studying the basic science, clinical application, and public policy of the plant. This includes the University of California-Los Angeles, Yale School of Medicine, Harvard Medical School, and Johns Hopkins University. In the neighboring state of Pennsylvania, autism is approved as a qualifying condition for medical cannabis and a total of nine of these research centers have been established at the following sites:

1. Drexel University (Medical Cannabis Research Center)
2. Geisinger Commonwealth School of Medicine (Geisinger Research Institute)
3. Lake Erie College of Osteopathic Medicine (Medical Marijuana Research Program)
4. Penn State College of Medicine (Center for Cannabis and Natural Product Pharmaceutics)
5. Philadelphia College of Osteopathic Medicine
6. Temple University (Center for Substance Abuse Research)
7. Thomas Jefferson University (Lambert Center for the Study of Medicinal Cannabis and Hemp)
8. University of Pennsylvania
9. University of Pittsburgh (Cannabis Multidisciplinary Education and Research)

There are multiple funding sources for the research that is ongoing, including the National Institutes of Health which dispensed almost $600 million for over 1000 projects between 2015 and 2024[28]. We hope that similar research endeavors will germinate in the state of Ohio, given the potential for cannabis to treat refractory ASD and other intractable health conditions that affect so many residents of the state.

There is understandable concern about the risk of cannabis in people with autism, but we also need to consider the risk of maintaining the status quo which is having little to no effect on the mounting burden of refractory ASD. Rejecting this application for refractory ASD as a qualifying condition for medical cannabis will not prevent families from utilizing this treatment as it is now readily available in Ohio and neighboring states without a medical recommendation. Approving it would signify to patients and families that the healthcare providers in this state are ready to collaborate with them in ascertaining optimal formulations and dosing regimens, learning how to avoid potential pitfalls and adverse effects, and exploring the significant potential of this medication in improving their welfare.

References:

1. Devane WA, Dysarz FA 3rd, Johnson MR, Melvin LS, Howlett AC. Determination and characterization of a cannabinoid receptor in rat brain. Mol Pharmacol. 1988 Nov;34(5):605-13. PMID: 2848184.

2. Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. Nature. 1993 Sep 2;365(6441):61-5. doi: 10.1038/365061a0. PMID: 7689702.

3. Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science. 1992 Dec 18;258(5090):1946-9. doi: 10.1126/science.1470919. PMID: 1470919.

4. Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martin BR, Compton DR, et al. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem Pharmacol. 1995 Jun 29;50(1):83-90. doi: 10.1016/0006-2952(95)00109-d. PMID: 7605349.

5. Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K, Yamashita A, Waku K. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochem Biophys Res Commun. 1995 Oct 4;215(1):89-97. doi: 10.1006/bbrc.1995.2437. PMID: 7575630.

6. Simard M, Archambault AS, Lavoie JC, Dumais É, Di Marzo V, Flamand N. Biosynthesis and metabolism of endocannabinoids and their congeners from the monoacylglycerol and N-acyl-ethanolamine families. Biochem Pharmacol. 2022 Nov;205:115261. doi: 10.1016/j.bcp.2022.115261. Epub 2022 Sep 21. PMID: 36152677.

7. Pacher P, Kunos G. Modulating the endocannabinoid system in human health and disease--successes and failures. FEBS J. 2013 May;280(9):1918-43. doi: 10.1111/febs.12260. Epub 2013 Apr 22. PMID: 23551849; PMCID: PMC3684164.

8. Ligresti A, Bisogno T, Matias I, De Petrocellis L, Cascio MG, Cosenza V, D'argenio G, Scaglione G, Bifulco M, Sorrentini I, Di Marzo V. Possible endocannabinoid control of colorectal cancer growth. Gastroenterology. 2003 Sep;125(3):677-87. doi: 10.1016/s0016-5085(03)00881-3. PMID: 12949714.

9. Russo EB. Clinical Endocannabinoid Deficiency Reconsidered: Current Research Supports the Theory in Migraine, Fibromyalgia, Irritable Bowel, and Other Treatment-Resistant Syndromes. Cannabis Cannabinoid Res. 2016 Jul 1;1(1):154-165. doi: 10.1089/can.2016.0009. PMID: 28861491; PMCID: PMC5576607.

10. Sarchielli P, Pini LA, Coppola F, Rossi C, Baldi A, Mancini ML, Calabresi P. Endocannabinoids in chronic migraine: CSF findings suggest a system failure. Neuropsychopharmacology. 2007 Jun;32(6):1384-90. doi: 10.1038/sj.npp.1301246. Epub 2006 Nov 22. Erratum in: Neuropsychopharmacology. 2007 Jun;32(6):1432. PMID: 17119542.

11. Romigi A, Bari M, Placidi F, Marciani MG, Malaponti M, Torelli F, Izzi F, Prosperetti C, Zannino S, Corte F, Chiaramonte C, Maccarrone M. Cerebrospinal fluid levels of the endocannabinoid anandamide are reduced in patients with untreated newly diagnosed temporal lobe epilepsy. Epilepsia. 2010 May;51(5):768-72. doi: 10.1111/j.1528-1167.2009.02334.x. Epub 2009 Oct 8. PMID: 19817812.

12. Di Filippo M, Pini LA, Pelliccioli GP, Calabresi P, Sarchielli P. Abnormalities in the cerebrospinal fluid levels of endocannabinoids in multiple sclerosis. J Neurol Neurosurg Psychiatry. 2008 Nov;79(11):1224-9. doi: 10.1136/jnnp.2007.139071. Epub 2008 Jun 5. PMID: 18535023.

13. Hill MN, Bierer LM, Makotkine I, Golier JA, Galea S, McEwen BS, Hillard CJ, Yehuda R. Reductions in circulating endocannabinoid levels in individuals with post-traumatic stress disorder following exposure to the World Trade Center attacks. Psychoneuroendocrinology. 2013 Dec;38(12):2952-61. doi: 10.1016/j.psyneuen.2013.08.004. Epub 2013 Sep 10. PMID: 24035186; PMCID: PMC3870889.

14. Zou M, Li D, Li L, Wu L, Sun C. Role of the endocannabinoid system in neurological disorders. Int J Dev Neurosci. 2019 Aug;76:95-102. doi: 10.1016/j.ijdevneu.2019.03.002. Epub 2019 Mar 8. PMID: 30858029.

15. Aran A, Eylon M, Harel M, et al. Lower circulating endocannabinoid levels in children with autism spectrum disorder. Mol Autism. 2019 Jan 30;10:2. doi: 10.1186/s13229-019-0256-6. PMID: 30728928; PMCID: PMC6354384.

16. Karhson DS, Krasinska KM, Dallaire JA, et al. Plasma anandamide concentrations are lower in children with autism spectrum disorder. Mol Autism. 2018 Mar 12;9:18. doi: 10.1186/s13229-018-0203-y. PMID: 29564080; PMCID: PMC5848550.

17. Siniscalco D, Sapone A, Giordano C, et al. Cannabinoid receptor type 2, but not type 1, is up-regulated in peripheral blood mononuclear cells of children affected by autistic disorders. J Autism Dev Disord. 2013 Nov;43(11):2686-95. doi: 10.1007/s10803-013-1824-9. PMID: 23585028.

18. Jana A, Nath A, Sen P, Kundu S, Alghamdi BS, Abujamel TS, Saboor M, Woon-Khiong C, Alexiou A, Papadakis M, Alam MZ, Ashraf GM. Unraveling the Endocannabinoid System: Exploring Its Therapeutic Potential in Autism Spectrum Disorder. Neuromolecular Med. 2024 May 14;26(1):20. doi: 10.1007/s12017-024-08781-6. PMID: 38744725; PMCID: PMC11093854.

19. Sulak D. (2021). Cannabis Dosing. In *Handbook of Cannabis for Clinicians*. (pp. 117-140). W.W. Norton & Co.

20. Ponton JA, Smyth K, Soumbasis E, et al. A pediatric patient with autism spectrum disorder and epilepsy using cannabinoid extracts as complementary therapy: a case report. J Med Case Rep. 2020 Sep 22;14(1):162. doi: 10.1186/s13256-020-02478-7. PMID: 32958062; PMCID: PMC7507278.

21. Hjorthøj C, Compton W, Starzer M, Nordholm D, Einstein E, Erlangsen A, Nordentoft M, Volkow ND, Han B. Association between cannabis use disorder and schizophrenia stronger in young males than in females. Psychol Med. 2023 Nov;53(15):7322-7328. doi: 10.1017/S0033291723000880. Epub 2023 May 4. PMID: 37140715; PMCID: PMC10719679.

22. Chakrabarti B, Persico A, Battista N, et al. Endocannabinoid Signaling in Autism. Neurotherapeutics. 2015 Oct;12(4):837-47. doi: 10.1007/s13311-015-0371-9. PMID: 26216231; PMCID: PMC4604173.

23. Bar-Lev Schleider L, Mechoulam R, Saban N, Meiri G, Novack V. Real life Experience of Medical Cannabis Treatment in Autism: Analysis of Safety and Efficacy. Sci Rep. 2019 Jan 17;9(1):200. doi: 10.1038/s41598-018-37570-y. PMID: 30655581; PMCID: PMC6336869.

24. Silva EAD Junior, Medeiros WMB, Santos JPMD, et al. Evaluation of the efficacy and safety of cannabidiol-rich cannabis extract in children with autism spectrum disorder: randomized, double-blind, and placebo-controlled clinical trial. Trends Psychiatry Psychother. 2024;46:e20210396. doi: 10.47626/2237-6089-2021-0396. Epub 2022 May 26. PMID: 35617670; PMCID: PMC11332686.

25. Siani-Rose M, Cox S, Goldstein B, Abrams D, Taylor M, Kurek I. Cannabis-Responsive Biomarkers: A Pharmacometabolomics-Based Application to Evaluate the Impact of Medical Cannabis Treatment on Children with Autism Spectrum Disorder. Cannabis Cannabinoid Res. 2023 Feb;8(1):126-137. doi: 10.1089/can.2021.0129. Epub 2021 Dec 6. PMID: 34874191; PMCID: PMC9940806.

26. Quillet JC, Siani-Rose M, McKee R, Goldstein B, Taylor M, Kurek I. A machine learning approach for understanding the metabolomics response of children with autism spectrum disorder to medical cannabis treatment. Sci Rep. 2023 Aug 22;13(1):13022. doi: 10.1038/s41598-023-40073-0. PMID: 37608004; PMCID: PMC10444802.

27. Barchel D, Stolar O, Ziv-Baran T, et al. Use of Medical Cannabis in Patients with Gilles de la Tourette's Syndrome in a Real-World Setting. Cannabis Cannabinoid Res. 2024 Feb;9(1):293-299. doi: 10.1089/can.2022.0112. Epub 2022 Nov 7. PMID: 36342913; PMCID: PMC10874815.

28. "NIH-Supported Research on Cannabis, Cannabinoids, and Related Compounds". https://www.nccih.nih.gov/grants/nih-supported-research-on-cannabis-cannabinoids-and-related-compounds. Accessed 12/31/2024.

12/30/2024

Hello,

This letter is in support of the addition of "Autism Spectrum Disorder (ASD) that is refractory to traditional route therapies" as a qualifying condition for medical cannabis in the state of Ohio. (Refractory being defined as ASD that has failed known traditional route therapies)

I am a board certified Internal Medicine physician and have been providing medical cannabis recommendations in the state of Ohio since July 2019. I have several clients that have co-morbid refractory Autism Spectrum Disorder and have personally have seen great benefit by adding medical cannabis to their treatment regimen. Every client we have seen in this regard has been medically complex and referred to us by their pediatrician.

In this light, I would like to share a case study of a patient I have been working with since the the spring 2024 to present.

Patient O.C. is a 13 year old female with a complex past medical history of refractory autism spectrum disorder as well as epilepsy secondary to Lennox Dravet syndrome. She is non verbal and also suffers from cognitive impairment, with behavioral disturbances. She also suffers from severe insomnia. When we met this spring 24', her ASD and behavioral issues were being treated with high dose anti psychotic Risperdal and mood stabilizer Valproic acid. This culminated in a 40 lb wt gain and marked lethargy during the day with excessive drooling. Despite the medication, her sleep remained poor as did her behavior. She was diagnosed with pre diabetes mellitus during that time as well.

After initiation of medical cannabis with use of both CBD and lower amounts of THC in the form of a sublingual tincture (cannabis oil), she has been able to stop her anti psychotic Risperdal and mood stabilizer Valproic acid. With discontinuation of these medications and addition of the medical cannabis, her parents have seen substantial improvement. She has lost 30 lbs and in general is more awake with no further drooling. She has started to sing/hum throughout the day which she had not done prior to this. Her parents are very happy with the overall progress. Her multi disciplinary medical team along with myself have been following from the onset of her cannabis use to present.

As above, I concur with Dr Douglas Woo on the addition of ASD that is refractory to traditional route therapies.

Feel free to contact me with any thoughts or questions in this regard.

Kind regards,

James Weeks MD

MD

513 - 321 - 1242

BRIDGET COLE WILLIAMS,MD

MEDICAL DIRECTOR

DR. BRIDGET MD INTEGRATIVE OFFICES

DATE:12/24/2024

TO: TO WHOM IT MAY CONCERN

To Whom It May Concern,

As a board-certified physician and medical cannabis specialist, I strongly support adding Refractory Autism Spectrum Disorder (ASD) as a qualifying condition for medical cannabis use. This proposal is grounded in scientific evidence, patient case studies, and the urgent need for alternative therapeutic options.

ASD presents challenges that profoundly impact patients and their families. Over the past five years in my practice, I have encountered numerous families who turned to medical cannabis after exhausting all other resources. While traditional treatments can be helpful, many patients experience persistent symptoms or adverse effects, highlighting the need for integrative approaches.

Evidence Supporting Medical Cannabis for ASD

Medical cannabis, particularly CBD and THC formulations, has shown promise in addressing:

- Behavioral Challenges: Reduction in aggression, self-injury, and tantrums.[1]
- Anxiety and Emotional Regulation: Cannabinoids support emotional well-being through the endocannabinoid system.[1,2]
- Sleep Disturbances: Improved sleep patterns for patients and caregivers.[3]
- Seizure Control: Effective for those with comorbid epilepsy, as demonstrated by FDA-approved CBD medications.[2]

Patient Stories and Impact

In my book series, Courage in Cannabis, one impactful story features Amie and her son Jayden, diagnosed with Asperger's, ADHD, and Oppositional Defiant Disorder. Jayden's violent behaviors and seizures made life unmanageable. Amie was advised by health professionals to abandon her child to restore normalcy to her families life.

BRIDGET COLE WILLIAMS,MD

MEDICAL DIRECTOR

DR. BRIDGET MD INTEGRATIVE OFFICES

DATE:12/24/2024

TO: TO WHOM IT MAY CONCERN

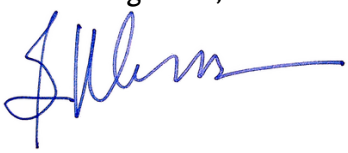
After trying traditional therapies without success, Amie turned to medical cannabis. At the age of nine, Jayden found peace for the first time, allowing him to learn, grow, and thrive. Now 17, he is a happy, energetic teen with a bright future. This transformation underscores the life-changing potential of medical cannabis for Refractory Autism Spectrum Disorder.

By approving ASD as a qualifying condition, the board can provide families with safe, regulated access to medical cannabis under physician supervision. This decision offers a compassionate, evidence-based solution for unmet clinical needs and prevents families from resorting to unregulated alternatives.

In closing, adding Refractory ASD to the list of approved conditions reflects our commitment to improving lives through innovation and equity in care. Children like Jayden deserve the chance to thrive, and families like Amie's deserve hope.

Thank you for considering this important matter. I am available to provide further insights or participate in discussions to support this initiative.

Best regards,



Dr. Bridget Cole Williams, M.D.
Board-Certified Family Physician
Medical Cannabis Specialist

1] Silva EAD Junior, Medeiros WMB, Torro N, et al. Cannabis and cannabinoid use in autism spectrum disorder: a systematic review. Trends Psychiatry Psychother. 2022;44:e20200149. Published 2022 Jun 13. doi:10.47626/2237-6089-2020-0149

2] Fusar-Poli L, Cavone V, Tinacci S, et al. Cannabinoids for People with ASD: A Systematic Review of Published and Ongoing Studies. Brain Sci. 2020;10(9):572. Published 2020 Aug 20. doi:10.3390/brainsci10090572

3] Holdman R, Vigil D, Robinson K, Shah P, Contreras AE. Safety and Efficacy of Medical Cannabis in Autism Spectrum Disorder Compared with Commonly Used Medications. Cannabis Cannabinoid Res. 2022;7(4):451-463. doi:10.1089/can.2020.0154

Vol. 215, No. 1, 1995 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 4, 1995 Pages 89-97

2-ARACHIDONOYLGLYCEROL: A POSSIBLE ENDOGENOUS CANNABINOID RECEPTOR LIGAND IN BRAIN

Takayuki Sugiura*, Sachiko Kondo, Akihiro Sukagawa, Shinji Nakane, Akira Shinoda, Kiyoko Itoh, Atsushi Yamashita and Keizo Waku

Faculty of Pharmaceutical Sciences, Teikyo University, Sagamiko, Kanagawa 199-01, Japan

Received August 25, 1995

Summary: The effects of anandamide, 2-arachidonoylglycerol and related compounds on the specific binding of a radiolabeled cannabinoid receptor ligand, [^{3}H]CP55940, to synaptosomal membranes were examined. Anandamide, an endogenous cannabinoid receptor ligand, reduced the specific binding of [^{3}H]CP55940 to synaptosomal membranes in a dose-dependent manner: the Ki value was 89 nM. 2-Arachidonoylglycerol was also shown to bind appreciably to the cannabinoid receptor in competitive inhibition experiments. The apparent binding affinity was markedly increased when the binding assay was carried out in the presence of the esterase inhibitor DFP or at 0^{o}C. Free arachidonic acid and N-palmitoylethanolamine were almost inactive in terms of binding to the cannabinoid receptor in synaptosomal membranes. 2-Arachidonoylglycerol may be an endogenous cannabinoid receptor ligand in the brain.

Anandamide (N-arachidonoylethanolamine) is an endogenous cannabinoid receptor ligand first described by Devane et al. (1) in 1992. Anandamide has been shown to possess potent cannabimimetic activity in various biological systems either in vitro or in vivo (1-5). The structure-activity relationship of anandamide has been explored extensively by Mechoulam and co-workers (6) and by others (2).

We have recently studied the actions of N-acylethanolamine phosphate on human platelets and found that this compound is a potent agonist toward lysophosphatidic acid receptor (7). In this series of experiments, we noticed that N-acylethanolamine phosphate and alkyllysophosphatidic acid have rather similar biological activity even though their chemical structures are

* To whom all correspondence should be addressed. FAX: 81-426-85-1345.

Abbreviations: DFP, diisopropyl fluorophosphate; BSA, bovine serum albumin; Fatty acids are designated in terms of number of carbon atoms:number of double bonds, e.g., 20:4 for arachidonic acid.

0006-291X/95 $12.00

considerably different from each other: the latter compound contains a glycerol backbone, while the former one contains the N-acylethanolamine structure and lacks a glycerol moiety. This observation prompted us to examine whether analogues of anandamide containing a glycerol backbone possess cannabimimetic activities. In a previous communication (8), we reported that 2-arachidonoylglycerol exhibits binding affinity toward cannabinoid receptor in rat brain synaptosomes and we suggested that this compound may function as an endogenous cannabinoid receptor ligand at some sites in the brain. However, its binding affinity estimated under our former experimental conditions (without inhibitors of esterases) was much weaker than that observed for anandamide estimated under the same experimental conditions, probably because of the susceptibility of 2-arachidonoylglycerol to lipase(s), which may degrade 2-arachidonoylglycerol to release arachidonic acid during the binding assay. In the present study, we compared in detail the binding affinities of anandamide, 2-arachidonoylglycerol and several other analogues to synaptosomal membranes in the presence or absence of diisopropyl fluorophosphate (DFP), which is a potent inhibitor of various types of serine esterases, including monoacylglycerol lipase (9-11), or at 0^{o}C. We established that 2-arachidonoylglycerol possesses relatively high binding affinity toward cannabinoid receptor in synaptosomal membranes and that its amount in brain is about a thousand times higher than that of anandamide. These results strongly suggest that 2-arachidonoylglycerol is another candidate for an endogenous cannabinoid receptor ligand.

MATERIALS AND METHODS

Materials: A non-classical cannabinoid receptor radiolabeled ligand [^{3}H]CP55940 (113 Ci/mmol) was purchased from Dupont-NEN (Boston, MA). Arachidonic acid (20:4), heptadecanoic acid (17:0), oleic acid (18:1), pentadecanoic acid (15:0) methyl ester and essentially fatty acid-free bovine serum albumin (BSA) were obtained from Sigma (St. Louis, MO). Palmitoyl chloride was prepared by treating palmitic acid with oxalyl chloride. Anandamide was purchased from Research Biochemicals International (Natick, MA). N-Palmitoylethanolamine was prepared by reacting palmitoyl chloride with ethanolamine and purified by TLC developed with petroleum ether:diethyl ether:acetone:acetic acid (30:40:20:1, v/v). DFP, polyethylenimine P-70 and other chemicals (analytical grade) were from Wako Pure Chem. Ind. (Osaka, Japan). Lipase (Rhizopus delemar) was from Seikagaku Kogyo (Tokyo, Japan). GF/B filters were purchased from Whatman (Maidstone, England). 2-Arachidonoylglycerol, 2-oleoylglycerol and 2-heptadecanoylglycerol were

prepared as follows. Fatty acids (100 mg) dissolved in 1 ml of chloroform (containing 2-methyl-2-butene as a stabilizer) were first converted to fatty acid anhydride by treatment with 100 mg of dicyclohexylcarbodiimide (12) and then mixed with 20 mg of glycerol, 20 mg of dimethylaminopyridine and 0.1 ml of pyridine in a tube sealed under N_2 gas. The mixture was stirred overnight. The resultant triacylglycerol was purified by TLC developed with petroleum ether:diethyl ether:acetic acid (80:20:1, v/v). Triacylglycerol (50 mg) was dispersed in 3 ml of 50 mM sodium acetate-acetic acid buffer (pH 5.6) containing 0.1 M NaCl and 10 mM $CaCl_2$ by brief sonication and then hydrolyzed by exposure to lipase (30 mg) for 60 min under vigorous stirring. Monoacylglycerol was purified by TLC developed with petroleum ether:diethyl ether:acetic acid (20:80:1, v/v). The content of monoacylglycerol was estimated by quantitating its fatty acyl moiety by GLC after conversion to fatty acid methyl esters by treatment with 0.5 M methanolic sodium methoxide. 15:0 Fatty acid methyl ester was used as an internal standard.

Preparation of synaptosomes: Rat brain synaptosomal membranes were prepared by the modified method of Whittaker et al. (13). Briefly, rats (Wistar, male, 350-400 g) were killed by decapitation and the brain was removed. The cerebrum was isolated and homogenized in 0.32 M sucrose/10 mM Tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 1000 x g for 10 min. The supernatant was taken and further centrifuged at 17000 x g for 55 min. This supernatant was aspirated and the pellet was dissolved in 0.32 M sucrose and carefully layered on a discontinuous sucrose gradient (upper, 0.8 M sucrose; lower, 1.2 M sucrose). Then, the tubes were centrifuged at 63000 x g for 120 min using a Hitachi swinging rotor (SRP28SA). The interfacial layer between 0.8 M sucrose and 1.2 M sucrose was carefully taken, and diluted with distilled water and further centrifuged at 105000 x g for 60 min. The pellet was dissolved in 16.6 mM sucrose/25 mM Tris-HCl buffer (pH 7.4) containing 1 mM EDTA and stored at $-80^{\circ}C$. This fraction was used as synaptosomal membranes. The protein content was estimated by the method of Lowry et al. (14).

Binding assay: The binding assay was carried out by the modified method of Devane et al. (15). Briefly, synaptosomal membranes (50 μg protein) were incubated with 50 fmol of [^{3}H]CP55940 (12500 dpm) in 500 μl of 50 mM Tris-HCl buffer (pH 7.4) containing 1 mM EDTA and 3 mM $MgCl_2$ in the presence or absence of varying concentrations of anandamide, 2-arachidonoylglycerol and other compounds (dissolved in 2.5 % BSA, the final concentration of BSA in the incubation mixture was 0.25 %) at $30^{\circ}C$ for 60 min. In some experiments, synaptosomal membranes (100 μg protein) were incubated in the same buffer at $0^{\circ}C$ for 240 min. In the case of the buffer containing 1 mM DFP, synaptosomal membranes (75 μg protein) were incubated with [^{3}H]CP55940 and various compounds at $30^{\circ}C$ for 60 min. Non-specific binding of the radiolabeled ligand was determined from the binding in the presence of 20 μM anandamide. After the incubation, 2.5 ml of 50 mM Tris-HCl buffer (pH 7.4) containing 0.05 % BSA was added to the tube and the mixture was immediately filtered on a GF/B filter which had been pre-treated with 0.1 % polyethylenimine. The filter was further washed with 2.5 ml of the same buffer 4 times and dried prior to measurement of the radioactivity.

Analysis of monoacylglycerol in brain: Rats were killed by decapitation and the brains were removed. The brains were homogenized in chloroform:methanol (1:2, v/v), and the total lipids were extracted by the method of Bligh and Dyer (16). 2-Heptadecanoylglycerol (25 nmol) was added as an internal standard. Lipids were applied to a silicic acid column and neutral lipids were eluted with chloroform. Neutral lipids were then separated by TLC developed with petroleum ether:diethyl ether:acetic acid (20:80:1, v/v). The monoacylglycerol fraction was extracted from the silica gel by the method of Bligh and Dyer (16) and further purified by TLC developed with the same solvent system. The fatty acyl moiety of monoacylglycerol was converted to fatty acid methyl ester with sodium methoxide and analyzed by GLC.

RESULTS AND DISCUSSION

First, we examined the kinetics of the binding of [^{3}H]CP55940 to synaptosomal membranes. The binding of [^{3}H]CP55940 to synaptosomal membranes was saturable as reported earlier (15). The Kd value estimated at 30^{o}C was 0.83 ± 0.06 nM and the Bmax was 2.25 ± 0.64 pmol/mg protein (the means ± SD of three separate experiments, with 10-12 determinations each (n=3)). The Kd value and the Bmax estimated in the presence of 1 mM DFP were 4.29 ± 1.20 nM and 2.05 ± 0.22 pmol/mg protein, respectively (the means ± SD of three separate experiments, with 10-12 determinations each (n=6)), and those estimated at 0^{o}C were 2.53 nM and 0.72 pmol/mg protein, respectively (the means of two separate experiments, with 10-12 determinations each (n=6)).

Next we examined the effects of anandamide and related lipid molecules on the specific binding of [^{3}H]CP55940. Fig. 1 shows the competitive inhibition of the binding of [^{3}H]CP55940 to synaptosomal membranes by various compounds. The specific binding of [^{3}H]CP55940 to synaptosomal membranes was markedly decreased with increased concentrations of anandamide (Fig. 1 (a)). The Ki value was calculated to be 89 nM. A similar value (99 nM) was observed when the binding experiments were carried out in the presence of 1 mM DFP (Fig. 1 (b)). It should be noted that 2-arachidonoylglycerol also inhibits the specific binding of [^{3}H]CP55940 in a dose-dependent manner. The Ki value for 2-arachidonoylglycerol estimated in the presence of 1 mM DFP was 2.4 μM (Fig. 1 (d)), indicating that 2-arachidonoylglycerol has a binding affinity which is about 24 times lower than that of anandamide estimated under the same experimental conditions. We also observed that the Ki value for 2-arachidonoylglycerol estimated at 0^{o}C was about 4.6 times higher than that for anandamide estimated under the same assay condition (Fig. 2). However, the Ki value for 2-arachidonoylglycerol was elevated to 48 μM (540 times higher than that for anandamide), if DFP was omitted from the assay system and the incubation was carried out at 30^{o}C (Fig. 1 (c)). We also found that arachidonoylethyleneglycol has a similar binding activity to that of 2-arachidonoylglycerol, while 2-oleoylglycerol fails to inhibit the specific

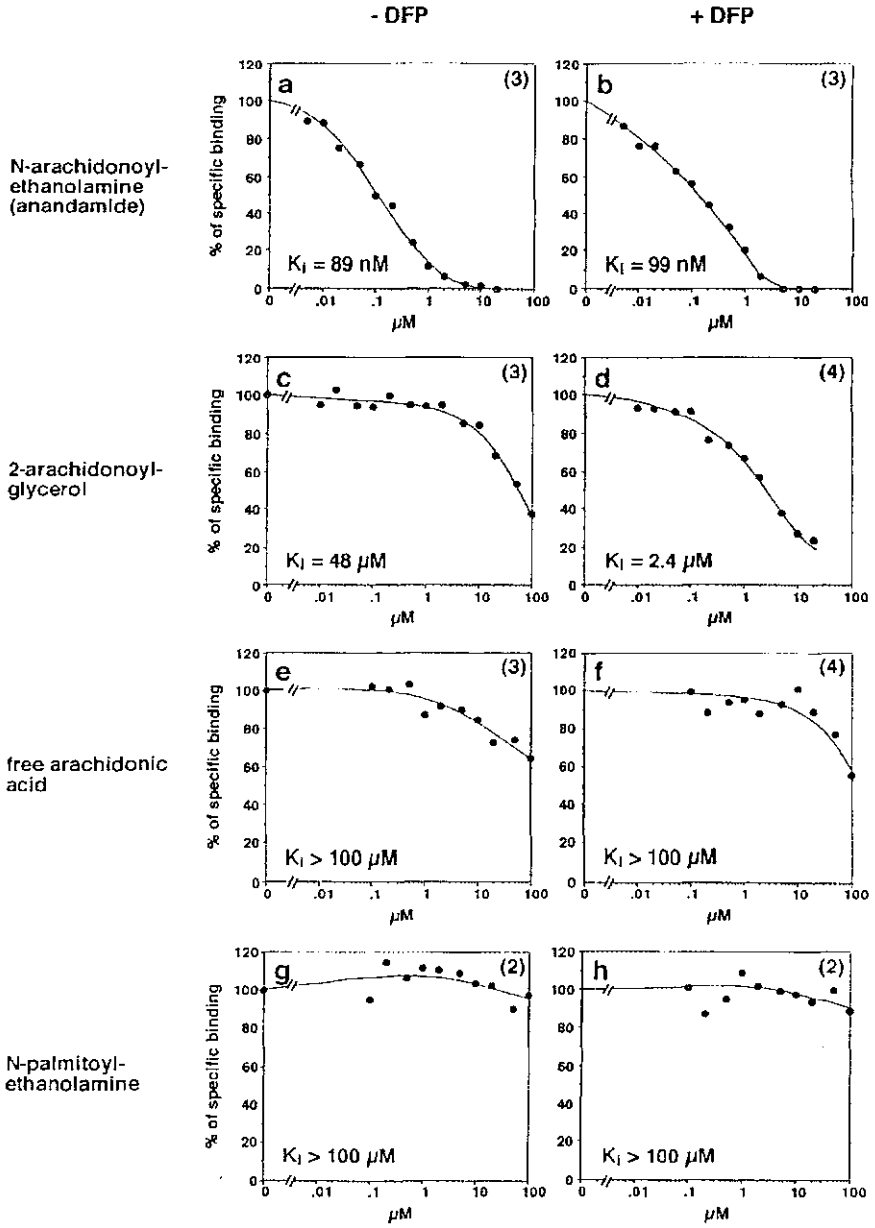


Fig. 1. Effects of anandamide, 2-arachidonoylglycerol and related molecules on the specific binding of [^{3}H]CP55940 to synaptosomal membranes in the presence or absence of 1 mM DFP at 30°C. The data are the means of two to four separate experiments (in parenthesis) each done in triplicate or quadruplicate.

binding of [^{3}H]CP55940 to synaptosomal membranes (data not shown). Free arachidonic acid itself did not exhibit high affinity for cannabinoid receptor, either in the presence or absence of DFP (Fig. 1 (e) and (f)). Further, N-

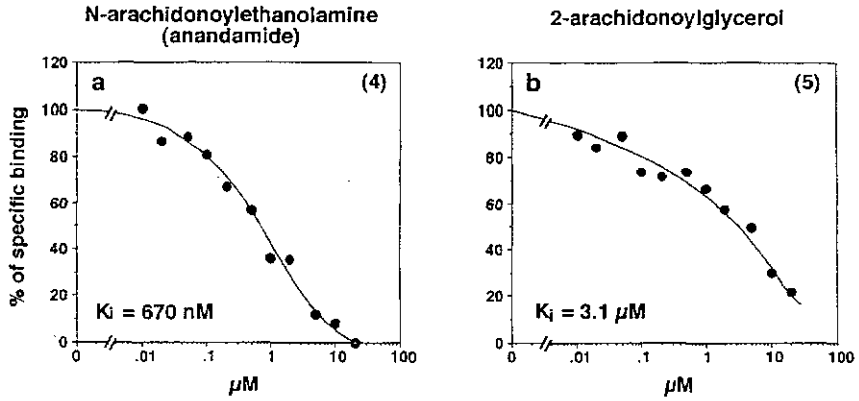


Fig. 2. Effects of anandamide and 2-arachidonoylglycerol on the specific binding of [^{3}H]CP55940 to synaptosomal membranes at 0°C. The data are the means of four to five separate experiments (in parenthesis) each done in quadruplicate.

palmitoylethanolamine (Fig. 1 (g) and (h)) was virtually inactive, at least in terms of binding to the cannabinoid receptor in synaptosomal membranes.

Then, we examined the level and the fatty acid profile of monoacylglycerol in brain. As shown in Table 1, rat brain contains monoacylglycerol at the level of 6.86 nmol/g wet tissue. Notably, the proportion of arachidonic acid-containing species was very high; arachidonic acid-containing species account for as much as 47 % of total monoacylglycerol. The concentration of arachidonoylglycerol in this tissue is, thus, approximately 4 μM, supposing

TABLE 1

Fatty acid composition of monoacylglycerol in rat brain

Fatty acyl moiety	%	nmol/g wet weight
16:0	11.2	0.77 ± 0.46
16:1	0.4	0.03 ± 0.01
18:0	5.5	0.38 ± 0.09
18:1	12.4	0.85 ± 0.44
18:2	0.9	0.06 ± 0.03
20:3	0.7	0.05 ± 0.01
20:4	47.4	3.25 ± 0.52
22:4	2.2	0.15 ± 0.04
22:5	1.8	0.12 ± 0.10
22:6	17.5	1.20 ± 0.56
total	100	6.86 ± 2.37

The data are the means ± SD of five separate experiments.

that 80 % of the tissue consists of water and that arachidonoylglycerol is homogeneously distributed in this tissue.

The binding affinity of 2-arachidonoylglycerol estimated in the absence of DFP at 30°C was considerably lower than that of anandamide. This can be attributed, at least in part, to possible hydrolysis of 2-arachidonoylglycerol by monoacylglycerol lipase, which is known to be present in brain tissues (17), during the incubation. In fact, in the presence of DFP, a potent inhibitor of monoacylglycerol lipase (9-11), the apparent binding affinity of 2-arachidonoylglycerol to cannabinoid receptor was increased (Fig. 1). Furthermore, we confirmed that the apparent binding activity of 2-arachidonoylglycerol was also markedly increased when the binding experiments were carried out at 0°C (Fig. 2). It is clear, therefore, that the actual activity of 2-arachidonoylglycerol is much higher than that observed in experiments where the activity of monoacylglycerol lipase is not blocked.

2-Arachidonoylglycerol can be formed through several metabolic pathways in brain (Fig. 3). First, inositol phospholipids are hydrolyzed by phospholipase C to release diacylglycerol enriched in arachidonic acid such as 1-stearoyl-2-arachidonoylglycerol. Then, this unique molecular species of diacylglycerol is hydrolyzed through the action of diacylglycerol lipase to release 2-arachidonoylglycerol. Another synthetic pathway involves the hydrolysis of phosphatidylinositol by phosphatidylinositol-specific phospholipase A_1, which

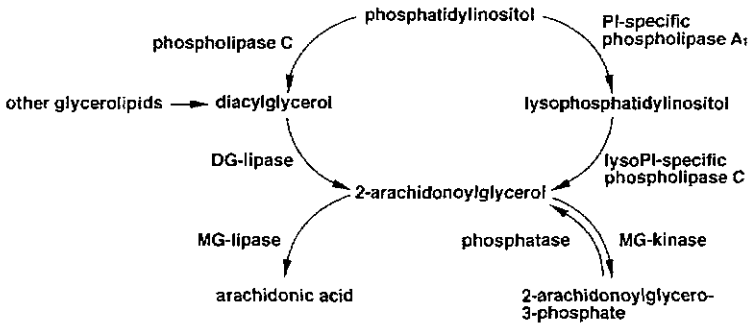


Fig. 3. Possible synthetic and metabolic pathways of 2-arachidonoylglycerol in brain.

is known to be present in brain (18,19). Alternatively, phosphatidylinositol may be hydrolyzed by a CoA-dependent degradation pathway to yield arachidonic acid-containing lysophosphatidylinositol in addition to stearic acid-containing species (20). Then, the resultant arachidonic acid-containing lysophosphatidylinositol is hydrolyzed by lysophosphatidylinositol-specific phospholipase C, which is also known to be present in brain (19), especially in synaptosomes (21). In both cases, the end product is 2-arachidonoylglycerol. The presence of such synthetic pathways is consistent with the observation that monoacylglycerol present in this tissue is particularly enriched in arachidonic acid-containing species (Table 1). Thus, it appears that 2-arachidonoylglycerol is not merely an end product of inositol phospholipid metabolism, but has some physiological role.

The observation that 2-arachidonoylglycerol has cannabimimetic binding activity is interesting in view of the possible linkage of enhanced inositol phospholipid metabolism within neuronal cells and the regulation of synaptic function through cannabinoid receptor expressed on the cell surface. It has already been demonstrated that arachidonoylglycerol is released from cells into the extracellular fluid upon stimulation (22). Recently, we found that the amount of anandamide in brain is of the order of pmol/g wet weight tissue (Sugiura, T. and Kondo, S., unpublished data), which is about a thousand times smaller than that of 2-arachidonoylglycerol found in the present study (Table 1), although little is so far known concerning the localization of these lipid molecules in tissues. In any case, the relative importance of anandamide and 2-arachidonoylglycerol in this tissue remains to be determined.

Not much attention has so far been paid to 2-arachidonoylglycerol, an interesting molecule from various biochemical and pharmacological viewpoints. Studies on possible physiological roles of this unique lipid mediator are in progress in our laboratory.

During the preparation of this manuscript, Mechoulam et al. (23) reported that 2-arachidonoylglycerol binds to cannabinoid receptors on rat spleen cells and receptor gene-transfected COS-7 cells.

REFERENCES

1. Devane, W.A., Hanus, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A. and Mechoulam, R. (1992) Science 258, 1946-1949.
2. Felder, C.C., Briley, E.M., Axelrod, J., Simpson, J.T., Mackie, K. and Devane, W.A. (1993) Proc. Natl. Acad. Sci. USA 90, 7656-7660.
3. Fride, E. and Mechoulam, R. (1993) Eur. J. Pharmacol. 231, 313-314.
4. Smith, P.B., Compton, D.R., Welch, S.P., Razdan, R.K., Mechoulam, R. and Martin, B.R. (1994) J. Pharmacol. Exp. Ther. 270, 219-227.
5. Crawley, J.N., Corwin, R.L., Robinson, J.K., Felder, C.C., Devane, W.A. and Axelrod, J. (1993) Pharmacol. Biochem. and Behav. 46, 967-972.
6. Mechoulam, R., Hanus, L. and Martin, B.R. (1994) Biochem. Pharmacol. 48, 1537-1544.
7. Sugiura, T., Tokumura, A., Gregory, L., Nouchi, T., Weintraub, S.T. and Hanahan, D.J. (1994) Arch. Biochem. Biophys. 311, 358-368.
8. Sugiura, T., Itoh, K., Waku, K. and Hanahan, D.J. (1994) Proceedings of Japanese Conference on the Biochemistry of Lipids 36, 71-74 (in Japanese).
9. Vaughan, M., Berger, J.E. and Steinberg, D. (1964) J. Biol. Chem. 239, 401-409.
10. Kupiecki, F. (1966) J. Lipid Res. 7, 230-235.
11. Yamamoto, M. and Drummond, G.I. (1967) Am. J. Physiol. 213, 1365-1370.
12. Selinger, Z. and Lapidot, Y. (1966) J. Lipid Res. 7, 174-175.
13. Whittaker, V.P., Michaelson, I.A. and Kirkland, R.J.A. (1964) Biochem. J. 90, 293-303.
14. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
15. Devane, W.A., Dysarz, F.A., III, Johnson, R., Melvin, L.S. and Howlett, A.C. (1988) Mol. Pharmacol. 34, 605-613.
16. Bligh, E.G. and Dyer, W.J. (1959) Can. J. Biochem. Physiol. 37, 911-917.
17. Vyvoda, O.S. and Rowe, C.E. (1973) Biochem. J. 132, 233-248.
18. Ueda, H., Kobayashi, T., Kishimoto, M., Tsutsumi, T., Watanabe, S. and Okuyama, H. (1993) Biochem. Biophys. Res. Commun. 195, 1272-1279.
19. Ueda, Kobayashi, T., Kishimoto, M., Tsutsumi, T. and Okuyama, H. (1993) J. Neurochem. 61, 1874-1881.
20. Sugiura, T., Kudo, N., Ojima, T., Mabuchi-Itoh, K., Yamashita, A. and Waku, K. (1995) Biochim. Biophys. Acta 1225, 167-176.
21. Tsutsumi, T., Kobayashi, T., Ueda, H., Yamauchi, E., Watanabe, S. and Okuyama, H. (1994) Neurochem. Res. 19, 399-406.
22. Hasegawa-Sasaki, H. (1985) Biochem. J. 232, 99-109.
23. Mechoulam, R., Ben-Shabat, S., Hanus, L., Ligumsky, M., Kaminski, N.E., Schatz, A.R., Gopher, A., Almog, S., Martin, B.R., Compton, D.R., Pertwee, R.G., Griffin, G., Bayewitch, M., Barg, J. and Vogel, Z. (1995) Biochem. Pharmacol. 50, 83-90.

scientific reports

Check for updates

OPEN

A machine learning approach for understanding the metabolomics response of children with autism spectrum disorder to medical cannabis treatment

Jean-Christophe Quillet, Michael Siani-Rose, Robert McKee, Bonni Goldstein, Myiesha Taylor & Itzhak Kurek✉

Autism spectrum disorder (ASD) is a neurodevelopmental condition impacting behavior, communication, social interaction and learning abilities. Medical cannabis (MC) treatment can reduce clinical symptoms in individuals with ASD. Cannabis-responsive biomarkers are metabolites found in saliva that change in response to MC treatment. Previously we showed levels of these biomarkers in children with ASD successfully treated with MC shift towards the physiological levels detected in typically developing (TD) children, and potentially can quantify the impact. Here, we tested for the first time the capabilities of machine learning techniques applied to our dynamic, high-resolution and rich feature dataset of cannabis-responsive biomarkers from a limited number of children with ASD before and after MC treatment and a TD group to identify: (1) biomarkers distinguishing ASD and TD groups; (2) non-cannabinoid plant molecules with synergistic effects; and (3) biomarkers associated with specific cannabinoids. We found: (1) lysophosphatidylethanolamine can distinguish between ASD and TD groups; (2) novel phytochemicals contribute to the therapeutic effects of MC treatment by inhibition of acetylcholinesterase; and (3) THC- and CBD-associated cannabis-responsive biomarkers are two distinct groups, while CBG is associated with some biomarkers from both groups.

Autism spectrum disorder (ASD) is a set of heterogeneous neurodevelopmental conditions that affect social interaction and communication with defined stereotyped patterns of behavior[1]. It is a lifelong condition with onset as early as the first or second trimester that is often co-occurring with intellectual disabilities, psychiatric conditions, neuro-inflammation, and/or gastrointestinal disorders[2–5].

Diagnosis and evaluation of treatment efficacy are challenging due to the clinical phenotypic heterogeneity of ASD and currently rely solely on subjective evaluation by developmental pediatricians, neurologists, or psychologists. As such, observational survey tool scores are not comparable among patients, and do not provide information regarding the underlying pathophysiology of ASD. Since the onset of ASD is triggered by both genetic and environmental factors via a cascade of biochemical events that leads to pleiotropic metabolic abnormalities with high variability among the individuals, it is challenging to identify ASD biomarkers[6]. The fact that the risk of having a second child with ASD is 25-fold higher for families that already have a child with ASD as compared to families with a typically-developing (TD) child strongly suggests the involvement of genetic factors[7]. However, genetic biomarkers associated specifically with ASD have not been identified or routinely used for screening. Abnormal levels of proteins and metabolites related to oxidative stress, inflammation, mitochondrial dysfunction and immune dysregulation have been identified and characterized in ASD in the last two decades[8]. Nonetheless, the high metabolic variability among individuals with ASD and comorbidity associated with other disorders have limited the development of reliable proteomic and metabolic biomarkers for diagnosis and treatment evaluation.

Machine learning (ML) is a subfield of artificial intelligence (AI) in which a variety of statistical and computational methods are applied to large and complex datasets in order to develop and/or fit predictive models by

Cannformatics, Inc., 3859 Cesar Chavez St, San Francisco, CA 94131, USA. ✉email: itzhak@cannformatics.com

imitating human pattern-recognition processes[9]. ML requires a training dataset consisting of data points each considered as a single observation from an experiment that is described by number of features. A sufficient number of trained features permits the development of a model that predicts the output. ML techniques have been successfully applied to metabolomics studies to identify the following: the metabolic signature of severe COVID-19 cases, the taxonomy of human gut microbiota, metabolic changes in human pregnancy, influenza infection, renal cell carcinoma (88% accuracy), diabetic kidney disease, head and neck paragangliomas (99.2% accuracy), early-stage bladder cancer (up to 95% accuracy) and the metabolomics signatures of major depressive disorder subtypes[10]. Chen et al.[11] combined GC/MS-based untargeted urine metabolomics of samples collected from a group of children with ASD and a TD control group with the XGBoost algorithm to identify 20 potential metabolic biomarkers to distinguish between the groups, which were mapped to a variety of metabolic pathways.

MC treatment is emerging as a promising solution for treatment of children with ASD, especially in the absence of approved medications that fail to treat the core symptoms of ASD. Improvements in the social communication skills of children and adolescents with ASD treated with CBD-rich cannabis were recently reported by Hacohen et al.[12], and improvement in behavioral outbursts, anxiety, and communication were reported for children with ASD treated with low tetrahydrocannabinol (THC) and high cannabidiol (CBD) formulations[13]. These studies used subjective evaluations, limiting the ability to quantify the impact of MC treatment. With the increase of MC treatment of children and adolescents with ASD, there is a growing need for objective, quantified data to determine the effectiveness, safety, mechanism of action and cellular targets of cannabinoids.

We have recently reported on a new pharmacometabolomics approach in which the levels of metabolites, specifically cannabis-responsive biomarkers, in children with ASD being treated with MC shifted toward the physiologic levels detected in untreated typically developing (TD) children[14,15]. In these investigations, we identified a possible link between cannabis-responsive biomarkers and mitochondrial dysfunction, alterations in neurotransmitters, abnormal neuronal development, neuroinflammation, bioenergy and oxidative stress. Importantly, the cannabis-responsive biomarkers quantified the impact of MC treatment on the children with ASD, shedding light on the underlying pathophysiology of ASD and indicating a possible mechanism of action (MOA) of cannabinoids.

In our previous studies[14,15] we used hard-coded algorithms to identify and rank biomarkers that solely shift toward physiological levels. Here we explore the potential of the cannabis-responsive biomarker database, which contains a large number of metabolites detected in a limited number of children with ASD, regardless of the physiological outcomes, and TD controls, for ML applications primarily yielding new biomarker candidates overlooked in previous studies. We trained Gradient Boosting models to: (1) distinguish individuals with ASD from the control group before and after MC treatment; (2) identify non-cannabinoid plant molecules (phytochemicals) with medicinal benefits that contribute to the synergistic effect known as the entourage effect[16]; (3) distinguish specific THC-, CBD- and cannabigerol (CBG)-responsive biomarkers; and (4) provide insights on the specific impact of cannabinoids on imbalanced metabolic pathways in children with ASD. Gradient Boosting-based ML models perform well with high dimensional data where the number of features exceeds the number of samples, and provide us with a straightforward method to rank these features, the metabolites, according to their relative contribution to the prediction for each task.

The preliminary results presented in this study demonstrate the potential of the cannabis-responsive biomarker database in conjunction with ML applications to provide insight into the pharmacokinetics, pharmacodynamics and MOA of cannabinoids and on targets in endocannabinoid system (ECS)-related disorders, and to identify new *Cannabis* phytoconstituents with potential therapeutic roles.

Results

Potential cannabis-responsive biomarker database characteristics. Fifteen children (average age 9.4 years) participated in the ASD group and 10 children with a similar age distribution (average age 9.3 years) participated in the TD untreated control group as previously described in detail[14,15]. Within the ASD group 11 children exhibited severe range, 2 children exhibited moderate range and 2 children exhibited a mild range of social impairment associated with ASD as reported by parent ratings (SRS-2), as described in detail by Siani-Rose et al.[14] (Suppl 2).

We applied ML applications to source data for each child containing the absolute values of 645 metabolites detected in the saliva collected from study participants by dual scan capillary electrophoresis time-of-flight-mass spectrometry (CE–TOF–MS) and rapid resolution liquid chromatography-time-of-flight-mass spectrometry (RRLC–TOF–MS)[14,15]. The samples were designated in children with ASD before MC treatment as PRE and approximately 90 min after MC treatment as PEAK, and a single sample from children in the untreated TD control group as (TD). For some children with ASD, we also collected samples designated Post-1 and Post-2 approximately 180 and 270 min after MC treatment, respectively. This data was supplemented with the numerical data shown in Table 1, in which the doses of the major cannabinoids used in the MC treatment were grouped onto scales ranging from 0–3 (THC), 0–4 (CBD) and 0–2 (CBG); and parent behavioral rating surveys at PRE and PEAK were grouped on a scale of 0–3. THC was part of the treatment for 80% of the children, CBD was part of the treatment for 67% of the children and CBG was part of the treatment for 33% of the children. All the children were taking THC or CBD, with 47% taking both THC and CBD, and 7% taking THC, CBD and CBG. This ranking included all the practical combinations of THC, CBD and CBG with sufficient statistical power. According to behavioral rating surveys, parents of the ASD group reported full and partial improvement after MC treatment (PEAK vs PRE) in 80% of children.

Differentially-expressed potential cannabis-responsive biomarkers distinguish categories of children with ASD. Using ML Gradient Boosting for Multi-Class classification of the metabolomics sam-

Participant ID	Rating				
	Cannabinoid Dosage			Behavior	
	THC	CBD	CBG	PRE	PEAK
A01	1	0	0	0	1
A02	2	1	0	0	1
A03	2	0	1	0	2
A05	1	0	1	0	
A06	2	1	0	0	1
A08	1	3	1	0	1
A09	3	0	0	0	1
A11	1	2	0	0	
A12	0	2	2	0	2
A13	0	3	1	0	1
A14	2	3	0	0	1
A15	0		0	0	2
A16	2	1	0	0	1
A17	1	1	0	0	1
A18	1	0	0	0	

Table 1. Numerical scales of major cannabinoids and behavioral rating surveys used for datasets of children with ASD. Cannabinoid dosages were grouped according to the following concentrations: THC: (0) No; (1) 0.05–5.00 mg; (2) 5.05–15.00 mg; and (3) > 15.05 mg. CBD: (0) No; (1) 1–0 mg; (2) 31-84 mg; (3) 85–100 mg; and (4) > 100 mg. CBG: (0) No; (1) 1–49 mg; (2) > 50 mg. Color intensity represents the group ranking, with no color at 0. Behavior ranking at PRE and PEAK time points were numbered and color coded as follows: (1, blue) improved; (2, yellow) partially improved; and (3, red) worsened.

ples and the resulting importance ranking of the features for model prediction, we have generated 3 categories of potential biomarkers described below and in Fig. 1A.

(1) ASD PRE/ASD PEAK

Nine potential cannabis-responsive biomarkers were identified as candidates for distinguishing before MC treatment (PRE) or after MC treatment (PEAK), including the neuroactive compounds anandamide (AEA), lysophosphatidylethanolamine (LysoPE18:1), the neurodegenerative-associated lipids lysophosphatidylcholine (LysoPC18:0 and LysoPC16:0) and sphingosine, and the lipids/lipid pathway compounds hydroxy glutaric acid, acetyl sphingosine, diethanolamine (dETA), and ethanolamine phosphate (ETA-P).

(2) ASD PRE and ASD PEAK

Ten potential cannabis-responsive biomarkers were identified as candidates for distinguishing all children with ASD treatment where both PRE and PEAK are combined in a single string including the neuroactive compounds AEA, LysoPE(18:1), homovanilic acid (HVA), cortisol; the lipids palmitoyl-carnitine, arichidic acid, 2-hydroxy butyric acid (2-HBA), lactosyl ceramide; and the steroid/derivative lanosterol and Dehydroisoandrosterone 3-sulfate (DHEA-S).

(3) TD/ASD PRE/ASD PEAK

Seven potential cannabis-responsive biomarkers were identified as candidates for distinguishing the TD control group and the ASD children at PRE and PEAK, including the neuroactive LysoPE(18:1), sphingosine, the neurodegenerative-associated lipids LysoPC18:0 and LysoPC16:0, sphingomyelin, sphinganine and eicosatrienoic acid (DGLA).

LysoPE (18:1) was the only potential cannabis-responsive biomarker for distinguishing all 3 categories, as indicated by the Venn diagram (Fig. 1A). The overall high LysoPE (18:1) levels and high sample variability (Fig. 1B) found in children with ASD at PRE (blue) decreased at PEAK (light blue) but did not reach the low levels and low variability detected in the TD group (green). This expression pattern fits the criteria of a biomarker

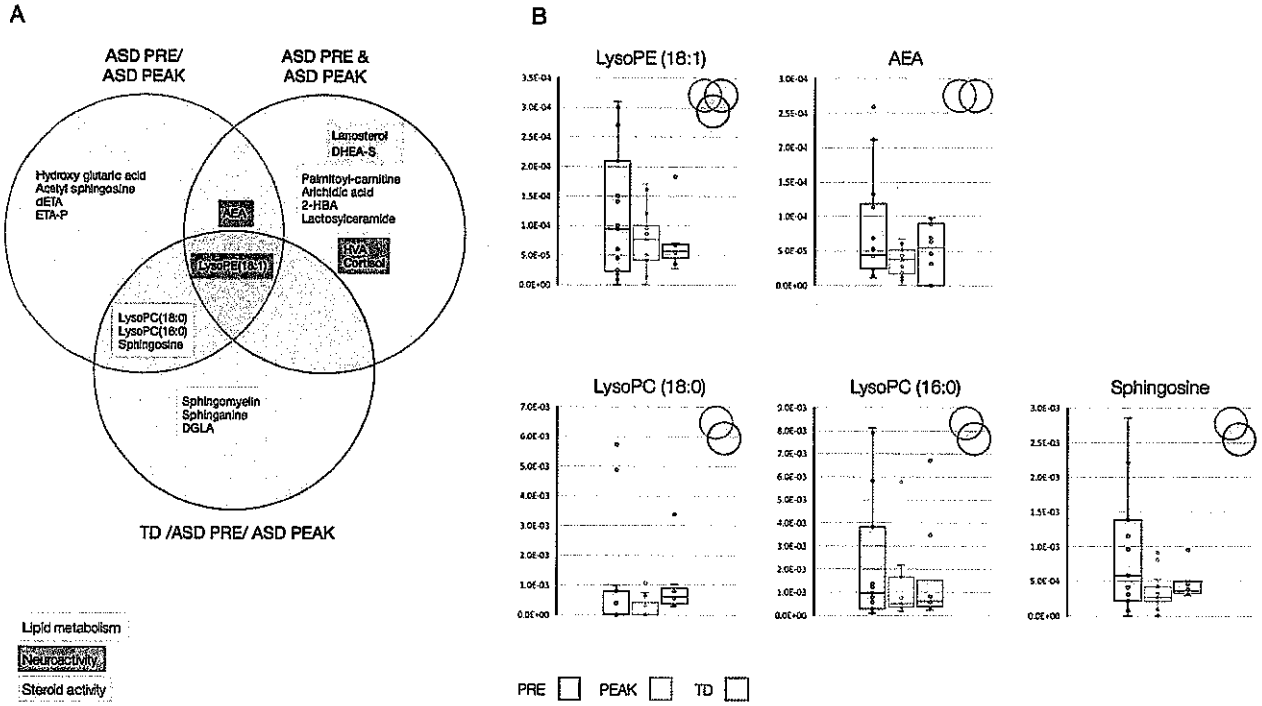


Figure 1. Identification of potential ASD cannabis-responsive biomarkers that distinguish categories of patients. (**A**) Venn diagram illustrating the unique and overlapping differentially-expressed cannabis-responsive biomarkers found in the categories of patients with ASD PRE/ASD PEAK, ASD PRE and ASD PEAK and TD/ASD PRE/ASD PEAK. The biomarker roles (lipid metabolism, neuroactivity and steroid activity) are color coded (white, orange and yellow, respectively). (**B**) Levels of potential cannabis-responsive biomarkers found in children with ASD at PRE (blue) and PEAK (light blue), and TD group (green) in the overlapping categories described in (**A**). Each box plot horizontally enclosed by the lower and upper quartiles and median (solid horizontal line within the box) is indicated. The overlapping categories are indicated in the upper right corner.

that can be used to distinguish all three categories, namely ASD PRE/ASD PEAK, ASD PRE and ASD PEAK and TD/ASD PRE/ASD PEAK.

The endocannabinoid AEA, which functions as a neurotransmitter and is produced and released "on demand"[14], was found to overlap the categories ASD PRE/ASD PEAK and ASD PRE and ASD PEAK, with the overall lowest levels and lowest variability in children with ASD at PEAK.

The potential lipid-based cannabis-responsive biomarkers LysoPC (18:0), LysoPC (16:0) and sphingosine overlapped in the ability to distinguish the ASD PRE/ASD PEAK and TD /ASD PRE/ASD PEAK categories by reducing the high levels and high sample variability at PRE to low levels and low sample variability at PEAK, reaching a range and sample variability closer to the levels obtained in the TD control group.

Differentially-expressed plant metabolites distinguish categories of patients. The approach described above successfully identified plant metabolites for distinguishing categories of children with ASD (Fig. 2A). Seven dietary phytochemicals including flavone, rutin (quercetin-3-rutinoside), vitexin (Apigenin 8-glucoside), naringenin, zeaxanthin, corosolic acid and sitosterol were detected in children with ASD at PEAK (Fig. 2B). Sitosterol was the most abundant dietary phytochemical detected in the saliva of 10 children with ASD. Rutin, vitexin and naringenin were less abundant and were each detected in only 5, 4 and 4 children (respectively). Corosolic acid was the only dietary phytochemical detected in both the ASD and TD control group and exhibited a slightly increased amount in PEAK vs PRE toward the levels seen in TD subjects.

As indicated in the Venn diagram in Fig. 2A, rutin overlapped the ASD PRE/ASD PEAK and TD/ASD PRE/ASD PEAK categories, and sitosterol overlapped the ASD PRE and ASD PEAK and TD/ASD PRE/ASD PEAK. Time dependent detection of vitexin in saliva samples of child A18 and rutin in saliva samples of child A16 (Fig. 2C,D respectively) indicated a different bioavailability pattern in which vitexin degrades faster than rutin. Therefore, the detected levels of rutin in the saliva of children with ASD at PRE could be a result of previous treatment.

The impact of major cannabinoids on differentially-expressed potential cannabis-responsive biomarkers distinguishing categories of children with ASD. Using ML Gradient Boosting for Multi-Class classification of the metabolomics samples and the resulting importance ranking of the features, we have analyzed the specific contribution of THC, CBD and CBG found in the MC treatment described in Table 1 in a data set of 645 metabolites detected in 30 saliva samples from 15 children with ASD at PRE and PEAK time points. As shown in Fig. 3, THC was associated with the response of 11 potential cannabis-responsive biomark-

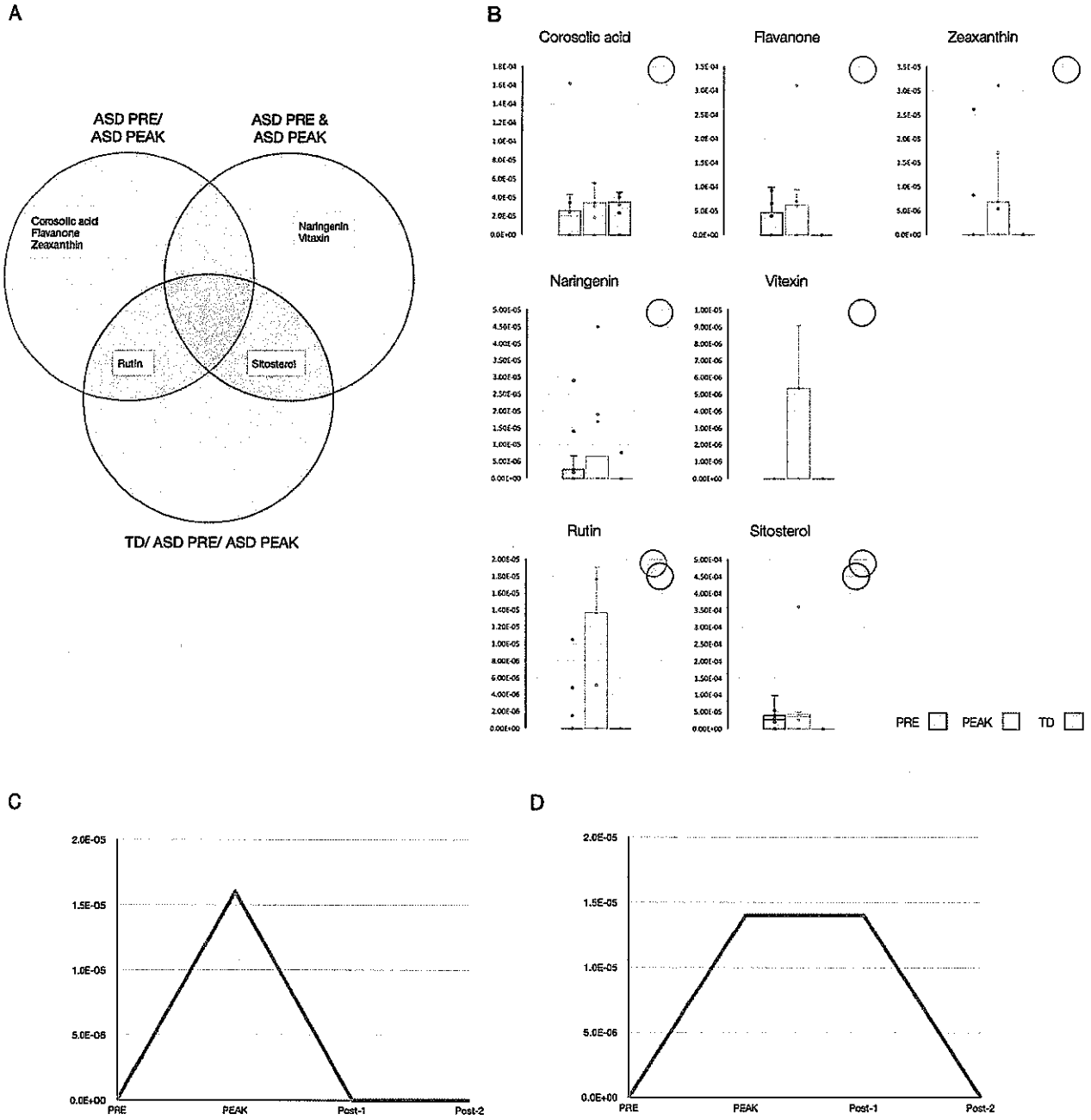


Figure 2. Identification of plant non-cannabinoid secondary metabolites (dietary phytochemical) that distinguish categories of patients. (**A**) Venn diagram illustrating the unique and overlapping dietary phytochemicals found in the categories of patients ASD PRE/ASD PEAK, ASD PRE and ASD PEAK and TD/ ASD PRE/ASD PEAK. The dietary phytochemical functions (lipid, neuroactive and steroid) are color coded (white, orange and yellow, respectively). (**B**) Levels of dietary phytochemicals found in children with ASD at PRE (blue) and PEAK (light blue), and TD group (green) in the overlapping categories described in (**A**). Each box plot horizontally enclosed by the lower and upper quartiles and median (solid horizontal line within the box) is indicated. The overlapping categories are indicated in the upper right corner. (**C**) Time dependent levels of vitexin (apigenin 8-glucoside) detected at time points PRE (10 min before MC treatment), PEAK, Post-1 and Post-2 (90, 180 and 270 min after MC treatment, respectively) in child ID A18. (**D**) Time dependent levels of rutin (quercetin 3-rutinoside) detected at time points described in (**C**) in child ID A16.

ers representing lipids, neuroactive molecules and steroids (5, 5 and 1 respectively), including the endocannabinoids AEA and 2-arachidonoylglycerol (2-AG). CBD was associated with 7 potential cannabis-responsive biomarkers with roles in lipid metabolism, neuroactivity and protein metabolism (5, 1, and 1 respectively). The 11

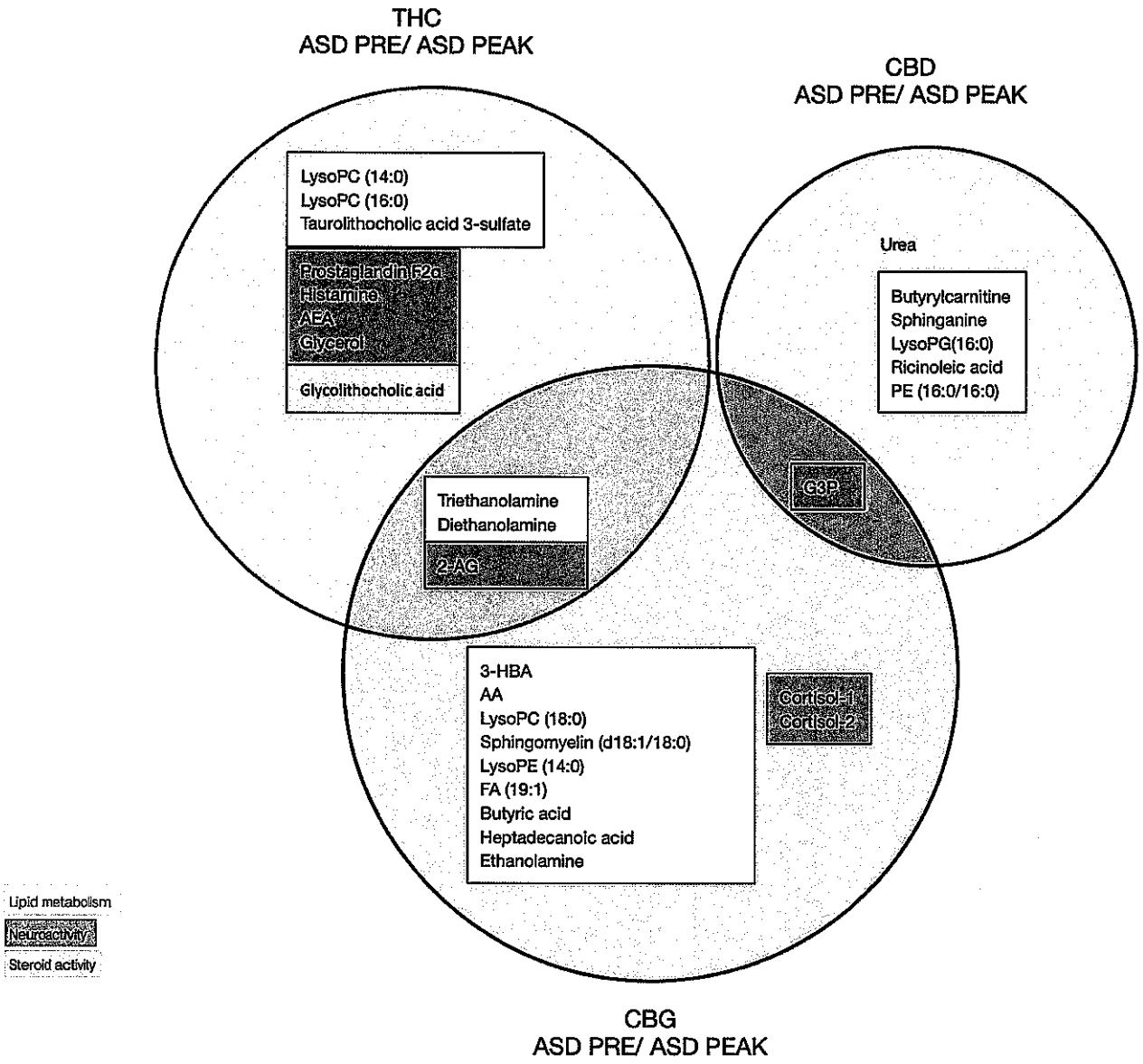


Figure 3. Identification of potential THC-, CBD- and CBG-responsive biomarkers that distinguish patients with ASD at PRE vs PEAK. Venn diagram illustrating the unique and overlapping cannabis-responsive biomarkers that respond (PRE/PEAK) to THC, CBD and CBG treatment. The biomarker functions (lipid metabolism, neuroactivity and steroid activity) are color coded (white, orange and yellow, respectively).

THC potential cannabis-responsive biomarkers did not overlap with the 7 CBD potential cannabis-responsive biomarkers. CBG was associated in the response of 15 potential cannabis-responsive biomarkers with roles in lipid metabolism and neuroactivity (11, and 4 respectively). Three CBG potential cannabis-responsive biomarkers overlapped with THC, including the endocannabinoid 2-AG, di- ethanolamine (dETA) and tri-ethanolamine (tETA); and one CBG potential cannabis-responsive biomarker, glycerol 3-phosphate (G3P), overlapped with CBD.

Discussion

Metabolic biomarkers are dynamic components of the omics disciplines (genomics, transcriptomics, proteomics, and metabolomics) closest to phenotype that can quantify physiological changes[10]. These biomarkers are being successfully used for patient stratification, diagnosis, monitoring, pharmacodynamic/response, and predictive tools[17]. We have recently reported on salivary cannabis-responsive biomarkers, metabolites that objectively measure the response to MC treatment in children with ASD and indicating the impact in comparison to targeted values determined in a TD population[14,15]. Cannabis-responsive biomarkers that provide a high-resolution snapshot of cannabinoid-dependent metabolic changes are the closest quantifiable step to the phenotypic evaluation currently used in children with ASD. Using insights from Gradient Boosting-based ML predictors with the limitations of a small patient dataset, we were able to successfully utilize the complex cross-features (metabolites)

and determine the possible impact of MC treatment on the role of known and annotated metabolic pathways in children with ASD. In addition, we were able to associate new plant-based non-cannabinoid metabolites with the therapeutic impact in our ASD treatment group, which has not been previously reported.

Biomarkers for distinguishing categories of children with ASD based on response to MC treatment. In our previous studies we used different algorithms on the same dataset in order to identify cannabis-responsive biomarkers using a screening and sorting methodology for beneficial outcomes[14,15]. The previous algorithms screened for metabolites that meet the following therapeutic importance criteria, namely: (1) changes in response to cannabis in 60% or more children with ASD; (2) levels after MC treatment (PEAK) significantly different from PRE; and (3) the highest number of children in which the levels shifted toward the physiological range determined as 2 standard deviations (SDEVs) from the average found in the TD group. In this study we applied ML tools to distinguish between categories of children with ASD at PRE, PEAK and the TD control focusing on unique metabolites that differentiate categories (i.e. PRE, PEAK, TD) regardless of the positive or negative outcome.

Within the TD/ASD PRE/ASD PEAK category, the ML predictors can include biomarkers that were identified in the previous study that moved toward the TD range (therapeutically beneficial) and/or new biomarkers that moved away from the TD range (therapeutically harmful) after MC treatment. We identified 7 metabolites in the TD /ASD PRE/ASD PEAK category that have been previously characterized as potential cannabis-responsive biomarkers (Siani-Rose et al.[14], Suppl 1) with roles in lipid metabolism[15] and linked to ASD. Moreover, we could not find any evidence of negative impact of MC, namely a metabolite in which the sample distribution in the PRE group was similar to the TD group and subsequently increased (shifted away from TD range) at PEAK (Fig. 1B). This could be explained by the selection of children for the ASD group that were successfully treated with MC under physician supervision.

Among the three categories described in Fig. 1, the neuroactive lipid-based cannabis-responsive biomarker LysoPE(18:1) was the only distinguishing factor in all 3 categories (ASD PRE/ASD PEAK, ASD PRE and ASD PEAK and TD/ASD PRE/ASD PEAK), which may indicate a partial impact of MC treatment on important ASD underlying conditions. Abnormal levels of LysoPE(18:1) detected in the hippocampus of rats were previously suggested as an indicator of postischemic cognitive impairment[18]. The endocannabinoid AEA was the only metabolite distinguishing factor in the categories ASD PRE/ASD PEAK and ASD PRE and ASD PEAK but not the TD group. AEA responds to MC similarly to all the other metabolites and overlapped at least 2 categories described in Fig. 1B by decreasing the large sample distribution range detected at PRE to the small distribution range obtained in the TD group. This tighter distribution at PEAK suggests down-regulation of the endocannabinoid AEA in response to cannabinoid treatment, potentially suggesting improved ECS tone. Our observations further support Di Marzo et al.[19] in which repeated treatment with THC reduced the AEA biosynthetic precursor N-arachidonoylphosphatidylethanolamine (NArPE) content and signaling in the striatum of rats.

Within the unique metabolites obtained specifically in the ASD PRE/ASD PEAK or the ASD PRE and ASD PEAK category, only palmitoyl-carnitine, HBA, HVA and cortisol met the cannabis-responsive biomarker criteria previously reported (Siani-Rose et al.[14], Supp 1), while all the others were identified in our previous study but did not meet the first criteria, namely present in 60% or more children[14]. For example, DHEA-S associated with aggression in psychiatric disorders[20] was detected at high levels only in young adolescents (11–12 years old boys).

Non-cannabinoid plant phytochemicals with potential entourage effects. The ability to identify metabolites distinguishing TD/ASD PRE/ASD PEAK allowed us to associate known plant phytochemicals with successful MC treatment observed by parental evaluation and previously reported in Siani-Rose et al.[14] Supp 3. Phytochemicals are secondary plant metabolites such as polyphenols (e.g. flavonoids), terpenoids (e.g.carotenoids) and phytosterols (e.g. sterols) with medicinal properties such as anti-inflammatory, antioxidant and antibacterial[21]. The cannabis monoterpenes such as limonene, myrcene, and linalool, that share the common C-10 precursor geranyl diphosphate (GPP) with CBGA, are considered to provide a phytocannabinoid-terpenoid synergistic effect known as the entourage effect[16]. In this study, we have identified 7 plant-based molecules, all dietary phytochemicals from 3 groups all previously reported to inhibit acetylcholinesterase: polyphenols (flavone[22], rutin[23], vitexin[24] and naringenin[25]), terpenoids (zeaxanthin[26] and corosolic acid[27]) and phytosterols (sitosterol[27]) (Fig. 2A,B). Since 6 phytochemicals (flavone, rutin, vitexin, naringenin, zeaxanthin and sitosterol) were not detected in the TD group and exhibited increased levels at PEAK, it is possible to characterize this acetylcholinesterase (AChE) inhibition activity as an entourage effect. In this respect, AChE inhibition by the drug galantamine was previously reported to effectively reduce irritability and lethargy/social withdrawal in children with ASD[28].

Biomarkers for distinguishing association with major cannabinoids in children with ASD based on response to MC treatment. Cannabis-responsive biomarkers specifically categorized by THC, CBD and CBG can indicate the metabolic pathways that are affected by cannabinoids in children with ASD (Fig. 3). This is a preliminary step in understanding the MOA of cannabinoids and a path to personalized MC treatment. We found that THC interacts with the retrograde cannabinoid signaling pathway (KEGG map 04723; Kyoto Encyclopedia of Genes and Genomes, https://www.kegg.jp) by changing the levels of 2-AG and AEA. THC therefore plays an important role in signaling across the synaptic cleft, and in the binding and activating CB1 receptor found in both the neural membranes at the synapse and in the mitochondrial membrane in the excitatory and inhibitory terminals[29]. CBG was found to affect the levels of 2-AG only, while CBD did not affect any of the endocannabinoids, suggesting a different MOA. Moreover, THC, CBD and CBG trigger changes in the glycerophospholipid metabolism pathway (KEGG map00564), and CBD and CBG affect sphingolipid metabo-

lism (KEGG map00600). While THC changes the levels of lipid-, neuroactive- and steroid-based biomarkers, CBD mainly affected lipid-based biomarkers as suggested by Veilleux et al. via the extended ECS or the endocannabinoidome (eCBome)[30]. CBGA, the acidic form to CBG, is also the precursor to THCA and CBDA, which convert to THC and CBD via decarboxylation at elevated temperature. CBG was less specific and can possibly function as a "bridge" between THC and CBD based on overlapping biomarkers obtained in this study; this adds support to the findings by Nachnani et al.[31], namely that "CBG seems to reside, pharmacologically, in between THC and CBD."

ML and expanding knowledge of metabolic pathways. By taking together the ML data obtained in this study and the known metabolic pathways of endocannabinoids[32], lysoglycerophospholipids[33], sphingolipid[34], and fatty acid oxidation[35] in ASD[36], markers of depression[37] and pathways containing histamine[38], we were able to assemble a preliminary simplified THC-, CBD- and CBG- responsive metabolic pathway in children with ASD (Fig. 4). We also introduced general cannabis-responsive biomarkers previously described in Siani-Rose[14,15] to this simplified metabolic pathway. The endocannabinoid metabolic pathway was strongly linked with THC, including with both AEA and 2-AG. CBG was associated with 2-AG, arachidonic acid (AA) and ethanol amine (ETA), while tETA and diETA were associated with both THC and CBG.

Studies suggested that ASD[36] and Alzheimer's Disease[40] are linked to neuroinflammation-associated increased activity of the brain phospholipase A2 (PLA2) that specifically converts cell membrane phospholipids to arachidonic acid (AA). Similarly, Esvap and Ulgen[34] reported increased PLA2 hydrolysis activity of phospholipids into AA and polyunsaturated free fatty acids (PUFA) in children with ASD. In this respect, the association of CBG with AA may provide an insight into its anti-inflammatory role by reducing AA levels in children with ASD (PRE/PEAK) possibly via PLA2. This CBG-associated AA was also linked with the THC-associated metabolic pathway that includes 2-AG, prostaglandin F2α and glycerol, all of which are involved in ASD[36] and in depression[37].

Lysoglycerophospholipids are hydrolyzed glycerophospholipidlipids involved in signaling and membrane biosynthesis. Our preliminary data showed that members of lysoglycerophospholipid pathways are possibly associated with THC (LysoPC), CBD (LysoPG) and CBG (LysoPE and LysoPC), all of which are products of the phospholipase A (PLA) enzymes sPLA2, PLA2 and PLA1 and 2, respectively[33]. A significant increase in PLA2 activity was previously reported by Bell et al.[39] and by Qasem et al.[41], who showed a decrease in the mean concentrations PE and PC, the substrates of LysoPE and LysoPC respectively. This is in agreement with our observations showing increased levels of LysoPE and LysoPC at PRE (Fig. 1) that were reduced in response to MC treatment containing THC, CBD and/or CBG. Qasem et al.[41] suggested that inflammation increases PLA2 levels via mediators in a response to oxidative stress in children with ASD. It is possible that THC, CBD and/or CBG prevent inflammation and therefore reduce the levels of PLA2.

The sphingolipid biosynthesis pathway was associated with CBD (sphinganine) and CBG (sphingomyelin). Three lipids in the sphingolipid biosynthesis pathway, namely, sphingosine, lactosyl-ceramide and acetyl sphingosine, detected in high levels at PRE in children with ASD, were not associated with any cannabinoid (Fig. 1). While low/no detectable levels of ceramide were obtained in our study[14,15], the high levels detected in its direct product sphingosine (Fig. 1B) and sphingomyelin (not shown) could be explained by the high production of ceramide that quickly converted to other derivatives including sphingosine and sphingomyelin. Esvap and Ulgen[34], using transcriptomics data to develop an ASD-specific genome-scale metabolic model (GEM), have suggested that ceramide biosynthesis is induced by oxidative stress in children with ASD and quickly hydrolyzed. Our data support this hypothesis as MC treatment in general[14,15] and CBD and CBG in this study were linked with reduced oxidative stress.

ML and cannabis-responsive biomarker database. Developing cannabis-responsive biomarkers is the first step to objectively quantify the impact of MC treatment. It also presents an opportunity to better understand the relationships between these biomarkers and cannabinoids using ML applications, and to obtain insight into the MOA of active cannabinoids on the underlying conditions of ASD. Cannabis-responsive biomarkers provide a dynamic, high-resolution and rich feature dataset in several aspects, as shown in this study: (1) the broad range of detection levels, in which the highest detectable levels of the metabolite lactic acid were 19,000-fold higher than the oxidized glutathione acid levels on a molar basis; (2) the large number of features, i.e. the metabolites and potential biomarkers in each sample; (3) each child is a dynamic (before and after treatment) independent study; and (4) the ability to analyze large numbers of correlations, both linear and complex, among a large number of features and samples. These four aspects, which create a dynamic, high-resolution and rich feature dataset for ML applications, are the key to identifying new potential biomarkers, even with low-distinguishing factors or only in combination with any number of other biomarkers. This dataset also supports the personalized medicine approach, especially for MC treatment, as metabolic changes reflect both genetic and environmental factors, with samples before and after treatment, forming the basis of accurate ML predictions for therapy in the future.

Conclusions

While further large sample size studies are needed to develop a large statistically-robust database, cannabis-responsive biomarkers combined with Gradient Boosting-based ML techniques can successfully personalize ECS-related MC therapy. It can also provide a metabolic snapshot in which MC treatment can be used as a probe to highlight ASD-related metabolic pathways by temporarily switching ASD pathophysiology to homeostasis. Additionally, our preliminary results suggest that ML applications can identify the specific MOA of cannabinoids and entourage effect of phytochemicals without the need to test each one separately.

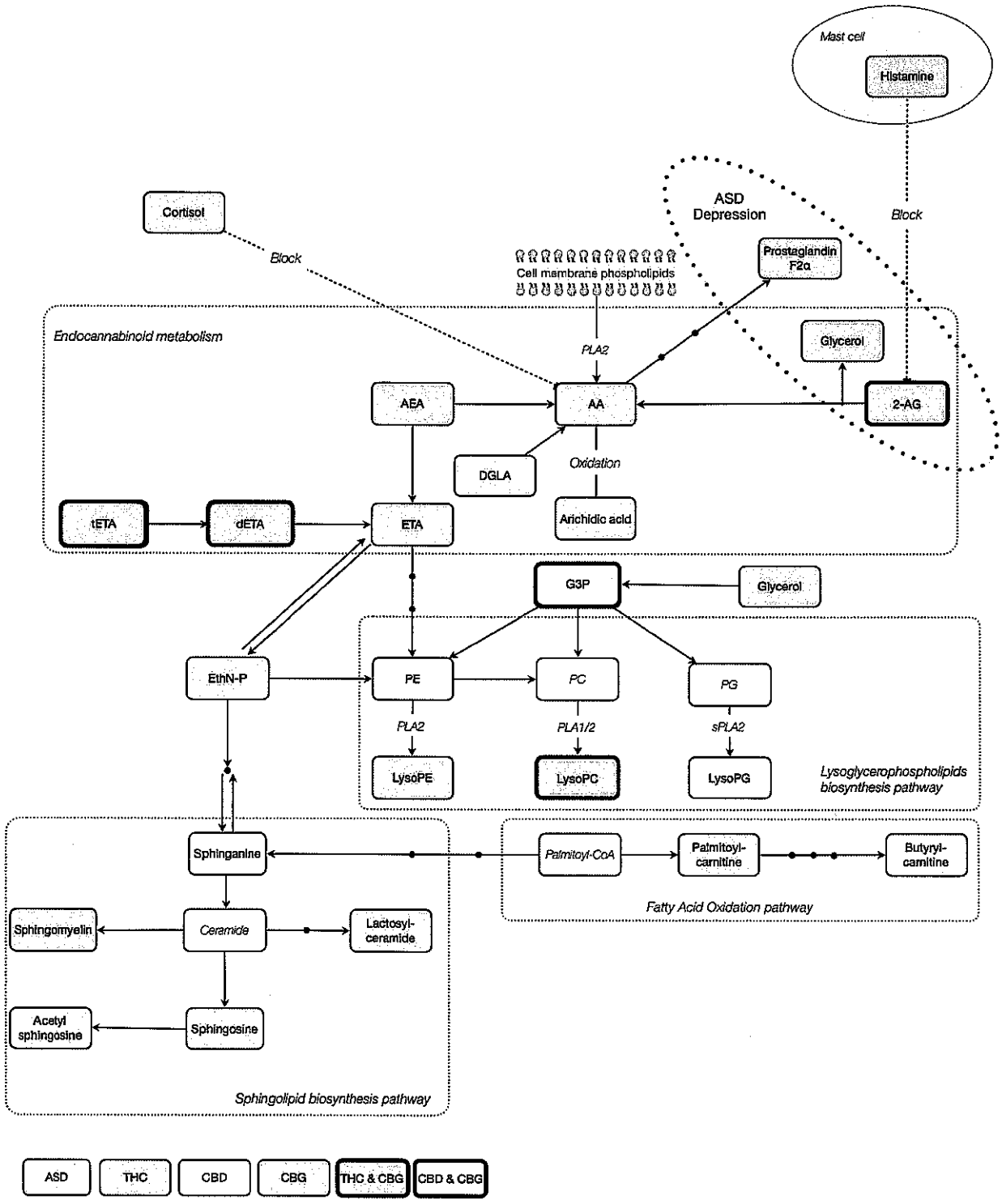


Figure 4. Simplified metabolic pathways associated with the differential expression of potential ASD cannabis-responsive biomarkers after THC, CBD and CBG treatment. Potential ASD cannabis-responsive biomarkers directly respond to THC (green), CBD (blue) and CBG (brown) found in the metabolic pathways of lysoglycerophospholipids, sphingolipid, fatty acid oxidation, anandamide and ethanolamine-phosphate (EthN-P), and their impact on ASD and depression, are indicated. Potential ASD cannabis-responsive biomarkers previously only identified and described in Siani-Rose (2021 and 2022) are in gray, with metabolites in white.

Limitations. Although the limitations to our pilot observational study were previously discussed in Siani-Rose et al.[14] and Siani-Rose et al.[15], we consider several additional limitations relevant to this study. First, the small sample size of the ASD group does not represent the full clinical phenotype heterogeneity found in the entire ASD population and thus we cannot suggest that MC treatment is relevant to all individuals with ASD. Second, each child was considered as single case treated with unique cannabinoid content and regimen. Thus, association of biomarkers with cannabinoid potency is limited to a range of concentrations arbitrarily determined and does not represent direct linear correlation. Third, each observational study was conducted on a single day and represents the child's behavior a single time point that may not reflect the full range of behaviors which are influenced by environmental factors and can vary from day to day. Forth, the biomarker population is biased toward children successfully treated with MC. Fifth, the parents used a dropper to treat the children with prescribed off-the-shelf MC, which may introduce inaccuracy in the levels of dose reported. Sixth, the authors did not verify the cannabinoid content and potency of the MC treatment reported by the parents. Seventh, since the biomarkers were detected in saliva, some may not represent their relevant physiological role. Eighth, the small size of the data set combined with the much larger number of features (molecules) and heterogeneity of regimen is not optimal for the training, and then validation and testing of ML prediction models. A future study to generate a dataset from a larger cohort of patients with more samples will be necessary to address this limitation and develop predictive models that can be generalized, with the purpose of classifying samples with robust accuracy levels.

Due to the complexity of metabolic pathways and current limitations of our collective knowledge as represented in the KEGG database, we expect our proposed simplified cross-pathway interactions (Fig. 4) to evolve as more information is uncovered through similar studies.

Methods

The observational study to assess the response of children with ASD to physician-directed MC treatment using saliva metabolomics and behavioral rating scales was conducted in 2020–2021. The study protocols were reviewed and approved by Ethical and Independent Review Services, an Association for the Accreditation of Human Research Protection Programs, Inc. (AAHRPP) certified institutional review board (ref 20114-01X). We confirm all methods were carried out in accordance with relevant guidelines and regulations. Parents/guardians of participating children signed an informed consent form and TD children from the control group signed an assent form. Participants, study design, data acquisition, and parental behavior assessment, were described in detail in Siani-Rose et al.[14] and Siani-Rose et al.[15], and briefly below.

Participants. ASD group participants (n = 15), average age 9.4 years, male:female ratio 8:1, were recruited through CannaCenters Wellness and Education (Lawndale, CA) and Whole Plant Access for Autism (WPA4A, a 501c3 nonprofit company, Canyon Lake, CA). The inclusion criteria were: (1) ASD diagnosed by a qualified health care professional; (2) MC treatment under physician supervision for at least a year; (3) age between 6 and 12; and (4) ability to donate up to four saliva samples (0.5 ml each) using the passive drool method without discomfort. The exclusion criteria were: (1) children who require cannabis more frequently than every 8 h; (2) traumatic brain injury with any known cognitive consequence or loss of consciousness for more than 5 min; and (3) diagnosed with epilepsy.

TD group participants (n = 10), average age 9.3 years, male:female ratio 9:1, were recruited through a San Francisco online parent group, and the inclusion criteria were as follows: (1) no special education needs and (2) no individual or immediate family member diagnosed with developmental disabilities.

Study design. All the participants in the TD group provided samples at the timepoints: PRE—morning, before MC treatment; and PEAK—when treatment was considered by parents to reach maximal impact based on their observations before the study, about 90 min after MC treatment. Some of the ASD group participants also provided samples at timepoints Post-1 and Post-2, about 180 and 270 min after MC treatment, respectively. The TD control group provided one saliva sample in the morning.

To ensure high reproducibility of the outcomes, the study was conducted as follows: (1) ASD group participants were not treated with MC for at least 8 h before PRE (washout period); (2) all participants did not consume high sugar, acid and caffeine content 1 h before any saliva sample collection; (3) all participants rinsed their mouth 20 min before saliva collection; (4) parents of all participants completed brief behavioral Likert scale 10 min before each saliva sample collection; and (5) all saliva samples were collected using the Passive Drool Collection Kit (Salimetrics, Carlsbad, CA), as previously described in detail in Siani-Rose et al.[14].

Untargeted metabolomic analysis. Immediately after collection all saliva samples were temporarily stored (up to 24 h) at − 20 °C, and then transferred to − 80 °C until the capillary electrophoresistime-of-flight-mass spectrometry (CE-TOF–MS) and rapid resolution liquid chromatography-time-of-flightmass spectrometry (RRLC–TOF–MS) analysis performed by Human Metabolome Technologies, Inc. (HMT, Tsuruoka, Japan) and processed as previously described in detail in Siani-Rose et al.[14].

Data source. Sample preparation, metabolite detection and identification, and quality control analysis were previously published in Siani-Rose et al.[14]. The metabolomics data consists of compounds detected in 40 saliva samples collected from: 15 children with ASD (15 samples at PRE, 15 matching samples at PEAK), and 10 samples from the TD control group.

Data pre-processing. In order to adapt the data to downstream ML analysis, the following preprocessing was applied:

(1) Each non-detected compound entry in a sample was replaced by 0. This allowed the use of algorithms that cannot process missing values, while still using all of the dataset; a significant part of the dataset presents such non-detected compound entries for one or more children. A non-detected compound indicates presence in a sample below the detection level, and therefore replacing it with 0 does not fundamentally change the results we expect to obtain from our analysis. (2) Values were normalized for each individual compound across the dataset in a range (0, 1).

Dataset preparation. Four different datasets were composed for the various ML analyses: (1) Full—a dataset consisting of all 40 samples; (2) ASD—a dataset consisting of only the 30 samples collected from children with ASD; (3) ASD PRE and ASD PEAK—a dataset of 15 entries consisting of the concatenation of the PRE data and a per-compound difference between PRE data and PEAK, referred to as the PRE + PEAK merged data. This dataset contains $2 \times 645 = 1290$ features (or molecules) per sample; and (4) ASD PRE/ASD PEAK—a dataset of 15 entries consisting of a per-compound difference between PRE data and PEAK data for each child in the ASD group.

The numerical datasets were completed with the addition of supplemental survey behavior data. This post-treatment survey data was converted into three outcome categories: (1) improved behavior, (2) partially improved behavior, and (3) worsening behavior. This survey data is used along with prior knowledge regarding which samples belong to children in the TD or ASD PRE group to create the prediction targets for training the ML models (Table 1). The data also included the concentrations of active cannabinoids in the treatment of each child in the ASD group in the form of numerical scales (Table 1) for each of the PEAK samples.

ML prediction tasks. The prediction tasks described below were designed to assess the predictability of the category of a sample from the dataset, as well as to identify potential candidate compounds of interest that enable those predictions.

The full dataset was applied for training models for the ML tasks of classifying samples as TD, ASD PRE, or one of the three ASD PEAK outcome categories (i.e., improved behavior, partially improved behavior, or worsening behavior). The ASD dataset was applied for training models for the ML tasks of classifying samples as ASD PRE or as one of the three ASD PEAK outcome categories. The ASD PRE and ASD PEAK merged dataset was applied for training models for the ML tasks of classifying atypical samples in one of the three ASD PEAK outcome categories. ASD PRE/ASD PEAK dataset was applied for training models for the ML tasks of classifying ASD samples according to the active cannabinoids present in the treatment and their dosage, based on the treatment composition information. The predicted active cannabinoids included THC ranging from 0 to 50 mg per treatment, CBD ranging from 0 to 200 mg per treatment and CBG ranging from 0 to 50 mg per treatment. The ASD PRE and ASD PEAK dataset was also applied for training models for the same ML task.

ML output analysis. The baseline algorithm used for the various prediction tasks described above was the Gradient Boosting[42] implementation from Scikit-learn package. This algorithm provides, after training a model for a prediction task, a score for the usefulness of each feature of the training dataset for the prediction tasks.

The limited number of samples (ASD or TD control) in the datasets combined with the much larger number of features (molecules) was not optimal for training, and then run validation and testing of the prediction models. Therefore, the relevance of the models trained on each task for future prediction is limited, as the models trained most likely overfit the dataset (F1-score equal to 1 for all models trained). A future study to generate a larger dataset with many more samples will be necessary to address this limitation and develop models with the purpose of classifying samples with robust accuracy levels. However, the models trained on the present dataset can provide insights on the high dimensional data, and identify specific metabolites (out of the 645) significant for prediction and information regarding the pathways involved. Therefore, we focused on the most significant metabolites identified for each of the prediction scenarios described above.

Importance ranking (Gini importance) is computed as the normalized total reduction of the criterion brought by that feature. This feature importance ranking represents the contribution of each feature to improving the predictive ability of the model through building the model's boosted decision tree. Though it reflects the importance of features in the final model, it is impossible to evaluate the relationship between the feature and the model predictions. Feature importance has been previously used successfully in the medical research context when using Gradient Boosting-based machine learning predictors. Previously reported applications include intensive care unit patient outcome prediction, where feature importance enabled the presentation of the top ranked features to clinicians to compare relevance of different models in and further characterize the performance of the prediction models[43], as well as acute coronary syndrome risk prediction, to perform feature selection for machine learning model training through an iterative process to select the optimal set of features[44].

In this study, features corresponding to the 645 distinct metabolites (identified by CE–TOF–MS and RRLC–TOF–MS in saliva) were evaluated for all samples. Out of these features, the top 50 ranking features in importance for each prediction task (top 7.7% of all available features) were considered for identifying compounds of interest.

Ethics declarations. This study was approved by the Ethical and Independent Review Services (E&I; Lees Summit, MO), the methods were carried out in accordance with the relevant guidelines and regulations of human subjects research. Informed consent was obtained from parents/guardians of all participating children, and assent was obtained from typically-developing children.

Data availability

The datasets used and/or analyzed in this study are available from the corresponding author upon reasonable request.

Received: 16 April 2023; Accepted: 3 August 2023
Published online: 22 August 2023

References

1. American Psychiatric Association. Diagnostic and statistical manual of mental disorders: DSM-5. Arlington, VA The American Psychiatric Association APA (2013).
2. Parker, W. *et al.* The role of oxidative stress, inflammation and acetaminophen exposure from birth to early childhood in the induction of autism. *J. Int. Med. Res.* **45**, 407–438 (2017).
3. DeFilippis, M. Depression in children and adolescents with autism spectrum disorder. *Children* **5**, 112. https://doi.org/10.3390/children5090112 (2018).
4. Siniscalco, D., Schultz, S., Brigida, A. L. & Antonucci, N. Inflammation and neuro-immune dysregulations in autism spectrum disorders. *Pharmaceuticals* **11**, 56. https://doi.org/10.3390/ph11020056 (2018).
5. Regev, O. *et al.* Association between ultrasonography foetal anomalies and autism spectrum disorder. *Brain* **145**, 4519–4530 (2022).
6. Courchesne, E. *et al.* The ASD living biology: From cell proliferation to clinical phenotype. *Mol. Psychiatry* **24**, 88–107 (2019).
7. Frye, R. E. *et al.* Emerging biomarkers in autism spectrum disorder: A systematic review. *Ann. Transl. Med.* **7**, 792. https://doi.org/10.21037/atm.2019.11.53 (2019).
8. Ristori, M. V. *et al.* Proteomics and metabolomics approaches towards a functional insight onto autism spectrum disorders: phenotype stratification and biomarker discovery. *Int. J. Mol. Sci.* **21**, 6274. https://doi.org/10.3390/ijms21176274 (2020).
9. Greener, J. G., Kandathil, S. M., Moffat, L. & Jones, D. T. A guide to machine learning for biologists. *Nat. Rev. Mol. Cell. Biol.* **23**, 40–55 (2022).
10. Galal, A., Marwa, T. & Ahmed, M. Applications of machine learning in metabolomics: Disease modeling and classification. *Front. Genet.* **13**, 3340. https://doi.org/10.3389/fgene.2022.1017340 (2022).
11. Chen, Q., Qiao, Y., Xu, X. J., You, X. & Tao, Y. Urine organic acids as potential biomarkers for autism-spectrum disorder in Chinese children. *Front. Cell. Neurosci.* **13**, 150. https://doi.org/10.3389/fncel.2019.00150 (2019).
12. Hacohen, M. *et al.* Children and adolescents with ASD treated with CBD-rich cannabis exhibit significant improvements particularly in social symptoms: An open label study. *Transl. Psychiatry* **12**, 375. https://doi.org/10.1038/s41398-022-02104-8 (2022).
13. Aran, A., Cassuto, H., Lubotzky, A., Wattad, N. & Hazan, E. Brief report: cannabidiol-rich cannabis in children with autism spectrum disorder and severe behavioral problems-a retrospective feasibility study. *J. Autism Dev. Disord.* **49**, 1284–1288 (2019).
14. Siani-Rose, M. *et al.* Cannabis-responsive biomarkers: A pharmacometabolomics-based application to evaluate the impact of medical cannabis treatment on children with autism spectrum disorder. *Cannabis Cannabinoid Res.* **8**, 126–137 (2023).
15. Siani-Rose, M. *et al.* The potential of salivary lipid-based Cannabis-responsive biomarkers to evaluate medical cannabis treatment in children with autism spectrum disorder. *Cannabis Cannabinoid Res.* https://doi.org/10.1089/can.2021.0224 (2022).
16. Russo, E. B. Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. *Br. J. Pharmacol.* **163**, 1344–1364 (2011).
17. Califf, R. M. Biomarker definitions and their applications. *Exp. Biol. Med.* **243**, 213–221 (2018).
18. Sabogal-Guáqueta, A. M., Villamil-Ortiz, J. G., Arias-Londoño, J. D. & Cardona-Gómez, G. P. Inverse phosphatidylcholine/phosphatidylinositol levels as peripheral biomarkers and phosphatidylcholine/lysophosphatidylethanolamine-phosphatidylserine as hippocampal indicator of postischemic cognitive impairment in rats. *Front. Neurosci.* **12**, 989. https://doi.org/10.3389/fnins.2018.00989 (2018).
19. Di Marzo, V. *et al.* Enhancement of anandamide formation in the limbic forebrain and reduction of endocannabinoid contents in the striatum of Δ9-tetrahydrocannabinol-tolerant rats. *J. Neurochem.* **74**, 1627–1635 (2000).
20. Barzman, D. H., Patel, A., Sonnier, L. & Strawn, J. R. Neuroendocrine aspects of pediatric aggression: Can hormone measures be clinically useful?. *Neuropsychiatr. Dis. Treat.* **6**, 691–697 (2010).
21. Upadhyay, S. & Madhulika, D. Role of polyphenols and other phytochemicals on molecular signaling. *Oxid. Med. Cell Longev.* 504253. https://doi.org/10.1155/2015/504253 (2015).
22. Uriarte-Pueyo, I. & Calvo, M. I. Flavonoids as acetylcholinesterase inhibitors. *Curr. Med. Chem.* **18**, 5289–5302 (2011).
23. Amat-ur-Rasool, H. *et al.* Potential nutraceutical properties of leaves from several commonly cultivated plants. *Biomolecules* **10**, 1556. https://doi.org/10.3390/biom10111556 (2020).
24. Sheeja Malar, D., Beema Shafreen, R., Karutha Pandian, S. & Pandima Devi, K. Cholinesterase inhibitory, anti-amyloidogenic and neuroprotective effect of the medicinal plant *Grewia tiliaefolia*—an in vitro and in silico study. *Pharm. Biol.* **55**, 381–393 (2017).
25. Ali, M. Y. *et al.* Flavanone glycosides inhibit β-site amyloid precursor protein cleaving enzyme 1 and cholinesterase and reduce Aβ aggregation in the amyloidogenic pathway. *Chem. Biol. Interact.* **309**, 108707. https://doi.org/10.1016/j.cbi.2019.06.020 (2019).
26. El-Baz, F. K., Abdel Jaleel, G. A., Hussein, R. A. & Saleh, D. O. Dunalialla salina microalgae and its isolated zeaxanthin mitigate age-related dementia in rats: Modulation of neurotransmission and amyloid-β protein. *Toxicol. Rep.* **8**, 1899–1908 (2021).
27. Bahadori, M. B., Dinparast, L., Valizadeh, H., Farimani, M. M. & Ebrahimi, S. N. Bioactive constituents from roots of *Salvia syriaca* L.: Acetylcholinesterase inhibitory activity and molecular docking studies. *S. Afr. J. Bot.* **106**, 1–4 (2016).
28. Ghaleiha, A. *et al.* Galantamine efficacy and tolerability as an augmentative therapy in autistic children: A randomized, double-blind, placebo-controlled trial. *J. Psychopharmacol.* **28**, 677–685 (2014).
29. Zou, S. & Ujendra, K. Cannabinoid receptors and the endocannabinoid system: signaling and function in the central nervous system. *Int. J. Mol. Sci.* **19**, 833 (2018).
30. Veilleux, A. D., Marzo, V. & Silvestri, C. The expanded endocannabinoid system/endocannabinoidome as a potential target for treating diabetes mellitus. *Curr. Diabetes Rep.* **19**, 1–12 (2019).
31. Nachnani, R., Raup-Konsavage, W. M. & Vrana, K. E. The pharmacological case for cannabigerol. *J. Pharmacol. Exp. Ther.* **376**, 204–212 (2021).
32. Maccarrone, M. Phytocannabinoids and endocannabinoids: Different in nature. *Rend. Lincei Sci. Fis. Nat.* **31**, 931–938 (2020).
33. Tan, S. T., Ramesh, T., Toh, X. R. & Nguyen, L. N. Emerging roles of lysophospholipids in health and disease. *Prog. Lipid Res.* **80**, 101068. https://doi.org/10.1016/j.plipres.2020.101068 (2020).
34. Esvap, E. & Ulgen, K. O. Neuroinflammation, energy and sphingolipid metabolism biomarkers are revealed by metabolic modeling of autistic brains. *Biomedicines* **11**, 583. https://doi.org/10.3390/biomedicines11020583 (2023).
35. Tracey, T. J., Steyn, F. J., Wolvetang, E. J. & Ngo, S. T. Neuronal lipid metabolism: Multiple pathways driving functional outcomes in health and disease. *Front. Mol. Neurosci.* **11**, 10. https://doi.org/10.3389/fnmol.2018.00010 (2018).
36. Yui, K., Imataka, G. & Yoshihara, S. Lipid-based molecules on signaling pathways in autism spectrum disorder. *Int. J. Mol. Sci.* **23**, 9803 (2022).
37. Yui, K., Imataka, G., Nakamura, H., Ohara, N. & Naito, Y. Eicosanoids derived from arachidonic acid and their family prostaglandins and cyclooxygenase in psychiatric disorders. *Curr. Neuropharmacol.* **13**, 776–785 (2015).

38. Pini, A. *et al.* The role of cannabinoids in inflammatory modulation of allergic respiratory disorders, inflammatory pain and ischemic stroke. *Curr. Drug Targets* **13**, 984–993 (2012).
39. Bell, J. G. *et al.* Essential fatty acids and phospholipase A2 in autistic spectrum disorders. *Prostaglandins Leukot. Essent. Fatty Acids.* **71**, 201–204 (2004).
40. Sanchez-Mejia, R. O. & Lennart, M. Phospholipase A2 and arachidonic acid in Alzheimer's disease. *BBA Mol. Cell Biol. Lipids* **1801**, 784–790 (2010).
41. Qasem, H. *et al.* Increase of cytosolic phospholipase A2 as hydrolytic enzyme of phospholipids and autism cognitive, social and sensory dysfunction severity. *Lipids Health Dis.* **16**, 1. https://doi.org/10.1186/s12944-016-0391-4 (2017).
42. Friedman, J. H. Greedy function approximation: A gradient boosting machine. *Ann. Stat.* **1**, 1189–1232 (2001).
43. Chen, T. *et al.* Prediction of extubation failure for intensive care unit patients using light gradient boosting machine. *IEEE. Access* **7**, 150960–150968 (2019).
44. Lin, H., Xue, Y., Chen, K., Zhong, S. & Chen, L. Acute coronary syndrome risk prediction based on gradient boosted tree feature selection and recursive feature elimination: A dataset-specific modeling study. *PLoS ONE* **17**, 11. https://doi.org/10.1371/journal.pone.0278217 (2022).

Acknowledgements
The study authors wish to acknowledge the contribution of Cannformatics cofounder Mr. Keneth Epstein for participant communications and sample collection. We are deeply grateful to the children and their families who participated in this study. We also thank Mr. Ray Mirzabegian from Canniatric, and Mrs. Rhonda Moeller and Mrs. Jenni Mai from Whole Plant Access for Autism (WPA4A), for their financial support and assistance.

Author contributions
J.C.Q., M.S.R. and I.K: developed the research concept, designed the study, and collected the data. J.C.Q., M.S.R., R.M. and I.K: processed and validated the data. J.C.Q., M.S.R., R.M., B.G. and I.K: analyzed the data. J.C.Q., M.S.R., R.M., B.G., M.T. and I.K.: drafted and reviewed the manuscript.

Competing interests
Mr. McKee and Dr. Kurek are co-founder and employees of Cannformatics. Mr. Siani-Rose is employee of Cannformatics. Mr. Quillet is consultant at Cannformatics. Dr. Goldstein and Dr. Taylor are scientific advisors to Cannformatics. The authors declare that they are bound by confidentiality agreements that prevent them from disclosing their financial interests in this work.

Additional information
Correspondence and requests for materials should be addressed to I.K.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abnormalities in the cerebrospinal fluid levels of endocannabinoids in multiple sclerosis

M Di Filippo,[1,2] L A Pini,[3] G P Pelliccioli,[4] P Calabresi,[1,2] P Sarchielli[1]

[1] Centre for the Study of Demyelinating Diseases, Section of Clinical Neurosciences, Department of Medical and Surgical Specialities and Public Health, Ospedale S Maria della Misericordia, University of Perugia, Perugia, Italy; [2] IRCCS Santa Lucia Foundation, Rome, Italy; [3] Institute of Toxicology and Clinical Pharmacology, University of Modena and Reggio Emilia, Modena, Italy; [4] Neuroradiology Unit, Ospedale S Maria della Misericordia, Perugia, Italy

Correspondence to: Paola Sarchielli, MD, Clinica Neurologica, Ospedale S Maria della Misericordia, Via S Andrea delle Fratte, Perugia, Italy; paola.sarchielli@gmail.com

Received 7 November 2007
Revised 19 February 2008
Accepted 3 March 2008
Published Online First 5 June 2008

ABSTRACT

Objective: Endocannabinoids (eCBs) play a role in the modulation of neuroinflammation, and experimental findings suggest that they may be directly involved in the pathogenesis of multiple sclerosis (MS). The objective of our study was to measure eCB levels in the cerebrospinal fluid (CSF) of patients with MS.

Patients and methods: Arachidonoylethanolamine (anandamide, AEA), palmotylethanolamide (PEA), 2-arachidonoylglycerol (2-AG) and oleoylethanolamide (OEA) levels were measured in the CSF of 50 patients with MS and 20 control subjects by isotope dilution gas-chromatography/mass-spectrometry. Patients included 35 patients with MS in the relapsing-remitting (RR) form of the disease, 20 in a stable clinical phase and 15 during a relapse, and 15 patients with MS in the secondary progressive (SP) form.

Results: Significantly reduced levels of all the tested eCBs were found in the CSF of patients with MS compared to control subjects, with lower values detected in the SP MS group. Higher levels of AEA and PEA, although below those of controls, were found in the CSF of RR MS patients during a relapse. Higher levels of AEA, 2-AG and OEA were found in patients with MRI gadolinium-enhancing (Gd+) lesions.

Discussion: The present findings suggest the presence of an impaired eCB system in MS. Increased CSF levels of AEA during relapses or in RR patients with Gd+ lesions suggest its potential role in limiting the ongoing inflammatory process with potential neuroprotective implications. These findings provide further support for the development of drugs targeting eCBs as a potential pharmacological strategy to reduce the symptoms and slow disease progression in MS.

The major components of the endocannabinoid (eCB) system are two endogenous lipids, α-*N*-arachidonoylethanolamine or anandamide (AEA) and 2-arachidonoylglycerol (2-AG), which are specific ligands of G-protein-coupled receptors named CB1 and CB2 receptors.[1–6] Further members of the same family, which are represented in the brain, are palmitoylethanolamide (PEA) and oleoylethanolamide (OEA).[7] In contrast to classical neurotransmitters, eCBs function as retrograde synaptic messengers travelling backward across synapses, activating presynaptic CB1 receptors and affecting memory, cognition and pain perception.[8]

The eCB system has been shown to modulate several aspects of the immune functions, including cytokine production, lymphocyte proliferation, and humoral and cell-mediated immune responses.[9 10]

Multiple sclerosis (MS) is known to be an immune-mediated disorder that is characterised by inflammatory demyelination of the central nervous system (CNS). Demyelinated areas in the CNS of patients with MS are characterised by inflammatory infiltrates that contain blood-derived myelin-specific T cells, B cells and non-specific, effector mononuclear cells.[11] Many cell types involved in CNS inflammation during MS express components of the cannabinoid signalling system that can be endogenously or pharmacologically controlled.

The potential role of the modulation of the eCB system in controlling either the symptoms or the evolution of experimental models of MS has been extensively demonstrated.[12–16]

In various CNS pathological conditions, an increase of eCB levels occurs in order to inhibit the molecular mechanisms that are involved in the production, release and diffusion of harmful mediators such as proinflammatory cytokines or excess glutamate. MS may disrupt itself in this eCB-mediated neuroprotective effect. In fact, contrary to the effects of the other brain diseases, such as cerebral ischaemia or traumatic brain injury, cell damage induced by experimental autoimmune encephalomyelitis (EAE) does not lead to enhancement of eCB levels.[17]

The present study aimed to investigate the levels of AEA, 2-AG, PEA and OEA in the cerebrospinal fluid (CSF) of patients with MS both in the relapsing remitting (RR) form and in the secondary progressive (SP) form of the disease in order to characterise the response of the human eCB system to the MS-related pathophysiological process.

PATIENTS AND METHODS

Patients

Patients with MS were recruited at the Centre for the Study of Demyelinating Diseases of the Neurologic Clinic of the University of Perugia.

The diagnosis of MS was established by clinical, laboratory and MRI parameters according to McDonald *et al* (2001) criteria.[18] The patients had no personal or family history of headache. At the moment of CSF withdrawal, patients were free of corticosteroids and MS-specific immunoactive therapies.

Disability was measured by Expanded Disability Status Scale (EDSS) according to Kurtzke (1983).[19]

According to Lublin *et al* (1996),[20] patients with MS were divided into two groups: patients in the relapsing-remitting form of the disease (RR MS, n = 35) and patients in the secondary progressive form of the disease (SP MS, n = 15). Of 35 RR MS patients, 20 were assessed during a stable phase of disease, whereas 15 were assessed during a relapse before intravenous administration of methylprednisolone. Of 20 RR MS patients assessed during a

stable clinical phase of the disease, 8 had gadolinium-enhancing (Gd+) MRI lesions.

The protocol was approved by the local ethics committee and patients gave their written informed consent.

Control CSF specimens were obtained from 20 age-matched subjects who were admitted to our neurological clinic for subjective symptoms and underwent lumbar puncture for diagnostic purposes. Neurological and general examinations were normal in all control subjects. All the control subjects underwent laboratory investigations (peripheral blood and CSF analysis) and brain MRI.

In all these subjects, blood tests, CSF analysis and MRI excluded CNS or systemic diseases. All control subjects were drug-free at the time of CSF sampling and they did not have a personal or family history of headache. None of the patients and controls developed post-lumbar puncture headache.

Details of patients and control subjects are reported in table 1.

Routine CSF determinations, both in patients and control subjects, included total cell count, total protein, measurement of the concentration of albumin and immunoglobulin G (IgG) in CSF and serum, determination of oligoclonal bands by isoelectric focusing, and extensive virological and microbiological testing. Samples were stored at −80°C until analysis.

Magnetic resonance imaging

Patients underwent brain MRI within ±3 days from undergoing lumbar puncture.

Brain MRI was performed using a 1.5 T system (GE) and consisted of an axial T1- and T2-weighted spin echo with 3 mm slice thickness and 1×1 mm in-plane resolution. Lesion load measures were analysed on a workstation (Sun, Montainview, CA) using a semiautomated seed-growing software developed inhouse based on local thresholding (Show-images). The total volume of hypointense lesions on T1 images, hyperintense lesions seen on the T2 images, brain volume and volume of Gd+ lesions, as well as their number, were calculated. Details of MRI results in the three patient groups are reported in table 1.

Routine CSF analysis

White cells (WCs) were counted and the upper limit of normal values was considered to be 4 per mm^3. IgG and albumin concentrations in serum and CSF were determined by rate-nephelometry on a Beckman Array*H* system using reagents and procedures provided by the manufacturer. The quantitative evaluation of intrathecal IgG synthesis was based on IgG index. An IgG index below 0.7 was considered normal. CSF/serum albumin ratio (Qalb, albumin quotient) expressed the condition of blood–CSF barrier function. A normal blood–CSF barrier was indicated as $Qalb < 8 \times 10^{-3}$.

Analysis for the presence of oligoclonal bands was performed by isoelectric focusing in agarose and immunoblotting.

CSF determination of endocannabinoids

Laboratory personnel was blinded for clinical and MRI data. Determination of eCB levels in the CSF was performed according to the method of Giuffrida and Piomelli (1998),[21] adapted to CSF by Giuffrida *et al* (2004).[22]

Standards for $[^2H_4]$ AEA, $[^2H_4]$ OEA and $[^2H_4]$ PEA were synthesised by the reaction of fatty acyl chlorides with unlabelled or $^2H_4$-labelled ethanolamine, provided by Cambridge Isotope Laboratories (Andover, MA). $[^2H_8]$-2-AG was custom-synthesised by Deva Biotech (Hatboro, PA). Fatty acyl chlorides in dichloromethane (10 mg/ml) were mixed with 1 equivalent of ethanolamine, and allowed to react for 15 min at 0–4°C. Reactions were stopped by adding water. After vigorous mixing, the upper aqueous phases were discarded to remove unreacted ethanolamine. The organic phases were washed twice with water, concentrated to dryness under a stream of N_2, and the reaction products were reconstituted in methanol. Identity and chemical purity (>98%) of the synthesised acylethanolamides and $[^2H_4]$ acylethanolamides were determined by gas chromatography/mass spectrometry (GC/MS).

These standards were added to 3 aliquots of CSF (1.2 nmol in 1.5 ml) to improve recovery and allow for quantification. After acetone precipitation of plasma proteins in CSF samples, the supernatants were collected and subjected to lipid extraction with methanol/chloroform. Enough of each solvent was added to reach a final ratio buffer/methanol/chloroform of 1:1:2 (v/v/v). The chloroform phases were recovered, evaporated to dryness under N_2, reconstituted in chloroform (150 ul), and analysed by high-performance liquid chromatography (HPLC). HPLC fractionations were performed on a Hewlett-Packard 1090 Liquid Chromatograph, equipped with a normal-phase Resolve Silica column (3.9 mm×15 cm, 5 um; Waters Associates, Milford, MA), eluted with a gradient of isopropyl alcohol (B) in *n*-hexane (A) (100% A initial; 90% A, 10% B for 1 min; 60% A, 40% B for 7 min, 50% A, 50% B for 12 min) at a flow rate of 1.7 ml/min. Under these conditions, all acylethanolamides were eluted from the HPLC column between 4.7 and 5.3 min. The acylethanolamide-containing fractions

Table 1 Clinical and MRI details of patients with MS and control subjects

	RR MS patients, stable clinical phase				
	Gd+	Gd–	RR MS patients, during relapse	SP MS patients	Control subjects
No. patients	8	12	15	15	20
Females/males (n)	6/2	9/3	11/4	13/2	15/5
Age (years)	35.8±6.1	33.0±7.6	36.4±6.2	46.8±8.2	43.4±10.2
Prior 2-year relapse rate	2.3±1.4	2.1±1.2	2.4±1.5	–	
EDSS score	2.8±1.7	2.2±1.5	4.1±2.7	5.5±1.6	
T_2-hyperintense lesion volume (ml)	22.2±14.2	20.4±13.4	24.2±15.3	29.2 ±12.7	
T_1-hypointense lesion volume (ml)	4.5±3.0	4.1±2.8	4.9±3.1	5.7±2.9	
Gd+ lesions (n) *	2.0±1.27	–	2.75±0.89	–	
Gd+ volume (ml)	0.9±0.7	–	1.2±0.6	–	
Brain volume (ml)	303.4±25.6	303.8±23.8	302.3±22.6	293.6±28.6	

Please note that none of the SP MS patients had Gd+ lesions at the time of MRI examination. RR MS patients in a stable clinical phase are subdivided according to the presence (Gd+) or absence (Gd–) of active MRI lesions.
Gd+, gadolinium-enhancing; Gd–, absence of gadolinium; RR MS, relapsing remitting multiple sclerosis; SP MS, secondary progressive multiple sclerosis.

were collected in glass reaction vessels, dried under N_2 and converted to trimethylethers by treatment with *Bis*(trimethylsilyl)trifluoroacetamide (BSTFA) for 30 min at room temperature. The trimethylsilylether (TMS) derivatives produced in this reaction were dried under N_2, reconstituted in *n*-hexane and injected in the splitless mode into a Hewlett-Packard 5890 GC equipped with an HP-5MS capillary column (30 m; internal diameter, 0.25 mm) and interfaced with a Hewlett-Packard 5972 MS.

Further details on isotope dilution and GC/MS methods are reported elsewhere (Giuffrida and Piomelli, 1998).[21] The concentrations of analytes in the CSF samples were expressed as pmol/mL and calculated in three separate determinations. Intra-assay variability was 2.5% for AEA and 2-AG, and 3% for OEA. Inter-assay variability was 3% for AEA, 4% for 2-AG and 5% for OEA.

Statistical analysis

Demographic, clinical and MRI characteristics of patients with MS were compared using two-tailed *t*-test for the continuous variables and Chi-square test or Fisher's exact test for the categorical variables. All variables were tested for normal distribution (Kolmogorov and Smirnov normality test), and most of them failed the test with $p<0.05$. Therefore, the significance of the within-patient and control group changes of CSF eCB levels was analysed using ordinary ANOVA with Least Significant Difference as *post hoc* analysis. As far as the eCB levels were concerned, there were no missing values because all the measurements were complete for all patients. The correlations between brain volume indices and number of Gd+ lesions and CSF eCB levels for each patient group were assessed using Spearman rank correlation coefficient.

RESULTS

Among the three patient groups, a significant difference emerged between age, EDSS and brain volume, total T2 hyperintense, T_1 Gd+ and T_1 hypointense lesion volumes. Eight patients in the RR MS group in the stable phase of disease and all RR MS patients assessed during a relapse, but none of the SP MS patients, had one or more Gd+ lesions in T1 scans.

CSF findings in patients with MS and controls are shown in table 2.

Values are expressed as median (min–max)

WC ≥ 5 were detected in 15.0%, 26.6% and 13.3% of RR MS patients in a stable clinical phase, RR MS patients during a relapse and SP MS patients, respectively. No cells were found in all control samples. A Qalb $\geq 8 \times 10^{-3}$) was found in 20% of RR MS patients in a stable clinical phase, 33.3% of RR MS patients during a relapse and 13.3% of SP MS patients but in none of control subjects. A total of 55% of RR MS patients in a stable clinical phase, 66% of RR MS patients during a relapse and 53% of SP MS patients had an IgG index >0.7. Oligoclonal bands were detected by isoelectric focusing in the CSF of all RR and SP MS patients.

Significantly lower values of all the tested eCBs were found in the CSF of patients with MS compared with control subjects, with a trend towards lower values detected in the SP MS group (table 3).

Although levels of AEA and PEA were significantly reduced in the RR MS group assessed during a relapse with respect to controls, these patients showed higher CSF values of both AEA and PEA compared with RR MS patients in a stable clinical phase ($p<0.0001$ and $p<0.008$, respectively) (figs 1 and 2). A trend towards higher levels of 2-AG and OEA was also found in RR MS CSF during a relapse but they did not differ, from a statistical point of view, from those of RR MS patients assessed during a stable phase.

No significant correlations were observed between values of all eCBs and routine CSF parameters, including IgG index in all patients and control groups, with the exception of a weak negative correlation between AEA and PEA and Qalb values ($R = 0.28$, $p<0.03$; and $R = 0.23$, $p<0.05$, respectively) in RR MS patients assessed during a relapse.

Patients with MS in a stable clinical phase with neuroradiological evidence of disease activity (presence of Gd+ lesions) displayed levels of AEA, 2-AG and OEA significantly greater than those found in RR MS patients without evidence of disease activity at MRI (table 3). A trend towards higher values of PEA was also observed but the difference between the means did not reach statistical significance.

In the RR MS group, there was a statistically significant correlation between number of Gd lesions and levels of AEA ($R = 0.84$, $p<0.003$ and 0.79, $p<0.0003$) but not those of PEA, 2-AG and OEA. No relationship was found between T1 and T2 lesional volume and all eCBs in the patient and control groups.

DISCUSSION

A growing body of evidence suggests that eCBs may potentially play a role in the modulation of neuroinflammation and that pharmacological compounds with cannabinoid-like activity may be effective either in ameliorating the clinical course of EAE or in alleviating spasticity, pain, tremor or other disabling signs of this autoimmune disease.[12–16]

AEA protects neurons from inflammatory damage by a CB (1/2) receptor-dependent mechanism, suggesting that its release by the injured CNS tissue might represent a key mechanism of neuroimmune communication during CNS injury, controlling and limiting the immune response after primary CNS damage.[23]

Accordingly, the activation of cannabinoid receptors seems to be effective in reducing the neurological impairment during

Table 2 Routine CSF findings in MS patients and controls

	RR MS patients, stable clinical phase	RR MS patients, during relapse	SP MS patients	Control subjects
No. patients	20	15	15	20
WBC (/mm^3)	1.8 (0–10)	2.1 (0–13)	1.9 (0–11)	0. 3 (0–4)
Protein (mg/dl)	28.3(18.1–43.7)	30.0 (19–41)	27 (16–37)	26 (17–31)
Q Alb ($\times 10^{-3}$)	5.8 (2.7–11.3)	6 (3.1–13.4)	5.4 (2.5–11.2)	3.8 (2.2–7.5)
Gammaglobulin, %	12 (3–18)	14 (5–21)	13 (5–19)	11 (3–16)
Ig Index	0.5 (0.2–1.4)	0.7 (0.3–1.7)	0.6 (0.4–1.5)	0.3 (0.2–0.5)
Oligoclonal bands (>2) %	100	100	100	0

Values are expressed as median (min–max). Ig, immunoglobulin; RR MS, relapsing remitting multiple sclerosis; SP MS, secondary progressive multiple sclerosis.

Table 3 Cerebrospinal fluid levels of AEA, PEA, 2-AG and OEA in patients with multiple sclerosis and control subjects

	AEA (pmol/mL)	PEA (pmol/mL)	2-AG (pmol/mL)	OEA (pmol/mL)
Controls	0.0085 ± 0.0019	5.28 ± 0.750	1.068 ± 0.123	1.173 ± 0.113
RR MS patients, stable phase of disease	0.0048 ± 0.0009*¶	4.82 ± 0.771†¶	0.824 ± 0.104‡	0.929 ± 0.146§¶
with Gd+ lesions	0.0054 ± 0.0007°**	5.09 ± 0.684	0.982 ± 0.112°	1.009 ± 0.136°
without Gd+ lesions	0.0034 ± 0006***	4.67 ± 0.742†	0.766 ± 0.096‡	0.87 ± 0.115§
RR MS patients, during a relapse	0.0068 ± 0009 ****	5.12 ± 0.8234	0.997 ± 0.127	1.078 ± 0.134
SP MS patients	0.0036 ± 0.0006*****	4.203 ± 0.73†	0.762 ± 0.0981‡	0.821 ± 0.146§

2-AG, 2-arachidonoylglycerol; AEA, anandamide; Gd+, gadolinium-enhancing; OEA, oleoylethanolamide; PEA, palmitoylethanolamide; RR MS, relapsing remitting multiple sclerosis; SP MS, secondary progressive multiple sclerosis.
Please note that data are expressed as mean ± SD.
Statistical significance is as follows:
MS patients vs controls
AEA: * = p<0.004; ** = p<0.008; *** = p<0.0001; **** = p<0.001; ***** = p<0.0002
PEA: † = p<0.03; † = p<0.02; ‡ = p<0.004
2-AG: ° p<0.04; °° p<0.02; °°°p<0.01
OEA: § p<0.05; § p<0.03; §p<0.01
RR MS patients in a stable phase of the disease vs SP MS patients
AEA ¶ = p<0.03; PEA ¶ = p<0.04; 2-AG = ns; OEA ¶ = p<0.05
RR MS patients during a relapse vs RR MS patients in a stable clinical phase
AEA: p<0.0001; PEA: p<0.008
RR MS patients with Gd+ lesions vs RR MS patients without Gd+ lesions
AEA ° = p<0001; 2-AG ° = p<0.004; OEA ° = p<0.03

EAE,[12 13] and mice deficient in the cannabinoid receptor CB1 tolerate inflammation and excitotoxic insults poorly, developing substantial neurodegeneration after immune attack in EAE.[24] Cannabinoids suppress CNS autoimmune inflammation occurring during EAE, acting both at neuronal CB1 receptors and at CB2 receptors expressed by encephalitogenic T cells.[25]

There is also evidence from clinical trials that cannabinoid receptor agonists can ameliorate some of the characteristic signs and symptoms of MS. Some of these studies have been performed with Δ^9-tetrahydrocannabinol (Δ^9-THC) or nabilone, a synthetic analogue of Δ^9-THC, whereas others have been conducted with cannabis extracts administered either in capsules or by a pump-action oromucosal spray. Nevertheless, the conclusions from these clinical studies have been unclear and confusing compared with experimental data, with both positive and negative results.[26–32]

In particular, in the largest clinical study into use of cannabinoids for treating symptoms related to MS (CAMS), 667 people were given capsules of THC, capsules of cannabis extract containing cannabidiol (CBD) and THC or placebo

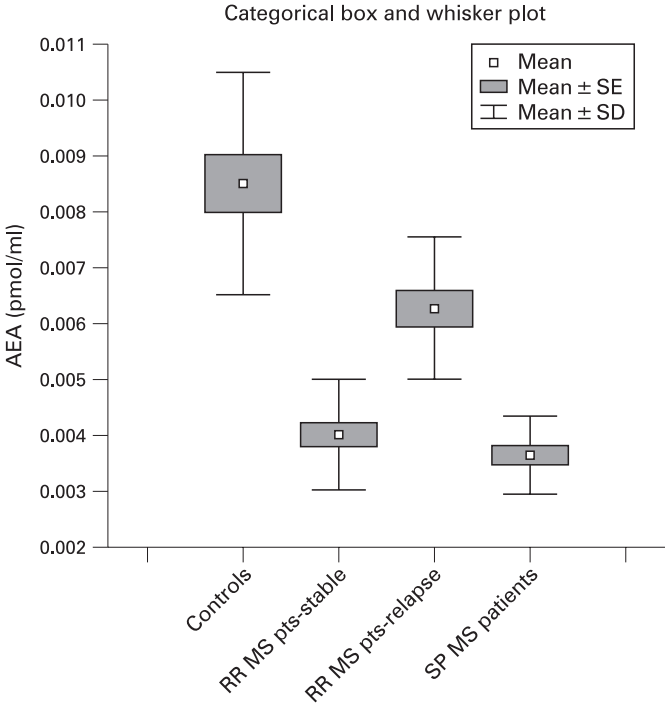


Figure 1 Plot of the levels of AEA, expressed as pmol/ml in the control subjects, in RR MS patients (both in a stable phase and during a relapse) and in SP MS patients. AEA, arachidonoyl-ethanolamine; RR MS, relapsing remitting multiple sclerosis; SE, standard error; SD, standard deviation; SP MS, secondary progressive multiple sclerosis.

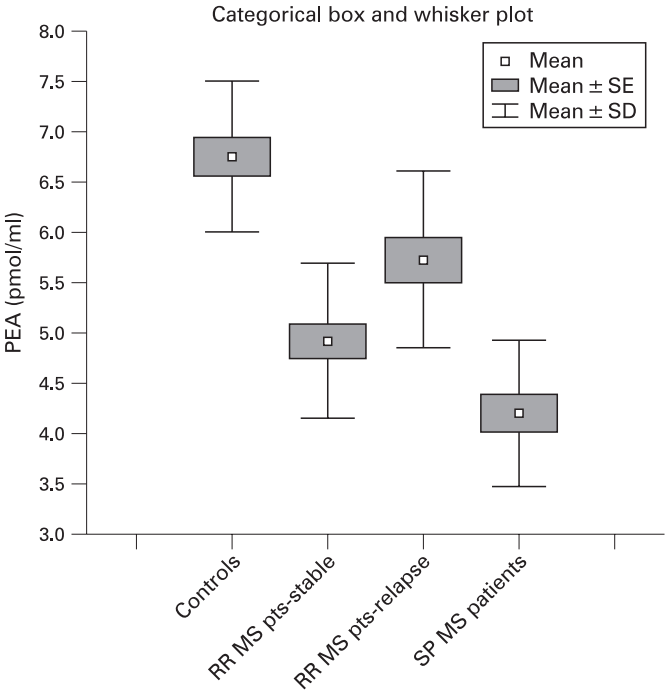


Figure 2 Plot of the levels of PEA, expressed as pmol/ml in the control subjects, in RR MS patients (both in a stable phase and during a relapse) and in SP MS patients. PEA, palmitoylethanolamide; RR MS, relapsing remitting multiple sclerosis; SE, standard error; SD, standard deviation; SP MS, secondary progressive multiple sclerosis.

capsules.[32] No overall improvement in spasticity, the primary outcome measure, was reported.[32] More encouraging is the 1-year follow-up of the same study in which overall objective improvements on both spasticity and general disability indices have been reported.[33] In some trials, including the CAMS trial, patients reported a subjective perception of improvement of specific symptoms, suggesting that the possible beneficial effect of exogenous cannabinoids might not depend on their putative effect as "immunomodulators" but rather on a positive "symptomatic" effect.

Our data demonstrate the presence of significantly lower values of all the tested eCBs in the CSF of patients with MS compared with control subjects, with a trend towards lower values detected in the SP MS group. These data suggest the presence of an impaired eCB system in MS. We believe that the observed differences are not to be attributed to methodological drawbacks because freezing/thawing conditions, storage and treatment of samples, which are critical for these assays, were the same for both patients and controls.

In several CNS pathologies, healthy cells surrounding lesioned areas attempt to protect themselves by producing eCBs. ECBs activate both immune CB2 receptors reducing the expression of proinflammatory cytokines and enzymes involved in the generation of free radicals and neuronal CB1 receptors, inhibiting neurotransmission, preventing excitotoxicity and increasing the expression of growth factors.

It has recently been described by Witting and colleagues that an exception to this pattern is represented by EAE. The authors showed that EAE does not lead to an increase in eCB levels and they attribute this lack of eCB increase to an IFN-γ-dependent disruption of the purinergic $P2X_7$-dependent eCB production by microglia.[17]

Based on these results, it has been hypothesised that the MS-related pathological process causes the disruption of the eCB-mediated neuroprotection.[34]

Specifically, reduced eCB levels in CSF can result in a lack of their neuroprotective mechanisms mediated via B1 receptors and anti-inflammatory effects exerted via B2 receptors aimed to confine demyelinating lesions and prevent axonal damage. Reduced eCB levels in the CSF of MS patients probably reflect the lack of this neuroprotective mechanism in MS brains. Interestingly, our findings indicate that the impairment of the eCB system is particularly evident in the SP form of MS. This latter evidence could reflect either the absence of sustained CNS inflammmation in this phase of the disease or a tendency towards a greater impairment of the eCB system in the progressive, neurodegenerative phases of MS and can contribute to sustained disability in patients affected by this form.

Unfortunately, it is difficult to provide a mechanistic explanation for the presented evidence of an eCB system "failure" in MS. Moreover, the majority of the studies on MS and eCB metabolism have been carried out by utilising experimental models of the disease and human studies are lacking. As described above, Witting and colleagues have recently demonstrated that the cell damage induced by EAE does not lead to increase in eCBs and that this lack of eCB increase is probably due to IFN-γ.[17] Increased levels of several cytokines, including IFN-γ, have been demonstrated in the CSF and brain tissue from patients with MS,[35 36] suggesting a potential link between cytokine overexpression and eCB system failure in MS. Further experimental and clinical investigations remain necessary to investigate the validity of this hypothesis.

Our data also suggest that an active inflammatory process within the human CNS directly influences eCB CSF levels in patients with MS. In fact, although eCB levels in the CSF of RR MS patients remained lower than those measured in control subjects, we found higher CSF levels of AEA and PEA in the CSF of RR MS patients assessed during a relapse compared with RR MS patients in a stable clinical phase of the disease.

The same conclusion is supported by the correlation between the number of MRI Gd+ lesions and the concentration of AEA, 2-AG and OEA. Conversely, no relationship was found between the T1 and T2 lesional volume and all eCBs tested between patients with MS and control subjects.

These latter results could suggest that, even in the presence of an impaired eCB system in MS, cells surrounding new demyelinating lesions attempt to protect themselves by producing eCBs.

Accordingly, the results of a previous study investigating whether the endocannabinoid system is activated during inflammation within the brain parenchyma have demonstrated that, in inflammatory lesions of patients with active MS, a 3.7-fold higher concentration of AEA is present in comparison to healthy controls and that also, in lesions of patients with silent MS, a 1.9-fold higher concentration of the same eCB occurs.[23]

Conversely, our results concur, at least in part, with those of a recent study carried out by Centonze *et al* who also demonstrated increased levels of AEA in patients with MS assessed during a clinical relapse.[37] Indeed, in this paper, the reported values in patients with MS appear to be significantly greater than those of control subjects.[37] Furthermore, the values obtained both in patients and control subjects are much higher than those reported for other patient and control groups in previous papers.[22 38] Methodological differences, including extraction procedures, could account for this discrepancy. One possibility might also be that samples from patients and controls have been stored or treated differently (freezing conditions, freezing/thawing cycles, etc). Storage and sample treatment can, in fact, strongly affect results. Nevertheless, as far as it concerns the order of magnitude of our results, it is worth noting that they are comparable with those obtained with the same methodology in a different laboratory by Giuffrida and colleagues.[22]

The confusing results that have been obtained in clinical trials are probably explained by several factors, including (i) cannabinoid pharmacokinetics (delivery route of choice and extensive first pass liver metabolism), (ii) inadequate outcome measurements, and (iii) difficulty of performing double-blinded studies.[39]

Our results lead to the evidence that the human eCB system is impaired in patients suffering from MS and might further support the potential usefulness of compounds modulating the eCB system (agonists/antagonists of cannabinoid CB1 and CB2 receptors, or inhibitors of eCBs metabolism) in the control of both symptoms and disease progression in MS.

Acknowledgements: The authors express their gratitude to John A. Toomey for editing the English text and Marisa M. Morson for technical assistance.

Competing interests: None declared.

REFERENCES

1. **Walter L,** Stella N. Cannabinoids and neuroinflammation. *Br J Pharmacol* 2004;**141**:775–85.
2. **Devane WA,** Hanus L, Breuer A, *et al*. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 1992;**258**:1946–9.
3. **Mechoulam R,** Ben-Shabat S, Hanus L, *et al*. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol* 1995;**50**:83–90.

4. **Stella N,** Schweitzer P, Piomelli D. A second endogenous cannabinoid that modulates long-term potentiation. *Nature* 1997;**388**:773–8.
5. **Matsuda LA,** Lolait SJ, Brownstein MJ, *et al*. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* 1990;**346**:561–4.
6. **Munro S,** Thomas KL, Abu–Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 1993;**365**:61–5.
7. **Maccarrone M,** Attinà M, Cartoni A, *et al*. Gas chromatography-mass spectrometry analysis of endogenous cannabinoids in healthy and tumoral human brain and human cells in culture. *J Neurochem* 2001;**76**:594–601.
8. **Wilson RI,** Nicoll RA. Endocannabinoid signaling in the brain. *Science* 2002;**296**:678–82.
9. **Klein TW,** Newton C, Larsen K, *et al*. The cannabinoid system and immune modulation. *J Leukoc Biol* 2003;**74**:486–96.
10. **Ullrich O,** Merker K, Timm J, *et al*. Immune control by endocannabinoids—new mechanisms of neuroprotection? *J Neuroimmunol* 2007;**184**:127–35.
11. **Martino G,** Adorini L, Rieckmann P, *et al*. Inflammation in multiple sclerosis: the good, the bad, and the complex. *Lancet Neurol* 2002;**1**:499–509.
12. **Cabranes A,** Venderova K, de Lago E, *et al*. Decreased endocannabinoid levels in the brain and beneficial effects of agents activating cannabinoid and/or vanilloid receptors in a rat model of multiple sclerosis. *Neurobiol Dis* 2005;**20**:207–17.
13. **Malfitano AM,** Matarese G, Pisanti S, *et al*. Arvanil inhibits T lymphocyte activation and ameliorates autoimmune encephalomyelitis. *J Neuroimmunol* 2006;**171**:110–9.
14. **Ortega-Gutierrez S,** Molina-Holgado E, Arevalo-Martin A, *et al*. Activation of the endocannabinoid system as therapeutic approach in a murine model of multiple sclerosis. *FASEB J* 2005;**19**:1338–40.
15. **Baker D,** Pryce G, Croxford JL, *et al*. Cannabinoids control spasticity and tremor in a multiple sclerosis model. *Nature* 2000;**404**:84–7.
16. **Baker D,** Pryce G, Croxford JL, *et al*. Endocannabinoids control spasticity in a multiple sclerosis model. *FASEB J* 2001;**15**:300–2.
17. **Witting A,** Chen L, Cudaback E, *et al*. Experimental autoimmune encephalomyelitis disrupts endocannabinoid-mediated neuroprotection. *Proc Natl Acad Sci USA* 2006;**103**:6362–7.
18. **McDonald WI,** Compston A, Edan G, *et al*. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann Neurol* 2001;**50**:121–7.
19. **Kurtzke JF.** Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology* 1983;**33**:1444–52.
20. **Lublin FD,** Reingold SC. Defining the clinical course of multiple sclerosis: results of an international survey. National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis. *Neurology* 1996;**46**:907–11.
21. **Giuffrida A,** Piomelli D. Isotope dilution GC/MS determination of anandamide and other fatty acylethanolamides in rat blood plasma. *FEBS Lett* 1998;**422**:373–6.
22. **Giuffrida A,** Leweke FM, Gerth CW, *et al*. Cerebrospinal anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. *Neuropsychopharmacology* 2004;**29**:2108–14.
23. **Eljaschewitsch E,** Witting A, Mawrin C, *et al*. The endocannabinoid anandamide protects neurons during CNS inflammation by induction of MKP-1 in microglial cells. *Neuron* 2006;**49**:67–79.
24. **Pryce G,** Ahmed Z, Hankey DJ, *et al*. Cannabinoids inhibit neurodegeneration in models of multiple sclerosis. *Brain* 2003;**126**:2191–202.
25. **Maresz K,** Pryce G, Ponomarev ED, *et al*. Direct suppression of CNS autoimmune inflammation via the cannabinoid receptor CB1 on neurons and CB2 on autoreactive T cells. *Nat Med* 2007;**13**:492–7.
26. **Rog DJ,** Nurmikko TJ, Friede T, *et al*. Randomized, controlled trial of cannabis-based medicine in central pain in multiple sclerosis. *Neurology* 2005;**65**:812–9.
27. **Brady CM,** DasGupta R, Dalton C, *et al*. An open-label pilot study of cannabis-based extracts for bladder dysfunction in advanced multiple sclerosis. *Mult Scler* 2004;**10**:425–33.
28. **Wissel J,** Haydn T, Muller J, *et al*. Low dose treatment with the synthetic cannabinoid Nabilone significantly reduces spasticity-related pain: a double-blind placebo-controlled cross-over trial. *J Neurol* 2006;**253**:1337–41.
29. **Wade DT,** Makela PM, House H, *et al*. Long-term use of a cannabis-based medicine in the treatment of spasticity and other symptoms in multiple sclerosis. *Mult Scler* 2006;**12**:639–45.
30. **Collin C,** Davies P, Mutiboko IK, *et al*. Sativex Spasticity in MS Study Group. Randomized controlled trial of cannabis-based medicine in spasticity caused by multiple sclerosis. *Eur J Neurol* 2007;**14**:290–6.
31. **Killestein J,** Hoogervorst EL, Reif M, *et al*. Safety, tolerability, and efficacy of orally administered cannabinoids in MS. *Neurology* 2002;**58**:1404–7.
32. **Zajicek J,** Fox P, Sanders H, *et al*. UK MS Research Group. Cannabinoids for treatment of spasticity and other symptoms related to multiple sclerosis (CAMS study): multicentre randomised placebo-controlled trial. *Lancet* 2003;**362**:1517–26.
33. **Zajicek JP,** Sanders HP, Wright DE, *et al*. Cannabinoids in multiple sclerosis (CAMS) study: safety and efficacy data for 12 months follow up. *J Neurol Neurosurg Psychiatry* 2005;**76**:1664–9.
34. **Shohami E,** Mechoulam R. Multiple sclerosis may disrupt endocannabinoid brain protection mechanism. *Proc Natl Acad Sci USA* 2006;**103**:6087–8.
35. **Cannella B,** Raine CS. The adhesion molecule and cytokine profile of multiple sclerosis lesions. *Ann Neurol* 1995;**37**:424–35.
36. **Merrill JE,** Benveniste EN. Cytokines in inflammatory brain lesions: helpful and harmful. *Trends Neurosci* 1996;**19**:331–8.
37. **Centonze D,** Bari M, Rossi S, *et al*. The endocannabinoid system is dysregulated in multiple sclerosis and in experimental autoimmune encephalomyelitis. *Brain* 2007;**130**:2543–53.
38. **Sarchielli P,** Pini LA, Coppola F, *et al*. Endocannabinoids in chronic migraine: CSF findings suggest a system failure. *Neuropsychopharmacology* 2007;**32**:1384–90.
39. **Pryce G,** Baker D. Emerging properties of cannabinoid medicines in management of multiple sclerosis. *Trends Neurosci* 2005;**28**:272–6.

Journal of Autism and Developmental Disorders
https://doi.org/10.1007/s10803-024-06244-2

ORIGINAL ARTICLE

Accelerated Theta Burst Transcranial Magnetic Stimulation for Refractory Depression in Autism Spectrum Disorder

Elizabeth Blank[1] · Donald L. Gilbert[2] · Steve W. Wu[2] · Travis Larsh[2] · Rana Elmaghraby[1] · Rui Liu[1] · Elizabeth Smith[4] · Grace Westerkamp[1] · Yanchen Liu[1] · Paul S. Horn[2] · Ethan Greenstein[1] · John A. Sweeney[3] · Craig A. Erickson[1,3] · Ernest V. Pedapati[1,2,3]

Accepted: 9 January 2024

Abstract

Purpose Major depressive disorder (MDD) disproportionately affects those living with autism spectrum disorder (ASD) and is associated with significant impairment and treatment recidivism.

Methods We studied the use of accelerated theta burst stimulation (ATBS) for the treatment of refractory MDD in ASD (3 treatments daily x 10 days). This prospective open-label 12-week trial included 10 subjects with a mean age of 21.5 years, randomized to receive unilateral or bilateral stimulation of the dorsolateral prefrontal cortex.

Results One participant dropped out of the study due to intolerability. In both treatment arms, depressive symptoms, scored on the Hamilton Depression Rating Scale scores, diminished substantially. At 12 weeks post-treatment, full remission was sustained in 5 subjects and partial remission in 3 subjects. Treatment with ATBS, regardless of the site of stimulation, was associated with a significant, substantial, and sustained improvement in depressive symptomatology via the primary outcome measure, the Hamilton Depression Rating Scale. Additional secondary measures, including self-report depression scales, fluid cognition, and sleep quality, also showed significant improvement. No serious adverse events occurred during the study. Mild transient headaches were infrequently reported, which are expected side effects of ATBS.

Conclusion Overall, ATBS treatment was highly effective and well-tolerated in individuals with ASD and co-occurring MDD. The findings support the need for a larger, sham-controlled randomized controlled trial to further evaluate efficacy of ATBS in this population.

Keywords Autism · Depression · Fluid cognition · Transcranial Magnetic Stimulation · Brain Stimulation · Neuromodulation

Autistic individuals are disproportionately affected by major depressive disorder (MDD), which contributes to functional disability, including educational and vocational impairment, social withdrawal, and suicide across the lifespan (Cassidy et al., 2014; Hollocks et al., 2019; Hudson et al., 2019; Matson & Nebel-Schwalm, 2007). Suicidal ideation in ASD is also elevated, with over 72% of adults with ASD scoring above the recommended cut-off for suicide risk on the Suicide Behaviors Questionnaire-Revised (SBQ-R) which was significantly correlated with reported non-suicidal self-injury, camouflaging, and number of unmet support needs (Cassidy et al., 2018). Though MDD is prevalent in ASD regardless of cognitive ability, autistic individuals without intellectual disability disorder (IDD) are more likely to receive the diagnosis, likely due to clearer communication of

✉ Ernest V. Pedapati
ernest.pedapati@cchmc.org

1 Division of Child and Adolescent Psychiatry, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States

2 Division of Neurology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States

3 Department of Psychiatry, University of Cincinnati College of Medicine, Cincinnati, OH, United States

4 Division of Behavioral Medicine and Child Psychology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States

Published online: 15 May 2024

internal states and more canonical presentations (Pezzimenti et al., 2019).

A major challenge in treating MDD among those with ASD is the high rates of treatment recidivism and relapse (Hirvikoski et al., 2016). It is estimated that treatment-resistant MDD in ASD individuals likely exceeds that of the general population (> 30%) based on prevalence rates and polypharmacy (Feroe et al., 2021; Rosenberg et al., 2010; Zheng et al., 2021). This is consistent with observations that standard-of-care medications for mood disorders can be unpredictable in ASD and may at times even be counterproductive in ameliorating symptoms (McCracken et al., 2021; Williams et al., 2013). Despite the urgent need for support for this population, little research exists targeting novel interventions for depression and suicidality in ASD.

Repetitive transcranial magnetic stimulation (rTMS) is an evidence-based intervention for MDD in typically developing populations (Razza et al., 2018). rTMS involves brief, high-intensity electrical currents passing through a coil placed near the scalp. This induces a rapidly changing magnetic field that induces an electrical current in local brain parenchyma, leading to both local inhibitory or excitatory neuronal changes as well as changes in connected brain regions (Terao & Ugawa, 2002). Though meta-analysis of rTMS randomized sham-controlled trials demonstrate efficacy in treatment of MDD severity and remission rates, several factors limit the feasibility of the conventional form of treatment in autistic individuals (Sehatzadeh et al., 2019). First, although patients remain awake during the procedure and require no aftercare, traditional rTMS stimulation is delivered above resting motor threshold (RMT) and can lead to headaches, scalp pain, muscle twitching, and eye discomfort. In ASD cohorts, where sensory hypersensitivity is more common, the prevalence of AEs associated with TMS is estimated to be at 25% (Huashuang et al., 2022). Moreover, a conventional rTMS treatment course typically involves daily 45-minute treatment sessions spanning four to six weeks, requiring a significant investment of time and logistical coordination.

Recent advances in rTMS protocols, namely theta burst stimulation (TBS) may help improve tolerability in which sensory hypersensitivity or duration of treatment may be a limiting factor (Elmaghraby et al., 2022; Hong et al., 2015). Since TBS protocols use a higher frequency pulse (> 30 Hz), they only involve several minutes of stimulation and can be performed at or below RMT, minimizing overall sensation (Huang et al., 2005b). Additionally, multiple treatments of TBS or accelerated TBS (ATBS) can be performed in a single day which can dramatically shorten the overall duration of treatment to one to ten days (Duprat et al., 2016; Fitzgerald et al., 2020; Weissman et al., 2018a). So far, TBS protocols (with or without acceleration) are comparable in safety and efficacy to conventional rTMS, but they offer advantages in tolerability, treatment capacity, and cost-effectiveness (Blumberger et al., 2018; Cai et al., 2023). The US Food and Drug Administration (FDA, 2011) cleared the use of TBS in 2018 and ATBS in 2022 as an alternative to conventional rTMS for MDD (Neuteboom et al., 2023).

No RCTs evaluating the efficacy of any form of TMS treatment of MDD in individuals with ASD are available. However, a recent open-label trial of conventional rTMS for MDD in adults with ASD ($n = 10$) found that 70% of participants responded to treatment and 40% reached remission (Gwynette et al., 2020). Two participants withdrew due to intolerability. Participants with sensitivity to stimulation were started on a lower stimulation intensity and gradually titrated to the full dose or used a < 1 mm foam barrier at the stimulation site. While these results are promising, we hypothesized that the abbreviated course and reduced stimulation intensity of ATBS may be better suited for individuals with ASD.

We conducted a prospective open-label accelerated TBS on treatment-refractory MDD in transition-aged youth with ASD (ages 12–26 years). The Hamilton Depression Rating Scale (HRDS-17) was used as the primary outcome, and we assessed changes from baseline at 1-, 4-, and 12-weeks post-treatment. To investigate stimulation parameters, we randomized participants either unilateral (UL) left dorsolateral prefrontal cortex (DLPFC) stimulation or bilateral stimulation (BL) DLPFC based on recent literature suggesting potential advantages of bilateral stimulation (Blumberger et al., 2012; Chistyakov et al., 2015). Our hypothesis was that bilateral stimulation would enhance the treatment efficacy but may also negatively affect tolerability. Additionally, we administered NIH Cognitive Toolbox measures at each timepoint hypothesizing that changes in scores may reflect prefrontal cortex engagement and predict MDD treatment response (Crane et al., 2017).

Methods

Ethics Statement

This study was approved by the institutional review board at Cincinnati Children's Hospital Medical Center (CCHMC) and registered with ClinicalTrials.gov (NCT01609374). Recruitment took place between November 2021 and November 2022 through clinician referrals, community flyers, emails, and clinics at

a tertiary academic pediatric hospital. All participants provided written informed consent or assent for all study procedures.

Diagnosis of MDD and co-occurring conditions was determined by the Mini-International Neuropsychiatric Interview for Children and Adolescents (MINI-KID) for participants under 18 years of age (Sheehan et al., 1998) and the Structured Clinical Interview for DSM-5 Disorders (SCID-5) for those 18 and older (Spitzer et al., 1992). Treatment-resistant MDD was determined using the Antidepressant Treatment History Form (Sackeim et al., 2019). Diagnostic assessments were conducted by qualified experienced raters, including licensed clinical psychologists (ADOS-2, MINI-KID) or board-certified child and adolescent psychiatrists (MINI-KID, ATHF).

Participants

Inclusion criteria for participants included: (1) age 12–26 years, (2) diagnosed with ASD (and confirmed by Autism Diagnostic Observation Schedule, 2nd Edition (ADOS-2) (Lord et al., 2012), (3) currently meeting Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria for a unipolar major depressive disorder or persistent depressive disorder, 3) exhibiting treatment resistance to at least one evidence-based antidepressant medication, (4) Global Assessment of Function (GAF) score ≤ 60, (5) 17-item Hamilton Depression Rating Scale (HDRS-17) or Beck Depression Inventory II (BDI-II) score in the clinically depressed range (≥ 20) that was sustained over the two-week lead-in period.

Exclusion criteria included any of the following: (1) significant psychiatric or neurological disease unrelated to ASD or MDD within the last six months, (2) use of investigational drugs, (3) any contraindications to TMS (Rossi et al., 2021) (4) Intelligence Quotient < 80 per the Wechsler Abbreviated Scale of Intelligence, 2nd Edition (Wechsler, 1999), (5) active pregnancy (confirmed by urine test), (6) active suicidality, (7) history of epilepsy or use of antiepileptic drugs, (8) prior rTMS treatment, (9) changes in psychiatric medicines two weeks before TMS treatment, 11) substance use or substance dependence disorder (confirmed by urine toxicology).

Study Design

This open-label prospective clinical trial involved 30 TBS sessions over a period of ten days (Fig. 1). Following the screening visit, eligible participants were randomly assigned to receive either standard, unilateral intermittent TBS (iTBS) to the left dorsolateral prefrontal cortex (DLPFC) (FDA, 2011) or bilateral stimulation with iTBS to the left DLPFC and continuous TBS (cTBS) to the right DLPFC. Following randomization, participants had to maintain eligibility for a two-week lead-in period prior to the first treatment session. Additional assessments were conducted at days 5 and 10 of the intervention and 1-, 4-, and 12-weeks post-treatment.

Intervention

A Magstim Horizon Performance stimulator (Magstim, Whitland, UK) with a 70mmm figure-eight EZ cool coil was used for all treatment sessions. Coil placement was determined using the BEAM-F3 method (Beam et al., 2009). A separate figure-eight coil was used to establish RMT. RMT was defined as the lowest TMS intensity needed to produce a contralateral thumb twitch in at least three of six trials (Horvath et al., 2010). For participants in the BL group, RMT was determined for each hemisphere. All iTBS and cTBS sessions consisted of triplet 50 Hz pulses repeated in 5 Hz bursts for a total of 600 pulses per session at 90% of RMT (Huang et al., 2005a). During iTBS 20 trains were applied in 2-second bursts with 8-second pauses, while cTBS involved a continuous pulse train for a total duration of 53 s. TBS was delivered in three sessions daily over ten days, with 50 min intervals between sessions (Cai et al., 2023). To account for participants with sensory hypersensitivity we titrated up to target (90%) stimulation intensity over the first two treatment days, starting at 50% RMT and increasing by 10–20% each session, depending on each subject's tolerance.

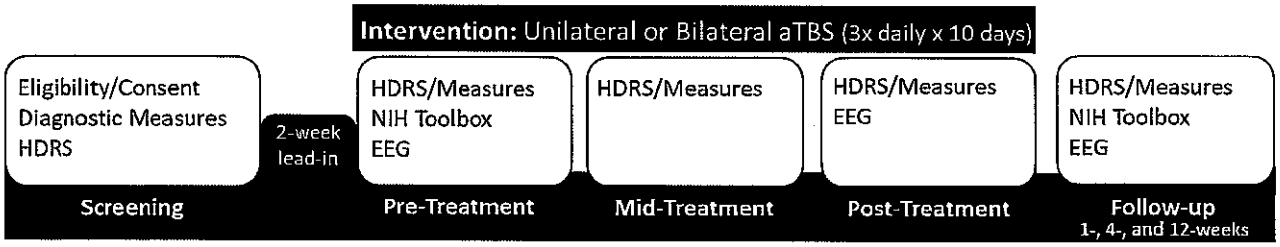


Fig. 1 Accelerated theta burst stimulation (aTBS) randomized control study design. Participants were assessed at seven timepoints up to 12-weeks following treatment

Outcome Measures

The primary outcome measure was change in scores on the HDRS-17 (Hamilton, 1986). Secondary depression measures (for validation) included BDI-II (Osman et al., 2004) and Quick Inventory of Depressive Symptomatology (QIDS) (Rush et al., 2003). Suicidal behavior was assessed by physician-administered Columbia-Suicide Severity Rating Scale (C-SSRS) (Posner et al., 2008) at screening and self-report Suicide Behavior Questionnaire (SBQ) (Osman et al., 2001) at screening, intervention days 5 and 10, and all follow-up visits. Changes in anxiety symptoms were assessed using the Generalized Anxiety Disorder-7 item (GAD-7) (Spitzer et al., 2006). Changes in sleep were measured using the Pittsburgh Sleep Quality Inventory (PSQI) (Buysse et al., 1989), and social functioning was measured using the Social Responsiveness Scale (SRS) (Bruni, 2014). Neurocognitive function was assessed using the NIH Cognitive Toolbox (processing speed, working memory, language, and executive function i.e., inhibitory control, set shifting) (Weintraub et al., 2013) and neuromuscular function using the Grip Strength Test from the NIH Toolbox Motor Battery (Reuben et al., 2013).

Safety Outcomes

To assess adverse events (AEs) related to TMS, a 16-point systematic review of systems was conducted at the beginning and end of each treatment day.

Statistical Analysis

Given the exploratory nature of this pilot study, we present data at the individual level and used streamlined statistical modeling to discern overarching trends and effects. No outliers were detected. For each outcome measure, we provide a three-panel figure depicting:

1. Main Effect Plot: This plot displays the mean values of the measure across different time points.
2. Group Interaction Plot: This plot illustrates the interaction effect between time and stimulation site. The mean values of the measure are plotted, stratified by treatment.
3. Subject-level Plot: This plot provides insights into individual variability by plotting each subject's mean value for the measure across time.

We conducted a linear mixed-effects analysis using the *lmerTest* package in R 4.3 to identify any main effects of time or treatment arm, as well as their potential interaction effect on each outcome measure. To account for the repeated measures design, we incorporated random intercepts for subjects.

Mathematically, the model can be represented as: $Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + \epsilon_{ijk}$

Where:

- Y_{ijk} is the dependent variable (e.g., a specific measure for a given subject at a particular time in a certain group).
- μ is the overall mean.
- α_i represents the effect of the ith level of factor A (Time).
- β_j denotes the effect of the jth level of factor B (Group).
- $(\alpha\beta)_{ij}$ stands for the interaction effect between the ith level of factor A and the jth level of factor B.
- γ_k is the random effect of the kth subject (or individual).
- ϵ_{ijk} is the random error associated with the kth observation under the ith level of factor A and jth level of factor B.

Following model estimation, we extracted the ANOVA table to ascertain if a main or interaction effect was present. Depending on the effect, post-hoc tests were carried out to assess pairwise differences between baseline and post-treatment time points, while also using false discovery rate (FDR) to adjust for multiple comparisons. An adjusted p value less than or equal to 0.05 was considered statistically significant.

Results

Ten participants (2 females; min age = 17, max age = 26.2, median age = 22) with ASD and treatment refractory MDD (mean failed antidepressant trials = 3.44 ± 1.7) were randomized to either UL or BL ATBS treatment. Demographics and baseline clinical measures (including MDD severity) were similar between treatment arms (Table 1). One subject disclosed additional history during the trial that supports a diagnosis of borderline personality disorder. While the subject was included in all the main analysis models, they were excluded from the exploratory correlation analysis. Consolidated Standards of Reporting Trials (CONSORT) flow diagram showing the selection of participants from initial screening to final analysis (Fig. 2).

Table 1 Baseline demographic and clinical characteristics of the study participants

Measure	Combined, n = 9	Unilateral, n = 5	Bilateral, n = 4	*p*-value
Age	21.5 ± 3.2	21.7 ± 3.9	21.2 ± 2.6	> .99
Sex				> .99
Male	7 (78%)	4 (80%)	3 (75%)	
Female	2 (22%)	1 (20%)	1 (25%)	
Race				.29
White	6 (67%)	2 (40%)	4 (100%)	
Black or African American	1 (11%)	1 (20%)	(0%)	
Asian	2 (22%)	2 (40%)	(0%)	
American Indian, Alaskan Native, Native Hawaiian, or other Pacific Islander	0 (0%)	(0%)	(0%)	
Ethnicity				.44
Hispanic or Latino	1 (11%)	(0%)	1 (25%)	
Not Hispanic or Latino	8 (89%)	5 (100%)	3 (75%)	
Full-Scale IQ	113.4 ± 12.5	113.4 ± 14.7	113.5 ± 11.4	> .99
Primary diagnosis				> .99
Dysthymia	2 (22%)	1 (20%)	1 (25%)	
MDD	7 (78%)	4 (80%)	3 (75%)	
# of failed antidepressant trials	3.4 ± 1.7	3.8 ± 2.2	3.0 ± 1.2	.46
SRS-II total	103.6 ± 25.8	103.8 ± 33.7	103.2 ± 16.3	> .99
Vineland-3 Composite Score	76.1 ± 8.1	76.8 ± 10.0	75.0 ± 5.0	> .99
Communication domain	80.3 ± 4.5	81.2 ± 3.7	78.7 ± 6.0	> .99
Receptive domain	12.0 ± 1.6	12.6 ± 1.1	11.0 ± 2.0	> .99
Daily Living Skills domain	83.8 ± 10.7	85.2 ± 12.0	81.3 ± 9.9	> .99
Socialization domain	69.1 ± 19.4	68.6 ± 25.4	70.0 ± 5.0	> .99
BRIEF, Global Executive Composite	143.3 ± 17.0	140.0 ± 20.8	147.5 ± 12.3	> .99
GAF	51.1 ± 6.1	52.6 ± 4.2	49.2 ± 8.3	.71
HDRS-17 total score	20.2 ± 3.4	19.6 ± 2.5	21.0 ± 4.5	.44
BDI-II total score	25.3 ± 14.3	23.4 ± 18.4	27.8 ± 8.9	> .99
QIDS total score	19.1 ± 4.6	19.2 ± 5.0	19.0 ± 4.8	.81
CGI-Severity	4.9 ± 0.3	4.8 ± .4	5.0 ± .0	> .99
EDI-Reactivity raw total	8.8 ± 5.6	8.8 ± 7.3	8.8 ± 3.7	.44
EDI-Dysphoria raw total	13.4 ± 7.8	14.0 ± 6.6	12.8 ± 10.2	.68
RRS total score	24.9 ± 5.4	26.0 ± 6.3	23.5 ± 4.5	> .99
GAD total score	12.1 ± 4.7	13.0 ± 4.7	11.0 ± 5.1	.68
PSQI global score	11.3 ± 4.3	11.6 ± 5.0	11.0 ± 3.9	.44
SBQ-R total score	9.9 ± 4.5	10.0 ± 4.0	9.8 ± 5.7	.44
Fluid Cognition Composite raw score	105.8 ± 10.4	103.0 ± 12.4	109.2 ± 7.3	> .99
Grip Strength (lbs. force)	66.6 ± 12.7	72.0 ± 6.8	59.7 ± 15.9	> .99

Data are presented as either mean ± SD or n (%) for Combined ($n = 9$), Unilateral ($n = 5$), and Bilateral ($n = 4$) groups. Measures include age, sex, race, ethnicity, IQ, primary diagnosis, and various clinical measures

Clinical and Behavioral Outcomes

Summary results of the effects from each LME model are displayed in Table 2. For models that showed a significant main or interaction effect, post-hoc testing results are presented in Table 3.

Depression: Primary and Secondary Outcomes

The main effect of time was significant for the primary outcome of interest, HRDS-17, $F(3, 21) = 28.49$, $p < 0.001$, EF = 1.976, indicating a large Cohen's treatment effect size (EF). For HRDS-17 Total Score, significant differences from baseline were observed at all three time points: Week 1, $t(21) = 7.09$, $p < 0.001$, estimated change = -11.43; Week 4, $t(21) = 7.80$, $p < 0.001$, estimated change = -12.58; Week 12, $t(21) = 7.68$, $p < 0.001$, estimated change = -12.38. By week 4, 7 out of 9 subjects showed a significant treatment response (≥ 50% reduction from baseline HDRS-17 score). Treatment effects were largely sustained through the 12-week follow-up period (Fig. 3); at this timepoint, five subjects

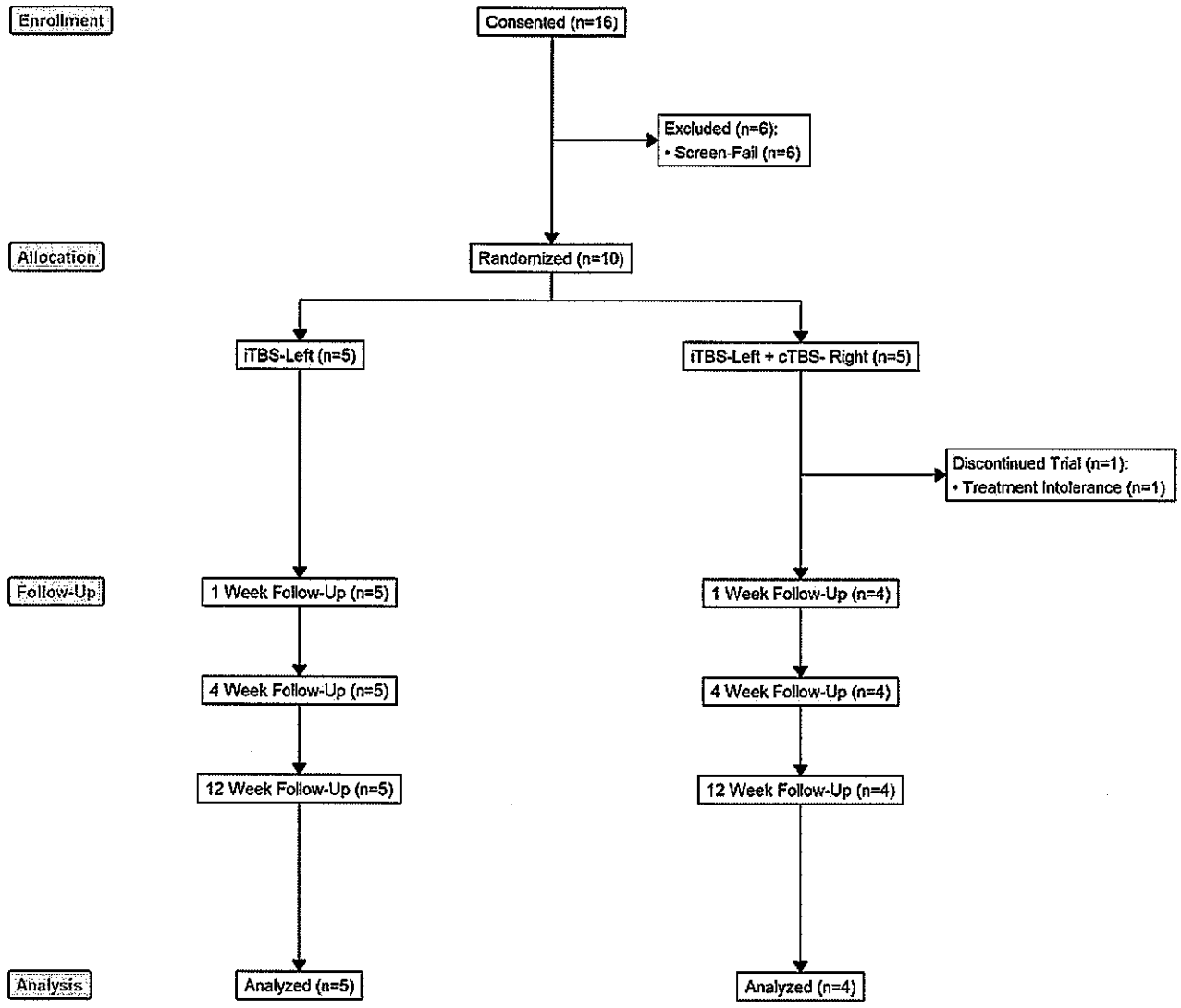


Fig. 2 CONSORT Flow Diagram

met the criteria for full remission of depression and three achieved partial remission (American Psychiatric Association & Association, 2013).

Only the main effect of time was significant for BDI-II Total, $F(3, 20) = 9.36$, $p < 0.001$, $EF = 1.181$, also indicating a large treatment effect. Similarly, for QIDS Total, only the main effect of time was significant, $F(3, 21) = 7.72$, $p = 0.001$, $EF = 1.037$. Post-hoc tests (Table 3) demonstrated that BDI and QIDS saw significant improvements from baseline at Weeks 1, 4, and 12 ($p < 0.01$ for each).

Anxiety

For GAD-7 Total, the main effect of time was significant, $F(3, 21) = 3.71$, $p = 0.028$, $EF = 0.721$ (Fig. 4). Post-hoc tests showed a significant difference from baseline observed at Week 12: $t(21) = 2.93$, $p = 0.039$, estimated change = 4.27, but not for Week 1 or 4.

Sleep

For PSQI Score, the main effect of time was significant, $F(3, 19) = 4.27$, $p = 0.018$, $EF = 0.799$ and demonstrated a large treatment effect (Fig. 4). Post-hoc tests demonstrated a significant improvement in sleep ratings at Week 4: $t(19) = 3.19$, $p = 0.023$, estimated change = 4.38 and trending improvement at Week 12.

Table 2 Results of mixed-effects linear models assessing the effects of time, group, and their interaction on clinical measures

Measure	Effect	Num DF	Den DF	MS	SS	F	*p*-value	Sig
HRDS-17 Total Score	Time	3	21	328.94	986.82	28.49	<0.001	***
	Group	1	7	1.67	1.67	0.14	0.715	
	Time*Group	3	21	9.68	29.04	0.84	0.488	
BDI-II Total	Time	3	20	202.79	608.38	9.36	<0.001	***
	Group	1	7	0.74	0.74	0.03	0.858	
	Time*Group	3	20	17.85	53.54	0.82	0.496	
QIDS Total	Time	3	21	155.50	466.50	7.72	0.001	**
	Group	1	7	1.05	1.05	0.05	0.826	
	Time*Group	3	21	8.83	26.50	0.44	0.728	
GAD-7 Total	Time	3	21	35.15	105.45	3.71	0.028	*
	Group	1	7	0.67	0.67	0.07	0.799	
	Time*Group	3	21	12.04	36.12	1.27	0.310	
PSQI Score	Time	3	20	32.66	97.99	3.66	0.030	*
	Group	1	7	1.31	1.31	0.15	0.713	
	Time*Group	3	20	9.06	27.18	1.02	0.406	
SRS Total	Time	3	21	68.62	205.86	0.80	0.507	
	Group	1	7	1.31	1.31	0.02	0.905	
	Time*Group	3	21	74.10	222.30	0.87	0.474	
Fluid Cognition Raw Score	Time	3	21	312.79	938.37	10.00	<0.001	***
	Group	1	7	69.56	69.56	2.22	0.180	
	Time*Group	3	21	21.23	63.70	0.68	0.575	
Grip Strength Measurement (lbs. force)	Time	3	21	93.28	279.84	1.19	0.339	
	Group	1	7	444.27	444.27	5.65	0.049	*
	Time*Group	3	21	67.48	202.43	0.86	0.478	

The table includes the degrees of freedom (Num DF for effects, Den DF for error), Mean Squares (MS), Sum of Squares (SS), F-statistic values (F), Effect Size (EF) as Cohen's F, and *p*-values (p) for each effect. The 'sig' column indicates significance levels (* $p<0.05$, ** $p<0.01$, *** $p<0.001$). Each row represents a different measure or interaction. The Cohen's F effect size (EF) correspond to small (0.10), medium (0.25), and large (0.40). The measures assessed include Hamilton Rating Scale for Depression (HRDS-17 Total Score), Beck Depression Inventory-II (BDI-II Total), Quick Inventory of Depressive Symptomatology (QIDS Total), Generalized Anxiety Disorder 7-item (GAD-7 Total), Pittsburgh Sleep Quality Index (PSQI Score), Social Responsiveness Scale (SRS Total), NIH Toolbox Fluid Cognition, and Grip Strength Measurement

Social

For SRS Total, neither the main effect of time, F(3, 21) = 0.80, $p = 0.51$, EF = 0.337, nor the interaction between time and group, F(3, 21) = 0.87, $p = 0.47$, EF = 0.350, were significant (Fig. 4). Neurocognitive.

For Fluid Cognition, only the main effect of time was significant, F(3, 21) = 10.00, $p < 0.001$, EF = 1.174, indicating a large effect size (Fig. 4). Post-hoc testing found a trending improvement in Fluid Cognition from baseline to Week 1 and significant improvement from baseline were observed at Week 4, t(21) = -3.03, $p = 0.031$, estimated change = -8.05, and Week 12, t(21) = -5.47, $p < 0.001$, estimated change = -14.50.

Neuromuscular

For Grip Strength, neither the main effect of time, F(3, 21) = 1.19, $p = 0.34$, EF = 0.394, nor the interaction between time and group, F(3, 21) = 0.86, $p = 0.48$, EF = 0.335, were significant, but the main effect of group was trending, F(1, 7) = 5.65, $p = 0.05$, EF = 0.497.

Exploratory Biomarker

An exploratory analysis was conducted to investigate the relationship between changes in Fluid Cognition scores (NIH Toolbox) and changes in depressive symptomatology (measured by HRDS-17) at Week 12. The results indicated a significant correlation between improvement in Fluid Cognition scores at week 4 and reduction in depression symptoms at 12 (Fig. 5).

Safety Outcomes

The ATBS intervention was completed by nine subjects (UL = 5; BL = 4). However, one participant in the bilateral group was unable to tolerate the stimulation and withdrew from treatment. Five subjects ($n = 2$ UL; $n = 3$ BL) required titration to reach target stimulation intensity. Overall, adverse events were mild and self-limiting. Over the 90 treatment days (10 days per subject), a total of 6 headaches were reported, resulting in a 7% incidence rate. All headaches were associated with bilateral

Table 3 Results of post hoc tests, conducted to investigate significant model effects

Measure	vs. Baseline	DF	Estimate	t	*p*-value	Sig
HRDS-17 Total Score	Week 1	21.00	11.42	7.09	< 0.001	***
	Week 4	21.00	12.58	7.80	< 0.001	***
	Week 12	21.00	12.38	7.68	< 0.001	***
BDI-II Total	Week 1	20.00	8.20	3.71	0.007	**
	Week 4	20.00	9.52	4.31	0.002	**
	Week 12	20.02	10.65	4.66	< 0.001	***
QIDS Total	Week 1	21.00	7.65	3.59	0.009	**
	Week 4	21.00	8.93	4.19	0.002	**
	Week 12	21.00	8.33	3.91	0.004	**
GAD-7 Total	Week 1	21.00	4.03	2.76	0.056	
	Week 4	21.00	3.45	2.36	0.123	
	Week 12	21.00	4.28	2.93	0.039	*
PSQI Score	Week 1	20.00	3.35	2.37	0.124	
	Week 4	20.00	4.38	3.09	0.028	*
	Week 12	20.17	3.51	2.34	0.130	
Fluid Cognition Raw Score	Week 1	21.00	-7.37	-2.78	0.053	
	Week 4	21.00	-8.05	-3.03	0.031	*
	Week 12	21.00	-14.50	-5.47	< 0.001	***

These tests examine differences between Baseline and subsequent time points (Week 1, Week 4, and Week 12) across various measures. All post-hoc tests underwent a 5% FDR *p*-value adjustment. The table provides information on degrees of freedom (DF), estimated differences from the baseline (Estimate), t-statistic values (t), adjusted *p*-values (Adj. p.) for controlling the family-wise error rate, and the significance (Sig) of the comparisons (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). The measured variables include the Hamilton Rating Scale for Depression (HRDS-17 Total Score), Beck Depression Inventory-II (BDI-II Total), Quick Inventory of Depressive Symptomatology (QIDS Total), Generalized Anxiety Disorder 7-item (GAD-7 Total), Pittsburgh Sleep Quality Index (PSQI Score), and NIH Toolbox Fluid Cognition

treatment and were described by participants as mild in severity and spontaneously resolved.

Discussion

This open-label trial demonstrates the efficacy and safety of ATBS in transition-aged autistic youth with treatment-refractory MDD. Treatment-resistant MDD in ASD is a challenging condition to manage, often necessitating polypharmacy or therapeutic approaches associated with higher side effects. Though the efficacy of rTMS for MDD is well established, adapting the intervention at scale for ASD individuals poses unique challenges. The primary purpose of this study was to identify optimal design parameters and outcome measures to advance into a larger pivotal RCT. We observed a robust and sustained treatment in most participants following ATBS regardless of stimulation site. The observed statistically significant improvement across various MDD scales immediately post-treatment suggests ATBS may elicit rapid antidepressant effects, especially in comparison to antidepressant medications or psychotherapy and especially notable in a treatment-refractory sample.

It is important to consider the limitations of our study design, non-controlled studies have shown large effects in depression trials (Wager & Atlas, 2015; Walsh et al., 2002). Nevertheless, recent research on the placebo effect has suggested that it may activate similar areas of the brain as actual antidepressant treatment, and strong placebo effects may indicate regression to the mean. To mitigate these potential confounding factors and identify potential mechanisms, we collected high-resolution electroencephalography to assess changes in brain activity (in preparation) and the inclusion of a lead-in period to account for spontaneous remission of depression symptoms. There is considerable existing evidence that TMS treatment in depression is superior to sham stimulation in typically developing cohorts, albeit with recent meta-analyses lowering the effect size in some populations. (Brini et al., 2023). As the current FDA-cleared rTMS protocol does not exclude individuals with ASD, it remains an evidence-based treatment for MDD when typical therapies are ineffective(Zemplenyi et al., 2022). ATBS may be particularly well suited in cases where there is a high risk of adverse outcomes associated with prolonged depressive episodes, intolerability to typical rTMS, or when the next proximal step is electroconvulsive therapy.

We observed no significant difference in efficacy between Unilateral (UL) and Bilateral (BL) stimulation. However, BL stimulation was associated with a higher incidence of side effects and involved a greater number of procedures. The question of relative effectiveness of BL

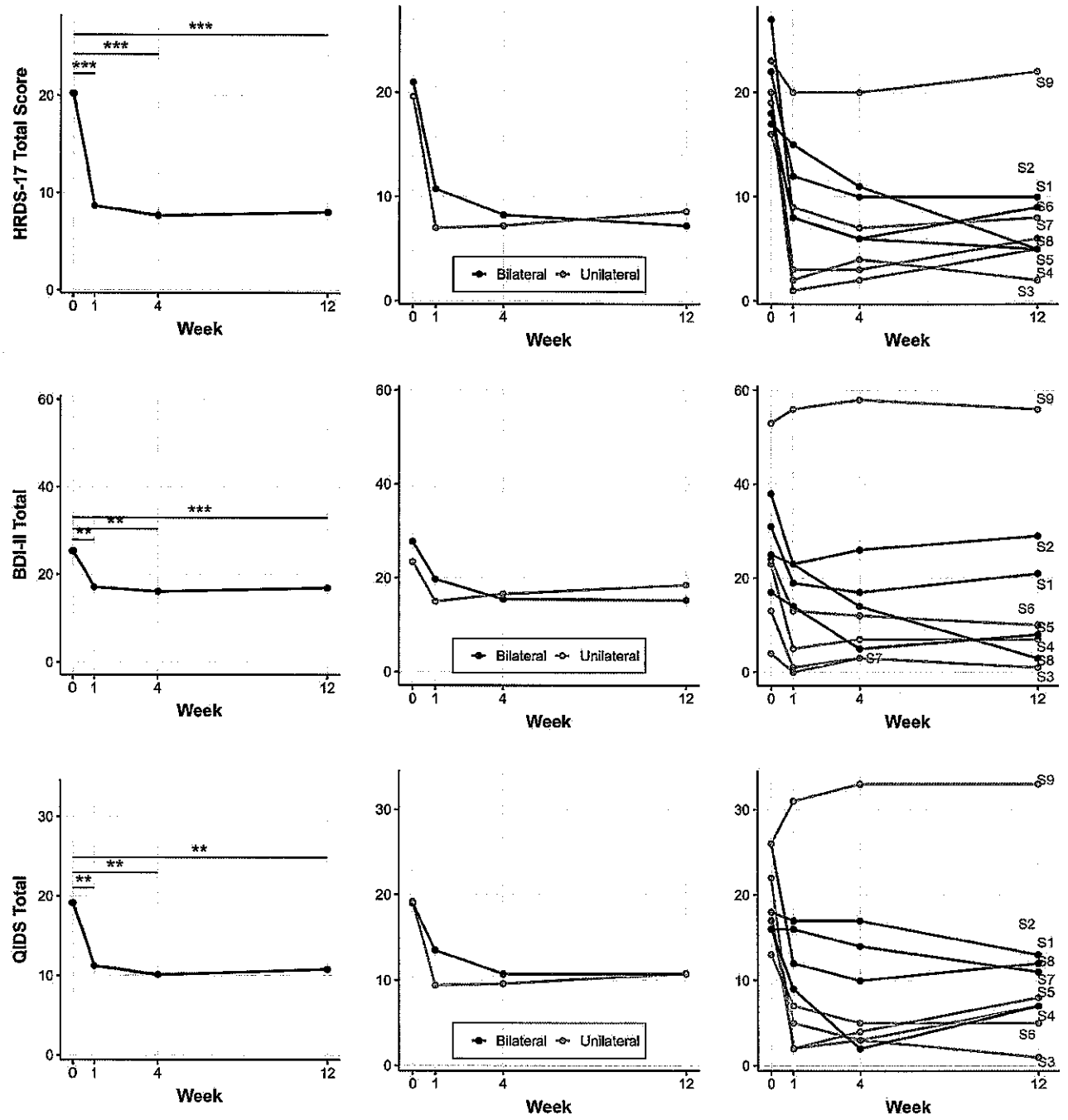


Fig. 3 Trajectories on depression scales (HDRS-17 Total Score, BDI-II Total, QIDS Total) over 12 weeks following ATBS treatment. Timepoint 0: baseline; Timepoints 1, 4, and 12: post-treatment follow-ups, numbered by week. Left: group means with significance; center: Bilateral vs. Unilateral group averages; right: individual progressions (S1-S9). Significance levels: * $p<0.05$, ** $p<0.01$, *** $p<0.001$

vs. unilateral rTMS in MDD treatment remains unclear; however, empirical data suggests that stimulation parameters, patient population, and tolerability should be considered (Blumberger et al., 2016; Fitzgerald et al., 2012, 2013; Trevizol et al., 2019; Weissman et al., 2018b). Taking into account the practical considerations which we observed and the present lack of evidence-based treatments, future RCTs may be well-served to focus on UL stimulation in ASD populations.

We administered serial computerized neurocognitive testing, hypothesizing that changes in performance on these tests could serve as biomarkers of prefrontal target

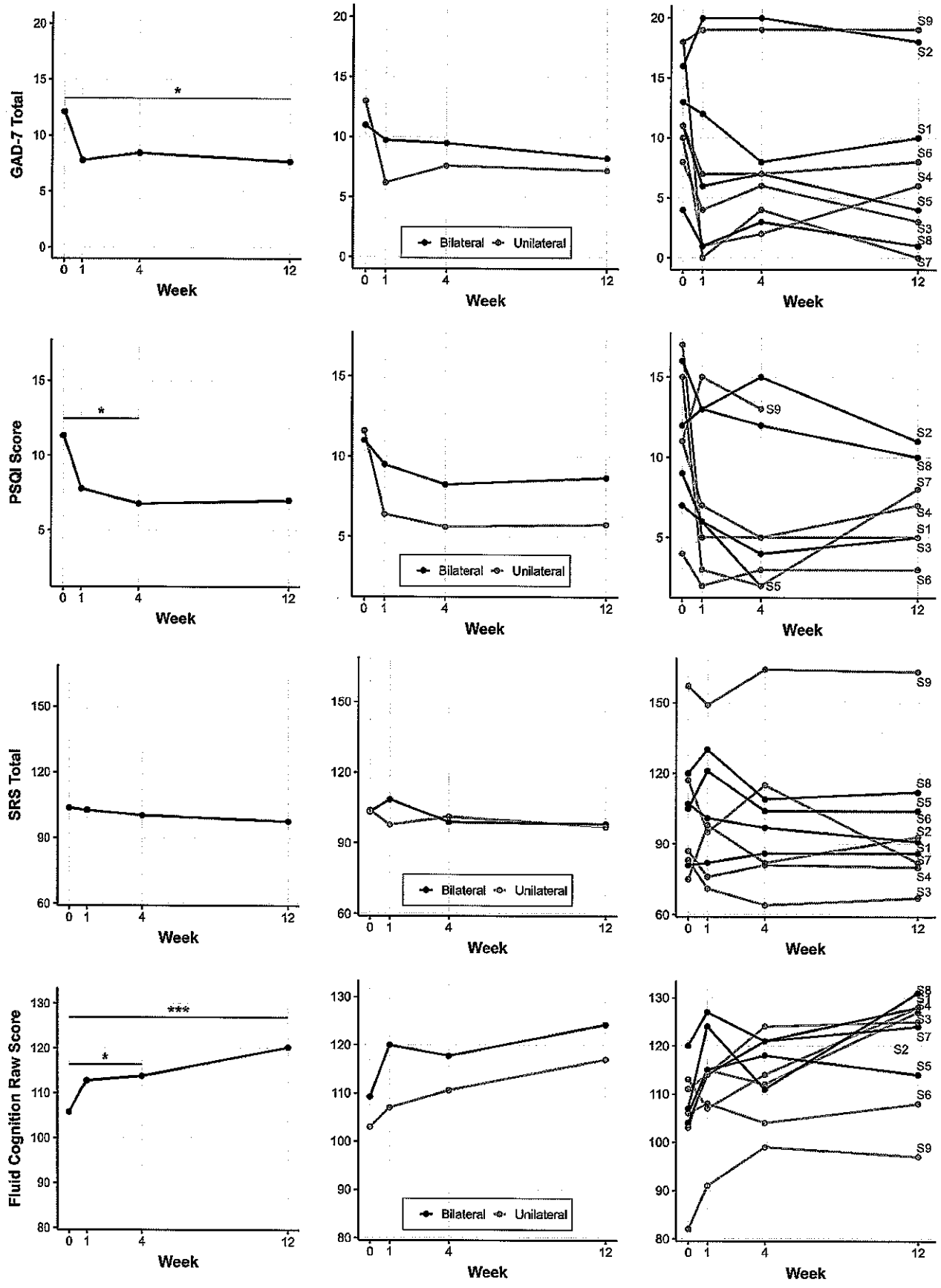

GAD-7 Total
PSQI Score
SRS Total
Fluid Cognition Raw Score
Week
Bilateral
Unilateral
*
***
S1
S2
S3
S4
S5
S6
S7
S8
S9

Fig. 4 Longitudinal score changes for various clinical measures (GAD-7 Total, PSQI Score, SRS Total, Fluid Cognition Raw Score) over 12 weeks following ATBS treatment. Timepoint 0: baseline; Timepoints 1, 4, and 12: post-treatment follow-ups, numbered by week. Left: group means with significance; center: Bilateral vs. Unilateral group averages; right: individual progressions (S1-S9). Significance levels: * $p<0.05$, ** $p<0.01$, *** $p<0.001$

engagement and predict later treatment effects (de Boer et al., 2021). Following ATBS treatment, we observed, in the majority of participants, a marked improvement in fluid cognition scores (NIH Toolbox) which was predictive of treatment response on the HRDS-17 at 12 weeks. The neurocognitive tests underlying these findings, such as the Flanker task and card sorting tasks, have been consistently linked to the efficiency of frontal-parietal cortical networks (Kim et al., 2017). However, in our sample, these findings are potentially confounded by concurrent improvements in MDD and associated pseudodementia (Kim et al., 2019).

Even if improvements of fluid cognition are unrelated to ATBS and instead secondary to the amelioration of MDD, assessing fluid cognition in ASD MDD may still serve as an indicator of the duration or response of treatment. Intriguingly, several studies have reported that, in comparison to typically developed individuals, those with ASD exhibit increased activity in temporal and occipital networks, but decreased activity in frontal-parietal networks during fluid reasoning tasks (Simard et al., 2015; Soulières et al., 2009). This suggests that independent studies of accelerated theta-burst stimulation for improving fluid cognition by engaging potentially underactive frontal-parietal networks in ASD may be worth exploring.

Limitations

Several limitations to our study must be considered in context with the strong treatment effects. First and foremost, the potential of expectancy effects, widely observed in rTMS studies, particularly with younger subjects (Oberman et al., 2021; Xu et al., 2023), is important to consider. However, the observed changes in fluid cognition provide an objective marker of treatment response, thus reducing the likelihood of expectancy effects significantly influencing our results. Second, sample size and the exclusion of autistic individuals with IDD reduces the power, generalizability of these results, and our ability to identify subgroups of patients who may benefit most from ATBS compared to other forms of treatment. For example, one participant disclosed during treatment additional history and symptoms consistent with co-occurring personality disorder and did not demonstrate any treatment effects. This result is consistent with the typical treatment refractoriness of personality disorders (Abraham & Calabrese, 2008). Another limitation is accurately diagnosing and assessing the severity of MDD in ASD populations, where clinical presentation may be atypical and standardized measures may not be validated. To assess depression severity, we used clinician and self-report measures, including input from caregivers when available. This approach aligns with previous studies that suggest a multi-informant assessment in ASD captures complex symptoms and experiences more effectively (Sandercock et al., 2020).

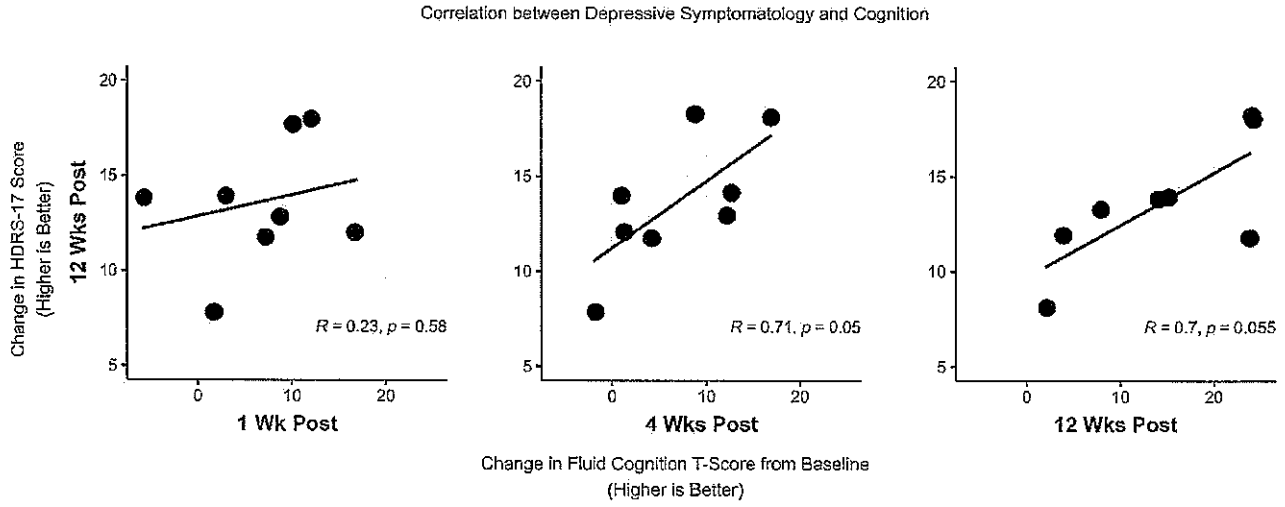


Fig. 5 Correlation between 12-week changes in HDRS-17 scores and changes in Fluid Cognition T-Scores at 1, 4, and 12 weeks post-treatment. Lines of best fit with Spearman coefficients and p-values indicate the strength and significance of the correlations

Conclusion

This exploratory study provides support for the safety and efficacy of ATBS in addressing treatment resistant MDD in autistic individuals. We observed rapid and large treatment effects across multiple domains that endured over time, with 56% (5/9) of subjects meeting criteria for remission at 12-weeks post-treatment. The distinctive treatment challenges posed by autistic populations, such as sensory hypersensitivities and the necessity of multi-informant outcome assessments, underscore the need for a nuanced approach to adapting ATBS protocols. Our findings suggest that neurocognitive testing could be an objective biomarker for predicting treatment response and potentially individualizing treatment. However, larger, sham-controlled studies are necessary to validate these findings. If successful, ATBS could emerge as an evidence-based intervention for MDD in ASD, an area that currently lacks effective treatments.

Author Contributions E.J.B.: writing (original draft and review), data curation, data analysis, and project administration. D.L.G.: conceptualization, investigation, and manuscript review. S.W.W.: conceptualization, investigation, and manuscript review. T.L.: manuscript review and project administration. R.E.: study design and manuscript review. R.L.: statistical analysis. E.S.: investigation and manuscript review. G.W.: manuscript review and project administration. Y.L.: manuscript review and project administration. P.S.H.: statistical analysis. E.G.: data curation, and manuscript review. J.A.S.: study design and conceptualization. C.A.E.: conceptualization, investigation, and manuscript review. E.V.P.: conceptualization, investigation, data analysis, writing (original draft and review), and project administration.
We must note the unfortunate passing of one of our esteemed co-authors, Dr. John Sweeney. Dr. Sweeney contributed significantly to the conception and design of our study prior to his untimely death. We include him as a co-author to acknowledge his valuable contributions and to honor his commitment to advancing our understanding of autism and developmental disorders. The remaining authors have assumed responsibility for the final content of the manuscript.

Code Availability The code is available at https://github.com/cincibrainlab.

Declarations

Conflict of interest The authors declare no competing interest (either financial or non-financial interest) with the present submitted manuscript described by the Journal of Autism and Developmental Disorders policy. The present study was federally funded by the Cincinnati Children's Hospital Research Foundation. We have opted to provide full disclosures of all authors' external commitments for the editor's review for transparency. The basis of our disclosure was made after review of after review of these activities.
The corresponding author, **Dr. Pedapati** reports no conflicts of interest and is supported by an NIH K23 Clinical Development Award (K23MH112936) and has received past research support from the American Academy of Child and Adolescent Psychiatry, and Cincinnati Children's Hospital Research Foundation and textbook royalties from Springer. In the past he has consulted for Eccrine Systems, Forge Therapeutics, P&G, and Autism Speaks. The senior author **Dr. Erickson** is the principal investigator of the human portion of the NIH FXS Center. He has received compensation from Confluence Pharma, Novartis, F. Hoffmann-La Roche Ltd., Seaside Therapeutics, Riovant Sciences, Inc., Fulcrum Therapeutics, Forge Therapeutics, Neuren Pharmaceuticals Ltd., Alcobra Pharmaceuticals, Neurotrope, Zynerba Pharmaceuticals, Inc., and Ovid Therapeutics Inc. to consult on trial design or development strategies and/or conduct clinical trials in neurodevelopmental disorders. Dr. Erickson is additionally the inventor or co-inventor on several patents held by Cincinnati Children's Hospital Medical Center and Indiana University School of Medicine describing methods of treatment of neurodevelopmental disorders. **Dr. Wu** receives research support from NIH, Quince Therapeutics, and Emalax Biosciences. **Dr. Sweeney** consults to VeraSci and has received support from NIH and Sichuan University. **Dr. Horn** has no conflicts of interest to disclose. **Dr. Gilbert** has received compensation for expert testimony for the U.S. National Vaccine Injury Compensation Program, through the Department of Health and Human Services. He has received payment for medical expert opinions through TeladocHealth International. He has served as a paid consultant for Emalex Biosciences. He has received research support from the United States National Institutes of Health (Tourette Syndrome, ADHD research) and the Department of Defense (Neurofibromatosis research). He has received salary compensation through Cincinnati Children's for work as a clinical trial site investigator from Emalex (clinical trial, Tourette Syndrome), PTC Therapeutics (registry and clinical trial, Amino Acid Decarboxylase Deficiency). He has received book/publication royalties from Elsevier and Wolters Kluwer. **Dr. Larsh** has received research and travel support from the Tourette Association of America (TAA), as well as travel support and honoraria from the Child Neurology Society (CNS). **Dr. Elmaghraby**, **Dr. Liu**, and **Dr. Smith** have no disclosures of funding or external compensation.
Finally, we acknowledge that Dr. Craig Erickson serves as an Associate Editor for the Journal of Autism and Developmental Disorders.

Ethics Approval and Consent This study was approved by the institutional review board at Cincinnati Children's Hospital Medical Center (CCHMC) and registered with ClinicalTrials.gov (NCT01609374). Recruitment took place between November 2021 and November 2022 through clinician referrals, community flyers, emails, and clinics at a tertiary academic pediatric hospital. All participants provided written informed consent or assent for all study procedures.

References

Abraham, P. F., & Calabrese, J. R. (2008). Evidenced-based pharmacologic treatment of borderline personality disorder: A shift from SSRIs to anticonvulsants and atypical antipsychotics? *Journal of Affective Disorders, 111*(1), 21–30.

American Psychiatric Association, D., &, & Association, A. P. (2013). *Diagnostic and statistical manual of mental disorders: DSM-5*, (Vol. 5). American Psychiatric Association Washington, DC.

Beam, W., Borckardt, J. J., Reeves, S. T., & George, M. S. (2009). An efficient and accurate new method for locating the F3 position for prefrontal TMS applications. *Brain Stimulation, 2*(1), 50–54. https://doi.org/10.1016/j.brs.2008.09.006

Blumberger, D. M., Mulsant, B. H., Fitzgerald, P. B., Rajji, T. K., Ravindran, A. V., Young, L. T., Levinson, A. J., & Daskalakis, Z. J. (2012). A randomized double-blind sham-controlled comparison of unilateral and bilateral repetitive transcranial magnetic stimulation for treatment-resistant major depression. *The World Journal of Biological Psychiatry : The Official Journal of the World Federation of Societies of Biological Psychiatry, 13*(6), 423–435.

Blumberger, D. M., Maller, J. J., Thomson, L., Mulsant, B. H., Rajji, T. K., Maher, M., Brown, P. E., Downar, J., Vila-Rodriguez, F., Fitzgerald, P. B., & Daskalakis, Z. J. (2016). Unilateral and bilateral MRI-targeted repetitive transcranial magnetic stimulation for treatment-resistant depression: A randomized controlled study. *Journal of Psychiatry and Neuroscience, 41*(4), E58–66. https://doi.org/10.1503/jpn.150265

Blumberger, D. M., Vila-Rodriguez, F., Thorpe, K. E., Feffer, K., Noda, Y., Giacobbe, P., Knyahnytska, Y., Kennedy, S. H., Lam, R. W., Daskalakis, Z. J., & Downar, J. (2018). Effectiveness of theta burst versus high-frequency repetitive transcranial magnetic stimulation in patients with depression (THREE-D): A randomised non-inferiority trial. *Lancet, 391*(10131), 1683–1692. https://doi.org/10.1016/S0140-6736(18)30295-2

Brini, S., Brudasca, N. I., Hodkinson, A., Kaluzinska, K., Wach, A., Storman, D., Prokop-Dorner, A., Jemioło, P., & Bala, M. M. (2023). Efficacy and safety of transcranial magnetic stimulation for treating major depressive disorder: An umbrella review and re-analysis of published meta-analyses of randomised controlled trials. *Clinical Psychology Review, 100*, 102236. https://doi.org/10.1016/j.cpr.2022.102236

Bruni, T. P. (2014). Test review: Social responsiveness scale–second edition (SRS-2). *Journal of Psychoeducational Assessment, 32*(4), 365–369. https://doi.org/10.1177/0734282913517525

Buysse, D. J., Reynolds, C. F. 3rd, Monk, T. H., Berman, S. R., & Kupfer, D. J. (1989). The Pittsburgh sleep quality index: A new instrument for psychiatric practice and research. *Psychiatry Research, 28*(2), 193–213. https://doi.org/10.1016/0165-1781(89)90047-4

Cai, D. B., Qin, Z. J., Lan, X. J., Liu, Q. M., Qin, X. D., Wang, J. J., Goya-Maldonado, R., Huang, X. B., Ungvari, G. S., Ng, C. H., Zheng, W., & Xiang, Y. T. (2023). Accelerated intermittent theta burst stimulation for major depressive disorder or bipolar depression: A systematic review and meta-analysis. *Asian Journal of Psychiatry, 85*, 103618. https://doi.org/10.1016/j.ajp.2023.103618

Cassidy, S., Bradley, P., Robinson, J., Allison, C., McHugh, M., & Baron-Cohen, S. (2014). Suicidal ideation and suicide plans or attempts in adults with Asperger's syndrome attending a specialist diagnostic clinic: A clinical cohort study. *Lancet Psychiatry, 1*(2), 142–147. https://doi.org/10.1016/S2215-0366(14)70248-2

Cassidy, S., Bradley, L., Shaw, R., & Baron-Cohen, S. (2018). Risk markers for suicidality in autistic adults. *Mol Autism, 9*, 42. https://doi.org/10.1186/s13229-018-0226-4

Chistyakov, A. V., Kreinin, B., Marmor, S., Kaplan, B., Khatib, A., Darawsheh, N., Koren, D., Zaaroor, M., & Klein, E. (2015). Preliminary assessment of the therapeutic efficacy of continuous theta-burst magnetic stimulation (cTBS) in major depression: A double-blind sham-controlled study. *Journal of Affective Disorders, 170*, 225–229. https://doi.org/10.1016/j.jad.2014.08.035

Crane, N. A., Jenkins, L. M., Bhaumik, R., Dion, C., Gowins, J. R., Mickey, B. J., Zubieta, J. K., & Langenecker, S. A. (2017). Multidimensional prediction of treatment response to antidepressants with cognitive control and functional MRI. *Brain, 140*(2), 472–486. https://doi.org/10.1093/brain/aww326

de Boer, N. S., Schluter, R. S., Daams, J. G., van der Werf, Y. D., Goudriaan, A. E., & van Holst, R. J. (2021). The effect of non-invasive brain stimulation on executive functioning in healthy controls: A systematic review and meta-analysis. *Neuroscience & Biobehavioral Reviews, 125*, 122–147. https://doi.org/10.1016/j.neubiorev.2021.01.013

Duprat, R., Desmyter, S., Rudi, D. R., van Heeringen, K., Van den Abbeele, D., Tandt, H., Bakic, J., Pourtois, G., Dedoncker, J., Vervaet, M., Van Autreve, S., Lemmens, G. M. D., & Baeken, C. (2016). Accelerated intermittent theta burst stimulation treatment in medication-resistant major depression: A fast road to remission? *Journal of Affective Disorders, 200*, 6–14. https://doi.org/10.1016/j.jad.2016.04.015

Elmaghraby, R., Sun, Q., Ozger, C., Shekunov, J., Romanowicz, M., & Croarkin, P. E. (2022). A systematic review of the safety and tolerability of theta burst stimulation in children and adolescents. *Neuromodulation: Technology at the Neural Interface, 25*(4), 494–503. https://doi.org/10.1111/ner.13455

FDA (2011). *Repetitive Transcranial Magnetic Stimulation (rTMS) Systems - Class II Special Controls Guidance for Industry and FDA Staff*. U.S. Food and Drug Administration Retrieved from https://www.fda.gov/medical-devices/guidance-documents-medical-devices-and-radiation-emitting-products/repetitive-transcranial-magnetic-stimulation-rtms-systems-class-ii-special-controls-guidance

Feroe, A. G., Uppal, N., Gutiérrez-Sacristán, A., Mousavi, S., Greenspun, P., Surati, R., Kohane, I. S., & Avillach, P. (2021). Medication use in the management of comorbidities among individuals with autism spectrum disorder from a large nationwide insurance database. *JAMA Pediatr, 175*(9), 957–965. https://doi.org/10.1001/jamapediatrics.2021.1329

Fitzgerald, P. B., Hoy, K. E., Herring, S. E., McQueen, S., Peachey, A. V., Segrave, R. A., Maller, J., Hall, P., & Daskalakis, Z. J. (2012). A double blind randomized trial of unilateral left and bilateral prefrontal cortex transcranial magnetic stimulation in treatment resistant major depression. *Journal of Affective Disorders, 139*(2), 193–198. https://doi.org/10.1016/j.jad.2012.02.017

Fitzgerald, P. B., Hoy, K. E., Singh, A., Gunewardene, R., Slack, C., Ibrahim, S., Hall, P. J., & Daskalakis, Z. J. (2013). Equivalent beneficial effects of unilateral and bilateral prefrontal cortex transcranial magnetic stimulation in a large randomized trial in treatment-resistant major depression. *International Journal of Neuropsychopharmacology, 16*(9), 1975–1984. https://doi.org/10.1017/S1461145713000369

Fitzgerald, P. B., Chen, L., Richardson, K., Daskalakis, Z. J., & Hoy, K. E. (2020). A pilot investigation of an intensive theta burst stimulation protocol for patients with treatment resistant depression. *Brain Stimulation, 13*(1), 137–144. https://doi.org/10.1016/j.brs.2019.08.013

Gwynette, M. F., Lowe, D. W., Henneberry, E. A., Sahlem, G. L., Wiley, M. G., Alsarraf, H., Russo, S. B., Joseph, J. E., Summers, P. M., Lohnes, L., & George, M. S. (2020). Treatment of adults with autism and major depressive disorder using transcranial magnetic stimulation: An open label pilot study. *Autism Research, 13*(3), 346–351. https://doi.org/10.1002/aur.2266

Hamilton, M. (1986). The Hamilton Rating Scale for Depression. In N. Sartorius & T. A. Ban (Eds.), *Assessment of Depression*, (pp. 143–152). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-70486-4_14

Hirvikoski, T., Mittendorfer-Rutz, E., Boman, M., Larsson, H., Lichtenstein, P., & Bolte, S. (2016). Premature mortality in autism spectrum disorder. *British Journal of Psychiatry, 208*(3), 232–238.

https://doi.org/10.1192/bjp.bp.114.160192S0007125000279385 [pii].

Hollocks, M. J., Lerh, J. W., Magiati, I., Meiser-Stedman, R., & Brugha, T. S. (2019). Anxiety and depression in adults with autism spectrum disorder: A systematic review and meta-analysis. *Psychological Medicine*, *49*(4), 559–572. https://doi.org/10.1017/S0033291718002283

Hong, Y. H., Wu, S. W., Pedapati, E. V., Horn, P. S., Huddleston, D. A., Laue, C. S., & Gilbert, D. L. (2015). Safety and tolerability of theta burst stimulation vs. single and paired pulse transcranial magnetic stimulation: A comparative study of 165 pediatric subjects. *Frontiers in Human Neuroscience*, *9*, 29. https://doi.org/10.3389/fnhum.2015.00029

Horvath, J. C., Mathews, J., Demitrack, M. A., & Pascual-Leone, A. (2010). The neurostar TMS device: conducting the FDA approved protocol for treatment of depression. *Journal of visualized experiments : JoVE*, (45), 2345. https://doi.org/10.3791/2345

Huang, Y. Z., Edwards, M. J., Rounis, E., Bhatia, K. P., & Rothwell, J. C. (2005a). Theta burst stimulation of the human motor cortex. *Neuron*, *45*(2), 201–206. https://doi.org/10.1016/j.neuron.2004.12.033

Huang, Y. Z., Edwards, M. J., Rounis, E., Bhatia, K. P., & Rothwell, J. C. (2005b). Theta burst stimulation of the human motor cortex. *Neuron*, *45*(2), 201–206. https://doi.org/10.1016/j.neuron.2004.12.033

Huashuang, Z., Yang, L., Chensheng, H., Jing, X., Bo, C., Dongming, Z., Kangfu, L., & Shi-Bin, W. (2022). Prevalence of adverse effects associated with transcranial magnetic stimulation for autism spectrum disorder: A systematic review and meta-analysis. *Frontiers in Psychiatry*, *13*, 875591. https://doi.org/10.3389/fpsyt.2022.875591

Hudson, C. C., Hall, L., & Harkness, K. L. (2019). Prevalence of depressive disorders in individuals with autism spectrum disorder: A meta-analysis. *Journal of Abnormal Child Psychology*, *47*(1), 165–175. https://doi.org/10.1007/s10802-018-0402-1

Kim, N. Y., Wittenberg, E., & Nam, C. S. (2017). Behavioral and neural correlates of executive function: Interplay between inhibition and updating processes. *Front Neurosci*, *11*, 378. https://doi.org/10.3389/fnins.2017.00378

Kim, T. D., Hong, G., Kim, J., & Yoon, S. (2019). Cognitive enhancement in neurological and psychiatric disorders using transcranial magnetic stimulation (TMS): A review of modalities, potential mechanisms and future implications. *Exp Neurobiol*, *28*(1), 1–16. https://doi.org/10.5607/en.2019.28.1.1

Lord, C., Rutter, M., DiLavore, P. C., Risi, S., Gotham, K., & Bishop, S. (2012). *Autism diagnostic observation schedule: ADOS-2*. Western Psychological Services Los Angeles.

Matson, J. L., & Nebel-Schwalm, M. (2007). Assessing challenging behaviors in children with autism spectrum disorders: A review. *Research in Developmental Disabilities*, *28*(6), 567–579. https://doi.org/10.1016/j.ridd.2006.08.001

McCracken, J. T., Anagnostou, E., Arango, C., Dawson, G., Farchione, T., Mantua, V., McPartland, J., Murphy, D., Pandina, G., & Veenstra-VanderWeele, J. (2021). Drug development for autism spectrum disorder (ASD): Progress, challenges, and future directions. *European Neuropsychopharmacology*, *48*, 3–31. https://doi.org/10.1016/j.euroneuro.2021.05.010

Neuteboom, D., Zantvoord, J. B., Goya-Maldonado, R., Wilkening, J., Dols, A., van Exel, E., Lok, A., de Haan, L., & Scheepstra, K. W. (2023). Accelerated intermittent theta burst stimulation in major depressive disorder: A systematic review. *Psychiatry Research*, 115429.

Oberman, L. M., Hynd, M., Nielson, D. M., Towbin, K. E., Lisanby, S. H., & Stringaris, A. (2021). Repetitive transcranial magnetic stimulation for adolescent major depressive disorder: A focus on neurodevelopment. *Frontiers in Psychiatry*, *12*, 642847. https://doi.org/10.3389/fpsyt.2021.642847

Osman, A., Bagge, C. L., Gutierrez, P. M., Konick, L. C., Kopper, B. A., & Barrios, F. X. (2001). The suicidal behaviors questionnaire-revised (SBQ-R): Validation with clinical and nonclinical samples. *Assessment*, *8*(4), 443–454. https://doi.org/10.1177/107319110100800409

Osman, A., Kopper, B. A., Barrios, F., Gutierrez, P. M., & Bagge, C. L. (2004). *Reliability and Validity of the Beck Depression Inventory–II With Adolescent Psychiatric Inpatients* [https://doi.org/10.1037/1040-3590.16.2.120]

Pezzimenti, F., Han, G. T., Vasa, R. A., & Gotham, K. (2019). Depression in youth with autism spectrum disorder. *Child and Adolescent Psychiatric Clinics of North America*, *28*(3), 397–409. https://doi.org/10.1016/j.chc.2019.02.009

Posner, K., Brent, D., Lucas, C., Gould, M., Stanley, B., Brown, G., Fisher, P., Zelazny, J., Burke, A., & Oquendo, M. (2008). *Columbia-suicide severity rating scale (C-SSRS)*. Columbia University Medical Center.

Razza, L. B., Moffa, A. H., Moreno, M. L., Carvalho, A. F., Padberg, F., Fregni, F., & Brunoni, A. R. (2018). A systematic review and meta-analysis on placebo response to repetitive transcranial magnetic stimulation for depression trials. *Progress in Neuropsychopharmacology and Biological Psychiatry*, *81*, 105–113. https://doi.org/10.1016/j.pnpbp.2017.10.016

Reuben, D. B., Magasi, S., McCreath, H. E., Bohannon, R. W., Wang, Y. C., Bubela, D. J., Rymer, W. Z., Beaumont, J., Rine, R. M., Lai, J. S., & Gershon, R. C. (2013). Motor assessment using the NIH toolbox. *Neurology*, *80*(11 Suppl 3), S65–75. https://doi.org/10.1212/WNL.0b013e3182872e01

Rosenberg, R. E., Mandell, D. S., Farmer, J. E., Law, J. K., Marvin, A. R., & Law, P. A. (2010). Psychotropic medication use among children with autism spectrum disorders enrolled in a national registry, 2007–2008. *Journal of Autism and Developmental Disorders*, *40*(3), 342–351. https://doi.org/10.1007/s10803-009-0878-1

Rossi, S., Antal, A., Bestmann, S., Bikson, M., Brewer, C., Brockmöller, J., Carpenter, L. L., Cincotta, M., Chen, R., Daskalakis, J. D., Di Lazzaro, V., Fox, M. D., George, M. S., Gilbert, D., Kimiskidis, V. K., Koch, G., Ilmoniemi, R. J., Lefaucheur, J. P., Leocani, L., & Hallett, M. (2021). Safety and recommendations for TMS use in healthy subjects and patient populations, with updates on training, ethical and regulatory issues: Expert guidelines. *Clinical Neurophysiology*, *132*(1), 269–306. https://doi.org/10.1016/j.clinph.2020.10.003

Rush, A. J., Trivedi, M. H., Ibrahim, H. M., Carmody, T. J., Arnow, B., Klein, D. N., Markowitz, J. C., Ninan, P. T., Kornstein, S., & Manber, R. (2003). The 16-item quick inventory of depressive symptomatology (QIDS), clinician rating (QIDS-C), and self-report (QIDS-SR): A psychometric evaluation in patients with chronic major depression. *Biological Psychiatry*, *54*(5), 573–583.

Sackeim, H. A., Aaronson, S. T., Bunker, M. T., Conway, C. R., Demitrack, M. A., George, M. S., Prudic, J., Thase, M. E., & Rush, A. J. (2019). The assessment of resistance to antidepressant treatment: Rationale for the antidepressant treatment history form: Short form (ATHF-SF). *Journal of Psychiatric Research*, *113*, 125–136. https://doi.org/10.1016/j.jpsychires.2019.03.021

Sandercock, R. K., Lamarche, E. M., Klinger, M. R., & Klinger, L. G. (2020). Assessing the convergence of self-report and informant measures for adults with autism spectrum disorder. *Autism*, *24*(8), 2256–2268. https://doi.org/10.1177/1362361320942981

Sehatzadeh, S., Daskalakis, Z. J., Yap, B., Tu, H. A., Palimaka, S., Bowen, J. M., & O'Reilly, D. J. (2019). Unilateral and bilateral repetitive transcranial magnetic stimulation for treatment-resistant depression: A meta-analysis of randomized controlled trials over 2 decades. *Journal of Psychiatry and Neuroscience*, *44*(3), 151–163. https://doi.org/10.1503/jpn.180056

Sheehan, D. V., Lecrubier, Y., Sheehan, K. H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., & Dunbar, G. C. (1998). The mini-international neuropsychiatric interview (M.I.N.I.): The development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *Journal of Clinical Psychiatry*, *59*(Suppl 20), 22–33quiz34. https://www.ncbi.nlm.nih.gov/pubmed/9881538

Simard, I., Luck, D., Mottron, L., Zeffiro, T. A., & Soulières, I. (2015). Autistic fluid intelligence: Increased reliance on visual functional connectivity with diminished modulation of coupling by task difficulty. *NeuroImage: Clinical*, *9*, 467–478. https://doi.org/10.1016/j.nicl.2015.09.007

Soulières, I., Dawson, M., Samson, F., Barbeau, E. B., Sahyoun, C. P., Strangman, G. E., Zeffiro, T. A., & Mottron, L. (2009). Enhanced visual processing contributes to matrix reasoning in autism. *Human Brain Mapping*, *30*(12), 4082–4107. https://doi.org/10.1002/hbm.20831

Spitzer, R. L., Williams, J. B., Gibbon, M., & First, M. B. (1992). The structured clinical interview for DSM-III-R (SCID): I: History, rationale, and description. *Archives of General Psychiatry*, *49*(8), 624–629.

Spitzer, R. L., Kroenke, K., Williams, J. B., & Lowe, B. (2006). A brief measure for assessing generalized anxiety disorder: The GAD-7. *Archives of Internal Medicine*, *166*(10), 1092–1097. https://doi.org/10.1001/archinte.166.10.1092

Terao, Y., & Ugawa, Y. (2002). Basic mechanisms of TMS. *Journal of Clinical Neurophysiology*, *19*(4), 322–343. https://doi.org/10.1097/00004691-200208000-00006

Trevizol, A. P., Goldberger, K. W., Mulsant, B. H., Rajji, T. K., Downar, J., Daskalakis, Z. J., & Blumberger, D. M. (2019). Unilateral and bilateral repetitive transcranial magnetic stimulation for treatment-resistant late-life depression. *International Journal of Geriatric Psychiatry*, *34*(6), 822–827. https://doi.org/10.1002/gps.5091

Wager, T. D., & Atlas, L. Y. (2015). The neuroscience of placebo effects: Connecting context, learning and health. *Nature Reviews Neuroscience*, *16*(7), 403–418. https://doi.org/10.1038/nrn3976

Walsh, B. T., Seidman, S. N., Sysko, R., & Gould, M. (2002). Placebo response in studies of major depression: Variable, substantial, and growing. *Journal of the American Medical Association*, *287*(14), 1840–1847.

Wechsler, D. (1999). *Wechsler Abbreviated Scale of Intelligence*. Psychological Corporation.

Weintraub, S., Dikmen, S. S., Heaton, R. K., Tulsky, D. S., Zelazo, P. D., Bauer, P. J., Carlozzi, N. E., Slotkin, J., Blitz, D., Wallner-Allen, K., Fox, N. A., Beaumont, J. L., Mungas, D., Nowinski, C. J., Richler, J., Deocampo, J. A., Anderson, J. E., Manly, J. J., Borosh, B., & Gershon, R. C. (2013). Cognition assessment using the NIH toolbox. *Neurology*, *80*(11 Supplement 3), S54–S64. https://doi.org/10.1212/WNL.0b013e3182872ded

Weissman, C. R., Blumberger, D. M., Brown, P. E., Isserles, M., Rajji, T. K., Downar, J., Mulsant, B. H., Fitzgerald, P. B., & Daskalakis, Z. J. (2018a). Bilateral repetitive transcranial magnetic stimulation decreases suicidal ideation in depression. *Journal of Clinical Psychiatry*, *79*(3). https://doi.org/10.4088/JCP.17m11692

Weissman, C. R., Blumberger, D. M., Brown, P. E., Isserles, M., Rajji, T. K., Downar, J., Mulsant, B. H., Fitzgerald, P. B., & Daskalakis, Z. J. (2018b). Bilateral repetitive transcranial magnetic stimulation decreases suicidal ideation in depression. *Journal of Clinical Psychiatry*, *79*(3). https://doi.org/10.4088/JCP.17m11692

Williams, K., Brignell, A., Randall, M., Silove, N., & Hazell, P. (2013). Selective serotonin reuptake inhibitors (SSRIs) for autism spectrum disorders (ASD). *Cochrane Database Systematic Review*, (*8*), CD004677. https://doi.org/10.1002/14651858.CD004677.pub3

Xu, Y., Zhang, Y., Zhao, D., Tian, Y., & Yuan, T. F. (2023). Growing placebo response in TMS treatment for depression: A meta-analysis of 27-year randomized sham-controlled trials. *Nature Mental Health*, *1*(10), 792–809. https://doi.org/10.1038/s44220-023-00118-9

Zemplenyi, A., Jozwiak-Hagymasy, J., Kovacs, S., Erdosi, D., Boncz, I., Tenyi, T., Osvath, P., & Voros, V. (2022). Repetitive transcranial magnetic stimulation may be a cost-effective alternative to antidepressant therapy after two treatment failures in patients with major depressive disorder. *Bmc Psychiatry*, *22*(1), 437. https://doi.org/10.1186/s12888-022-04078-9

Zheng, S., Kim, H., Salzman, E., Ankenman, K., & Bent, S. (2021). Improving social knowledge and skills among adolescents with autism: Systematic review and meta-analysis of UCLA PEERS® for adolescents. *Journal of Autism and Developmental Disorders*, *51*(12), 4488–4503. https://doi.org/10.1007/s10803-021-04885-1

Adverse childhood experiences in children with autism spectrum disorder

Daniel W. Hoover[a,b] *and Joan Kaufman*[a,b]

Purpose of review
Recent years have shown an uptick in studies assessing bullying and other adverse childhood experiences (ACEs) in children with autism spectrum disorder (ASD). This article reviews extant findings, and points to gaps in the literature.

Recent findings
Children with ASD are bullied by peers at a rate three to four times that of nondisabled peers with negative impacts on academic functioning and mental health symptoms, including increased risk for suicidality. Children with ASD are also at enhanced risk for other ACES, particularly parental divorce and income insufficiency, and as observed in the general population, children with ASD who experience an increased number of ACES are at elevated risk for comorbid psychiatric and medical health problems. Children with ASD with an elevated number of ACES also experience a delay in ASD diagnosis and treatment initiation. There is no evidence of increased risk of child maltreatment within the ASD population.

Summary
As bullying and other adverse experiences are common and associated with deleterious outcomes in children with ASD, there is a need for additional research on intervention strategies to prevent and mitigate the impact of these experiences. Ongoing work on the assessment of trauma experiences and PTSD symptoms in children on the spectrum is also needed.

Keywords
adverse childhood experiences, autism spectrum disorders, bullying

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disability characterized by impaired communication, social reciprocity, and rigid, repetitive behaviors [1]. The disorder is increasingly diagnosed, with an estimated 1 in 68 children affected [2]. This article reviews recent studies examining the rates of bullying, other adverse childhood experiences (ACEs), and reports of maltreatment among children with ASD. Issues of differential diagnosis with reactive attachment disorder are also discussed, together with directions for future research on the assessment and treatment of children with ASD who are exposed to trauma and other childhood adversities.

TYPES OF ADVERSE EXPERIENCES REPORTED IN CHILDREN WITH AUTISM SPECTRUM DISORDER

Bullying

Children with ASD are bullied more often than peers with other disabilities, their own nondisabled siblings [3,4], and those with intellectual disabilities alone [5]. In an international review of 17 studies of school bullying, Maïano *et al.* [6▪] reported children with ASD are bullied at a rate three times that of typically developing children. Physical, verbal, and relational school bullying (e.g. trying to hurt a peer and/or that peer's standing within a particular peer group) were reported in 33, 50, and 31% of ASD students, respectively. Bullying occurs both in and outside of special education settings, but is more likely in mainstream classrooms and unstructured areas such as the school bus. In addition, children

[a]Center for Child and Family Traumatic Stress, Kennedy Krieger Institute and [b]Department of Psychiatry and Behavioral Sciences, Johns Hopkins School of Medicine, Baltimore, Maryland, USA

Correspondence to Daniel W. Hoover, PhD, Center for Child and Family Traumatic Stress, Kennedy Krieger Institute, 1750 E. Fairmount Ave., Baltimore, MD 21231, USA. E-mail: hooverd@kennedykrieger.org.

Curr Opin Psychiatry 2018, 31:128–132
DOI:10.1097/YCO.0000000000000390

KEY POINTS

- Children with ASD are bullied at a rate three to four times that of nondisabled youth, with bullying associated with negative effects on children's academic functioning and mental health, including an increased risk for suicidality.
- Adverse childhood experiences (ACEs) are reported more frequently by families of children with autism, particularly experiences of parental divorce and income insufficiency.
- Extant studies do not show increased risk of child maltreatment in the ASD population.
- ACE exposure is associated with increased risk of comorbid mental health and medical problems, and later diagnosis and initiation of treatment in youth with ASD.

with limited social supports and less parent involvement are at the greatest risk for bullying [7▪].

In our clinical experience, incidents of physical victimization can be severe, and in extreme cases result in the need for emergency treatment medical care. Subsets of children that we see in our clinic have developed posttraumatic stress disorder (PTSD) secondary to these assaults. The prevalence of PTSD in ASD populations, however, has been little studied, with only 2 out of 86 studies assessing anxiety disorders in children with ASD including an assessment of PTSD [8].

Peer victimization of children with ASD has been shown to have serious negative impacts on children's academic and social functioning [9▪]. Mayes *et al.* [10] also found ASD youth who were teased were three times more likely than nonteased ASD youth to report suicidal ideation or to make a suicide attempt. Although youth with ASD are most often the victims of bullying, they have also been reported to become perpetrators of bullying violence [11].

Antibullying interventions have proliferated in recent years with a recent meta-analysis of 14 randomized controlled trials involving over 30 000 students demonstrating these interventions have moderate effects on reducing peer victimization rates in the schools [12]. None of these broad interventions, however, have focused on children with ASD. To date there has only been one small ($N = 3$) pilot antibullying intervention investigated with youth with ASD [13]. The pilot study suggests peer networks are a promising strategy for increasing youth's social interactions and reducing rates of bullying victimization of secondary students with ASD, but more work is needed in this area.

Adverse childhood experiences

Adults in the general population who report a range of adverse experiences in childhood have been shown to have poorer long-term health and mental health outcomes [14]. ACE are nonspecific risk factors for multiple psychiatric disorders, and several health risk behaviors, including smoking, overeating, and excessive alcohol and drug use. Above and beyond the effect of these health risk behaviors, ACE have been found to predict a multitude of medical health problems later in life, including: ischemic heart disease, stroke, respiratory problems, diabetes, and even cancer. In general, exposure to four or more ACEs is an established threshold for poor health, whereas those with one to three adverse experiences do not fare as well as those with none.

Several investigators have examined the number and effects of ACEs on children with ASD by analyzing data from the 2011–2012 US National Survey of Children's Health ($N = 95\,677$) [15▪]. Figure 1 lists the ACE questions included in this survey. Children with ASD were found to have experienced more ACEs than healthy control peers, with increased rates of the following ACES reported: income insufficiency (ASD = 40%, healthy control = 23%), parental divorce (ASD, 28%; healthy control, 20%), neighborhood violence (ASD, 11%; healthy control, 8%), and household mental health (ASD, 18%; healthy control, 7%) and/or substance use (ASD, 14%; healthy control, 10%) problems. Children with ASD were also twice as likely as the healthy control peers to have experienced four or more ACEs (10.2 versus 5.1%).

The effects of ACEs on timing of ASD diagnoses and receipt of therapies were also assessed using data from the 2011–2012 National Survey of Children's Health [16]. Compared with children without ACEs, the adjusted effects of one to two and at least three ACEs resulted in prolonged time to diagnoses, with children with no, one to two, and three or more ACEs diagnosed at a mean age of 4.3, 5.2, and 5.7 years, respectively. Report of one to two and at least three ACEs were also associated with a 22 and 27% increase in the median age of entry into services. As early and sustained intervention for children with ASD is associated with the best prognosis, the delay in diagnosis and initiation of treatment interventions associated with the presence of ACEs is clinically meaningful [17].

The effects of ACEs on comorbid psychiatric and medical health problems were also assessed using data from the 2011–2012 National Survey of Children's Health [18▪]. Consistent with research in the field with nondevelopmental disability populations, among children with ASD, an increased

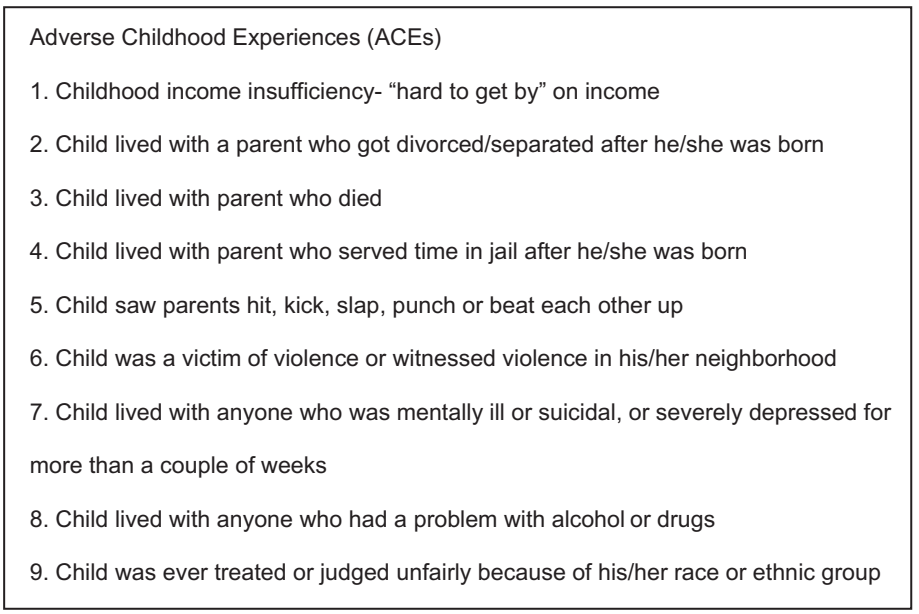


FIGURE 1. Adverse Childhood Experiences (ACE) Questions. The items included in the ACE survey of the National Survey of Children's Health and discussed in the text, are depicted. Data from source: National Center for Health Statistics, Maternal and Child Health Bureau. National Survey of Children's Health. Data Resource for Child and Adolescent Health, 2011/12. Available from: http://www.childhealthdata.org/learn/NSCH.

number of ACEs was associated with elevated risk for depression, anxiety, and a number of medical health problems.

Little research has been conducted to date in examining rates of ACE in ASD clinical samples, and interventions to address ACE and mitigate their negative effects have yet to be evaluated.

Child maltreatment

In a large-scale study ($N = 9536$), which linked child protective services (CPS) data with school data to determine rates of children with ASD who were referred to CPS because of suspicions of abuse and neglect, children with ASD were found to constitute 1.7% of the referrals [19▪]. This rate is consistent with the population prevalence for ASD and suggests children with ASD are not over-represented in the CPS system. A population-based record-linkage study of all children born in Western Australia between 1990 and 2010 ($N = 524\,534$) reported similar results, with youth with ASD having the same risk for allegations of maltreatment as children without disabilities.

In a sample of youth with ASD who were admitted to psychiatric hospitals ($N = 350$), Brenner *et al.* [20▪] found that 28% of the youth were reported to have experienced maltreatment by caregivers. This rate is about half the rate reported for psychiatrically hospitalized youth overall [21], again suggesting that ASD is not associated with an increased risk for child maltreatment.

The inpatient youth with ASD and reports of child maltreatment experienced typical trauma-related symptoms, including intrusive thoughts, distressing memories, irritability, and depressive affect, however, only 7% met full diagnostic criteria for PTSD [20▪]. Rates of PTSD among youth with ASD have also been assessed in two outpatient cohorts. One study ($N = 94$) reported no children with comorbid PTSD [22], and the other study ($N = 69$) reported 17% of the children with ASD met diagnostic criteria for PTSD [23]. This latter study is the only investigation to utilize child and parent report of PTSD symptoms; the other studies relied exclusively on parent report which may have contributed to the low rate of diagnosis in these studies.

There are no published assessment tools designed specifically to assess trauma experiences and symptoms in children with ASD. Particularly lacking at this point are well validated self-report measures that would be appealing, engaging, able to hold children's attention, and present material through more than one modality (e.g. visual, auditory, touch) to allow accessibility by individuals at different functional levels. We are currently developing an interactive app that can be used with children on the spectrum that appears to be promising in assessing trauma experiences and symptoms in children with ASD with borderline and higher IQs. Ongoing work on the assessment of trauma experiences and PTSD symptoms in children on the spectrum is needed.

DIFFERENTIAL DIAGNOSIS WITH REACTIVE ATTACHMENT DISORDER

Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) and International Statistical Classification of Diseases (ICD)-10 diagnostic criteria for reactive attachment disorder (RAD) rule out the diagnosis in cases where ASD is present. Autism is seen as qualitatively different from RAD and differentially diagnosed based on ASD-related restricted interests and ritualized behavior and marked social communications deficits [24]. Recent studies, however, show that children can meet diagnostic criteria for both disorders [10,25], and a significant subset of children with histories of institutional rearing and severe neglect present with what has been termed 'quasi-autism,' core autism features that resolve by age 11 in a quarter of the children with this diagnostic designation [26]. It is hypothesized that ASD and or quasi-autism clinical presentations may be overrepresented in samples of children adopted following neglect, abuse, or placement disruption because of prenatal, genetic, and family risk factors.

In a recent study with 58 children with autism and no known history of maltreatment and 67 children with RAD [27], the two groups could not be distinguished on most of the features of the inhibited subtype of RAD. Both groups avoided eye contact (RAD, 58%; ASD, 66%), displayed frozen watchfulness (RAD, 18%; ASD, 12%), and displayed unpredictable behavior upon reunion with their caregiver (RAD, 18%; ASD, 12%). The children in the RAD group, however, were more likely to show hypervigilance (RAD, 39%; ASD, 19%). Children with RAD and ASD, however, differed significantly on all the core features of disinhibited attachment disorder, including: cuddliness with strangers (RAD, 45%; ASD, 14%), indiscriminate adult relationships (RAD, 55%; ASD, 10%), comfort seeking from strangers (RAD, 20%; ASD, 0%), minimal referencing of the caregiver (RAD, 48%; ASD, 28%), and attention-seeking behaviors (RAD, 76%; ASD, 26%).

Thus, the extant literature suggests that ASD and attachment disorders are not mutually exclusive, and can be differentiated based on child and family history, developmental status, the presence or absence of cardinal ASD features, and the presence or absence of hypervigilance and disinhibited attachment symptoms.

Cognitive behavioral treatment

Trauma-Focused Cognitive Behavior Therapy (TF-CBT) is the psychotherapeutic intervention with the strongest empirical support for PTSD and other trauma-related symptoms in children and adolescents [28]. Cognitive behavioral therapy (CBT) has recently been adapted for treating comorbid anxiety disorders in ASD, with a randomized controlled trial of its effectiveness currently underway [29]. In a meta-analysis of CBT treatment studies for affective disorders with children on the autism spectrum, Weston *et al.* [30] found small-to-medium effect size ($g = 0.24$) on self-report measures, a significant medium effect size ($g = 0.66$) for informant-report measures, and a significant medium effect size ($g = 0.73$) for clinician-report measures of depression. We have developed adaptations for using TF-CBT with children with ASD and comorbid trauma-related psychopathology [31,32], but to date there have been no controlled studies of TF-CBT in this population.

CONCLUSION AND CLINICAL IMPLICATIONS

Children with ASD are bullied by peers at a rate of three to four times that of nondisabled peers with negative impacts on academic functioning and mental health symptoms, including increased risk for suicidality. Children with ASD are also at enhanced risk for ACES, particularly parental divorce and income insufficiency, and as observed in the general population, children with ASD who experience an increased number of ACES are at elevated risk for comorbid psychiatric and medical health problems. There is no evidence of increased risk of child maltreatment within the ASD population.

It is recommended that a thorough assessment of adverse childhood experiences and other potentially traumatic events be integrated in clinical evaluations of children with ASD [33▪]. The assessment and treatment of children with ASD exposed to trauma and other forms of adversity remains practically un-addressed in the literature. Going forward, it will be crucial to establish sensitive measures for detecting exposure and responses to trauma in order to inform both research and clinical practice.

Acknowledgements

None.

Financial support and sponsorship

This work was supported by a grant from the Substance Abuse and Mental Health Services Administration SM080003-01, and the National Institute of Health RO1 MH098073.

Conflicts of interest.

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

1. APA. Diagnostic and statistical manual of mental disorders: DSM-5™. 5th ed. Arlington, Virginiaa, USA: American Psychiatric Publishing, Inc; 2013.
2. Centers for Disease Control and Prevention. Prevalence of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 sites, United States, 2010. Morb Mortal Wkly Rep Surveill Summ 2014; 63:1–13.
3. Nowell KP, Brewton CM, Goin-Kochel R. A multirater study on being teased among children/adolescents with autism spectrum disorder (ASD) and their typically developing siblings: associations with ASD symptoms. Focus Autism Other Dev Disabil 2014; 29:195–205.
4. Sreckovic MA, Brunsting NC, Able H. Victimization of students with autism spectrum disorder: a review of prevalence and risk factors. Res Autism Spectr Disord 2014; 8:1155–1172.
5. Zeedyk SM, Rodriguez G, Tipton LA, *et al.* Bullying of youth with autism spectrum disorder, intellectual disability, or typical development: victim and parent perspectives. Res Autism Spectr Disord 2014; 8:1173–1183.
6. ■ Maïano C, Normand CL, Salvas MC, *et al.* Prevalence of school bullying among youth with autism spectrum disorders: a systematic review and meta-analysis. Autism Res 2016; 9:601–615.

A meta-analysis of 17 studies resulting in estimated prevalence of for physical, verbal, and relational school victimization of 33, 50, and 31%, respectively.

7. ■ Hebron J, Oldfield J, Humphrey N. Cumulative risk effects in the bullying of children and young people with autism spectrum conditions. Autism 2017; 21:291–300.

Factors that increase risk for bullying among youth with ASD include: individual factors –behavioral difficulties, poor peer relationships, and contextual factors – taking bus to school, being mainstreamed. Parent engagement decreases risk.

8. Kerns CM, Newschaffer CJ, Berkowitz SJ. Traumatic childhood events and autism spectrum disorder. J Autism Dev Disord 2015; 45:3475–3486.
9. ■ Adams R, Taylor J, Duncan A, *et al.* Peer victimization and educational outcomes in mainstreamed adolescents with autism spectrum disorder (ASD). J Autism Dev Disord 2016; 46:3557–3566.

Peer victimization negatively associated with a variety of educational outcomes.

10. Mayes SD, Calhoun SL, Waschbusch DA, Baweja R. Autism and reactive attachment/disinhibited social engagement disorders: Co-occurrence and differentiation. Clin Child Psychol Psychiatry 2016; 22:620–631.
11. Schrooten I, Scholte RHJ, Cillessen AHN, Hymel S. Participant roles in bullying among Dutch adolescents with autism spectrum disorders. J Clin Child Adolesc Psychol 2016; 53:1–14.
12. Jiménez-Barbero JA, Ruis Hernandez JA, Lior-Zaragoza L, *et al.* Effectiveness of antibullying school programs: a meta-analysis. Child Youth Serv Rev 2016; 61:165–175; Results show moderate effect sizes for the outcome measures bullying frequency. Studies of universal interventions, not programs developed specifically for children with ASD.
13. Sreckovic MA, Hume K, Able H. Examining the efficacy of peer network interventions on the social interactions of high school students with autism spectrum disorder. J Autism Dev Disord 2017; 47:2556–2574.
14. Felitti VJ, Anda RF, Nordenberg D, *et al.* Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults. The Adverse Childhood Experiences (ACE) Study. Am J Prev Med 1998; 14:245–258.
15. ■ Berg KL, Shiu CS, Acharya K, *et al.* Disparities in adversity among children with autism spectrum disorder: a population-based study. Dev Med Child Neurol 2016; 58:1124–1131.

ASD status among children was significantly associated with higher probability of reporting one to three ACEs.

16. Berg KL, Acharya K, Shiu CS, Msall ME. Delayed diagnosis and treatment among children with autism who experience adversity. J Autism Dev Disord 2017. [Epub ahead of print]
17. Volkmar F, Siegel M, Woodbury-Smith M, *et al.,* American Academy of Child and Adolescent Psychiatry (AACAP) Committee on Quality Issues (CQI). Practice parameter for the assessment and treatment of children and adolescents with autism spectrum disorder. J Am Acad Child Adolesc Psychiatry 2014; 53:237–257.
18. ■ Rigles B. The relationship between adverse childhood events, resiliency and health among children with autism. J Autism Dev Disord 2017; 47:187–202.

ACEs associated with increasd risk for depression, anxiety, and medical health problems.

19. ■ Hall-Lande J, Hewitt A, Mishra S, *et al.* Involvement of children with autism spectrum disorder (ASD) in the child protection system. Focus Autism Dev Disabil 2015; 30:237–248.

Data on 9536 children with an accepted case of alleged maltreatment linked with school records data to identify children with ASD and other disabilities. Prevalence of ASD in protective services comparable with population rates of ASD.

20. ■ Brenner J, Pan Z, Mazefsky C, *et al.* Behavioral symptoms of reported abuse in children and adolescents with autism spectrum disorder in inpatient settings. J Autism Dev Disord 2017. [Epub ahead of print]

A small proportion of youth with ASD and histories of child maltreatment (7%) met full diagnostic criteria for PTSD.

21. Boxer P, Terranova AM. Effects of multiple maltreatment experiences among psychiatrically hospitalized youth. Child Abuse Negl 2008; 32: 637–647.
22. de Bruin EI, Ferdinand RF, Meester S, *et al.* High rates of psychiatric comorbidity in PDD-NOS. J Autism Dev Disord 2007; 37:877–886.
23. Mehtar M, Mukaddes NM. Posttraumatic stress disorder in individuals with diagnosis of autistic spectrum disorders. Res Autism Spectr Disord 2011; 5:539–546.
24. APA. Diagnostic and statistical manual of mental disorders, fifth edition: DSM-5. Washington, D.C: American Psychiatric Association; 2013.
25. Green J, Leadbitter K, Kay C, *et al.* Autism spectrum disorder in children adopted after early care breakdown. J Autism Dev Disord 2016; 46:1392–1402.
26. Rutter M, Kreppner J, Croft C, *et al.* Early adolescent outcomes of institutionally deprived and nondeprived adoptees. III. Quasi-autism. J Child Psychol Psychiatry 2007; 48:1200–1207.
27. Davidson C, *et al.* Social relationship difficulties in autism and reactive attachment disorder: improving diagnostic validity through structured assessment. Res Dev Disabil 2015; 40:63–72.
28. Cohen JA, Mannarino AP. Trauma-focused cognitive behavior therapy for traumatized children and families. Child Adolesc Psychiatr Clin N America 2015; 24:557–570.
29. Kerns CM, Wood JJ, Kendall PC, *et al.* The treatment of anxiety in autism spectrum disorder (TAASD) study: rationale, design and methods. J Child Fam Stud 2016; 25:1889–1902.
30. Weston L, Hodgekins J, Langdon PE. Effectiveness of cognitive behavioural therapy with people who have autistic spectrum disorders: a systematic review and meta-analysis. Clin Psychol Rev 2016; 49:41–54.
31. Hoover DW, Gasior CC. Trauma focused cognitive behavior therapy for autism spectrum disorders: a treatment manual. Baltimore, Maryland: Kennedy Krieger Institute; 2017.
32. D'Amico P, Hoover D, Mannarino A. Adapting trauma-informed therapy for children with developmental disabilities. Washington, DC: National Child Traumatic Stress Network; 2017.
33. ■ Hoover DW. The effects of psychological trauma on children with autism spectrum disorders: a research review. Rev J Autism Dev Disord 2015; 2:287–299.

Review article describing studies assessing impact of various traumas including bullying, on ASD child adjustment and symptoms.

Psychological Medicine

cambridge.org/psm

Association between cannabis use disorder and schizophrenia stronger in young males than in females

Carsten Hjorthøj[1,2], Wilson Compton[3], Marie Starzer[1], Dorte Nordholm[1], Emily Einstein[3], Annette Erlangsen[1,4,5,6], Merete Nordentoft[1,7], Nora D. Volkow[3] and Beth Han[3]

[1]Copenhagen Research Center for Mental Health – CORE, Mental Health Center Copenhagen, Copenhagen University Hospital, Copenhagen, Denmark; [2]Department of Public Health, University of Copenhagen, Section of Epidemiology, Copenhagen, Denmark; [3]National Institute on Drug Abuse, National Institutes of Health, Bethesda, USA; [4]Danish Research Institute for Suicide Prevention, Mental Health Centre Copenhagen, Copenhagen, Denmark; [5]Department of Mental Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA; [6]Centre for Mental Health Research, Research School of Population Health, The Australian National University, Canberra, Australia and [7]Department of Clinical Medicine, University of Copenhagen, Faculty of Health and Medical Sciences, Copenhagen, Denmark

Original Article

Cite this article: Hjorthøj C, Compton W, Starzer M, Nordholm D, Einstein E, Erlangsen A, Nordentoft M, Volkow ND, Han B (2023). Association between cannabis use disorder and schizophrenia stronger in young males than in females. *Psychological Medicine* **53**, 7322–7328. https://doi.org/10.1017/S0033291723000880

Received: 19 December 2022
Revised: 23 March 2023
Accepted: 23 March 2023
First published online: 4 May 2023

Keywords:
Cannabis; epidemiology; gender; psychosis; schizophrenia; sex differences; sex

Corresponding author:
Carsten Hjorthøj,
E-mail: carsten.hjorthoej@regionh.dk

Abstract

Background. Previous research suggests an increase in schizophrenia population attributable risk fraction (PARF) for cannabis use disorder (CUD). However, sex and age variations in CUD and schizophrenia suggest the importance of examining differences in PARFs in sex and age subgroups.

Methods. We conducted a nationwide Danish register-based cohort study including all individuals aged 16–49 at some point during 1972–2021. CUD and schizophrenia status was obtained from the registers. Hazard ratios (HR), incidence risk ratios (IRR), and PARFs were estimated. Joinpoint analyses were applied to sex-specific PARFs.

Results. We examined 6 907 859 individuals with 45 327 cases of incident schizophrenia during follow-up across 129 521 260 person-years. The overall adjusted HR (aHR) for CUD on schizophrenia was slightly higher among males (aHR = 2.42, 95% CI 2.33–2.52) than females (aHR = 2.02, 95% CI 1.89–2.17); however, among 16–20-year-olds, the adjusted IRR (aIRR) for males was more than twice that for females (males: aIRR = 3.84, 95% CI 3.43–4.29; females: aIRR = 1.81, 95% CI 1.53–2.15). During 1972–2021, the annual average percentage change in PARFs for CUD in schizophrenia incidence was 4.8 among males (95% CI 4.3–5.3; $p < 0.0001$) and 3.2 among females (95% CI 2.5–3.8; $p < 0.0001$). In 2021, among males, PARF was 15%; among females, it was around 4%.

Conclusions. Young males might be particularly susceptible to the effects of cannabis on schizophrenia. At a population level, assuming causality, one-fifth of cases of schizophrenia among young males might be prevented by averting CUD. Results highlight the importance of early detection and treatment of CUD and policy decisions regarding cannabis use and access, particularly for 16–25-year-olds.

Introduction

Cannabis is among the most frequently used psychoactive substance in the world, and laws restricting cannabis use have been liberalized over the past 20 years (Compton, Han, Jones, Blanco, & Hughes, 2016). Based on the World Health Organization's 2021 World Drug Report, approximately 200 million people in the world used cannabis in 2019 (UNODC, 2021). Moreover, cannabis potency measured by the percentage of delta-9-tetrahydrocannabinol (THC) (main psychoactive component of cannabis) has increased dramatically, e.g. from −10% in 2009 to 14% in 2019 in the USA (ElSohly, Chandra, Radwan, Majumdar, & Church, 2021); and from 13% in 2006 to 30% in 2016 in Denmark (Freeman et al., 2019). Consistently, the prevalence of cannabis use disorder (CUD) has increased markedly. For example, past-year CUD rose significantly from 4.9% in 2014 to 5.9% in 2018 among US 18–25-year-olds.

THC may trigger and/or worsen schizophrenia, especially for those with a CUD or with regular and high THC use (Marconi, Di Forti, Lewis, Murray, & Vassos, 2016; Petrilli et al., 2022; Volkow et al., 2016). For example, in Denmark, the incidence of schizophrenia steadily increased from 2000 to 2012 (Kühl, Laursen, Thorup, & Nordentoft, 2016), and the schizophrenia population attributable risk fraction (PARF) for CUD increased three- to fourfold over the past two decades, parallel to increases in THC concentration (Hjorthøj, Posselt, & Nordentoft, 2021). The increased THC content may thus, along a potential increase in the

prevalence of CUD, be a main driver of the population-level increase in PARF between CUD and schizophrenia.

A growing body of evidence suggests that the relationship between CUD and schizophrenia may differ by sex (Arranz et al., 2015; Crocker & Tibbo, 2018). Male sex (Arranz et al., 2015; Veen et al., 2004) and early heavy or frequent cannabis use are associated with earlier onset of psychosis (Han, Compton, Einstein, & Volkow, 2021; Large, Sharma, Compton, Slade, & Nielssen, 2011). Although it has not been shown that there are sex differences in age of first cannabis use (Crane, Schuster, Mermelstein, & Gonzalez, 2015; SAMHSA, 2019), younger age of CUD onset was found in males compared to females (Haberstick et al., 2014). Past-year prevalence of daily or near daily cannabis use and CUD were consistently higher in males than females among US adults aged 18–34 in each year during 2008–2019 (Han et al., 2021). The same was seen in Denmark where schizophrenia incidence rates were consistently higher in males than females among patients aged 19 or older in each year during 2000–2012 (Kühl et al., 2016).

It has been proposed that the higher incidence of schizophrenia among males than females could reflect the higher prevalence and quantity of consumption of cannabis use in males (Ochoa, Usall, Cobo, Labad, & Kulkarni, 2012; Sommer, Tiihonen, van Mourik, Tanskanen, & Taipale, 2020). Consequently, it is pivotal to understand whether and how incidence of schizophrenia attributable to CUD varies by sex and age.

Thus, based on nationwide Danish registers, this current study aimed to investigate:

1. Do the associations between CUD and schizophrenia vary by sex?
2. Do the sex differences in the associations between CUD and schizophrenia change over time and by age?
3. Does the proportion of schizophrenia cases attributable to CUD vary by sex?
4. Does the sex-specific proportion of schizophrenia cases attributable to CUD change over time and by age?

The results of this study may inform ongoing policy discussions on legalization and regulation of cannabis use and highlight the importance of targeted public health prevention and intervention efforts. Because males, especially those with CUD, often have worse schizophrenia treatment outcomes than their female counterparts (Abel, Drake, & Goldstein, 2010; Arranz et al., 2015), our results may also have implications beyond policy, underscoring the need for clinicians to proactively screen for and diagnose CUD and schizophrenia and deliver sex-specific, high-quality, and patient-centered care.

Methods

Data sources

We used the nationwide Danish registers, full-linkage of which is made possible through the unique identification number in the Civil Registration System (Pedersen, 2011). We included all people born before 31 December 2005, and who were alive and aged between 16 and 49 (both inclusive) at some point during 1972–2021.

Cannabis use disorder and schizophrenia

Information on psychiatric disorders was obtained from the Psychiatric Central Research Register and the psychiatric section of the National Patient Register, which contains information on all psychiatric inpatient treatments in Denmark since 1969, supplemented with all outpatient treatments since 1995 (Lynge, Sandegaard, & Rebolj, 2011; Mors, Perto, & Mortensen, 2011). Within these registers, schizophrenia was defined as ICD-8 codes 295.X (except 295.7) and ICD-10 codes F20.X. CUD was identified in the same registers and supplemented with the somatic part of the National Patient Register, defined as ICD-8 code 304.5 and ICD-10 code F12.X.

Other variables

We also included information on alcohol use disorder (AUD) and other types of substance use disorders, using the same registers as described above, using ICD-8 codes 291.X, 303.X, 571.0, and 304.X (except 304.5) and ICD-10 codes F1X.X (except F12.X, E24.4, E52, G31.2, G62.1, G72.1, K29.2, K70, K86.0, O35.4, Y57.3, Z50.2, Z50.3, Z71.4, Z71.5, Z72.1, and Z72.2). We included information on whether an individual had been diagnosed with any other psychiatric disorder (remaining ICD-8 codes from 290 to 315, and remaining ICD-10 codes in the F chapter). Sex was defined from the civil registration system registry, which will include the sex assigned at birth, except in the (in Denmark rather rare) cases in which a person legally changed their registered sex or gender in this registry. We also examined information on parental history of the same disorders and whether a person was Danish-born.

Statistical analyses

First, we conducted Cox proportional hazards regression analyses (with age as the underlying time-variable) in the full population, treating alcohol and specific drug use disorders, including CUD, as time-varying covariates, and included an interaction term between CUD and sex. If this interaction term was statistically significant, stratified multivariable Cox proportional hazards regression analyses (adjusting for the aforementioned potential confounders) by sex were conducted. We estimated PARFs overall and for males and females, separately, using the formula pd×([HR−1]/HR), with pd being the prevalence of CUD among cases developing schizophrenia.

Next, to examine trends in adjusted PARFs within each sex, we estimated PARFs for each study year through applying the fully adjusted Cox proportional hazards regression models by year, for males and females separately. To test the assumption of proportional hazards, we visually inspected both plots of Schoenfeld residuals and log-minus-log plots, neither indicating important deviations from this assumption.

Based on adjusted PARF results for each study year, joinpoint Regression Program (version 4.8.01) was used to test for significant changes in trends using Bayesian information criterion and to estimate average annual percentage changes in PARFs of CUD in schizophrenia during 1972–2021, which are valid even if the joinpoint models indicate changes in trends during this study period. In particular, joinpoint Regression Program takes trend data and fits the simplest joinpoint model that the data allow, starting with the minimum number of joinpoints (e.g. 0 joinpoint, which is a straight line) and testing whether more joinpoints are statistically significant and must be added to the model (up to that maximum number). This enables us to test whether a change in trend is statistically significant. The tests of significance use the Bayesian information criterion, which finds the model with the best fit for the data.

https://doi.org/10.1017/S0033291723000880 Published online by Cambridge University Press

Table 1. Characteristics of the study population overall and by sex, *N* (%)

	Overall	Males	Females
Male	3 531 266 (51.1%)	3 531 266 (100%)	–
Females	3 376 593 (48.9%)	–	3 376 593 (100%)
Foreign born	626 671 (9.1%)	315 723 (8.9%)	310 948 (9.2%)
Parental schizophrenia	30 103 (0.4%)	15 590 (0.4%)	14 513 (0.1%)
Other parental psychiatric disorder	835 738 (12.1%)	434 776 (12.3%)	400 962 (11.9%)
Parental alcohol or substance use disorder	639 845 (9.3%)	329 495 (9.3%)	310 350 (9.2%)
Cannabis use disorder	60 563 (0.9%)	45 322 (1.3%)	15 241 (0.5%)
Alcohol use disorder	398 430 (5.8%)	265 578 (7.5%)	132 852 (3.9%)
Other substance use disorder	233 512 (3.4%)	126 910 (3.6%)	106 602 (3.2%)
Other psychiatric disorder	869 274 (12.6%)	397 389 (11.3%)	471 885 (14.0%)

Data source: the nationwide Danish registers, full-linkage of which is made possible through the unique identification number in the Civil Registration System.

Finally, we applied Poisson regression and evaluated the potential three-way interaction effect among CUD, sex, and age (as a time-varying covariate, coded as ages 16–20, 21–25, 26–30, 31–40, ⩾41) on the incidence of schizophrenia. The analyses allowed an interaction with the underlying time-scale (age). Except for Joinpoint regression, all other analyses were conducted in Stata/MP, version 16.1 (StataCorp LLC). For each analysis, $p < 0.05$ (two-tailed) was considered statistically significant.

Results

This study examined 6 907 859 individuals and 45 327 cases of incident schizophrenia during a follow-up of 129 521 260 person-years at risk. Table 1 shows characteristics of the study population, and online Supplementary Fig. S1 shows the distribution of date of birth of the cohort. The unadjusted hazard ratio (HR) for people with CUD to be diagnosed with schizophrenia was 30.18 (95% CI 29.31–31.08; $p < 0.001$). However, after adjusting for potential confounding factors in the pooled model, this was reduced to an adjusted HR (aHR) = 2.31 (95% CI 2.24–2.40; $p < 0.001$), and a significant interaction effect between sex and CUD was identified ($p < 0.001$). Thus, stratified analyses by sex were conducted, showing that the overall aHR for CUD on schizophrenia was slightly higher ($p < 0.001$) among males (aHR = 2.42, 95% CI 2.33–2.52) than females (aHR = 2.02, 95% CI 1.89–2.17) (Table 2). Time since CUD and incident schizophrenia is depicted in online Supplementary Fig. S2.

Figure 1 shows the aHR for CUD on incident schizophrenia by year for males and females, separately. For males, the aHR increases gradually from ∼2 to ∼3, whereas for females, there is no such clear pattern. The corresponding aHR for AUD and other substance use disorder are shown in online Supplementary Figs S3 and S4, respectively. Note that for AUD, the estimates were not stable until 1994, which is consequently the first year included in online Supplementary Fig. S3. The lifetime prevalence of CUD for males and females is presented in online Supplementary Fig. S5.

Moreover, during 1972–2021, among males, the annual average percentage change in the PARFs of CUD on the incidence of schizophrenia was 4.8 (95% CI 4.3–5.3; $p < 0.0001$; no joinpoint identified); whereas among females, it was 3.2 (95% CI 2.5–3.8; $p < 0.0001$; no joinpoint identified) (Fig. 2). These results suggest that during 1972 throughout 2021, the annual average percentage change in the PARFs of CUD on the incidence of schizophrenia was consistently higher in males than in females ($p < 0.0001$). Assuming causality, approximately 15% of recent cases of schizophrenia among males in 2021 would have been prevented in the absence of CUD; by contrast, among females, 4% of recent cases

Table 2. Adjusted hazard ratios of cannabis use disorder CUD on schizophrenia by sex and adjusted incidence rate ratios of CUD on schizophrenia by sex and age group

	No CUD	Males with CUD adjusted hazard ratio (95% CI)	Females with CUD adjusted hazard ratio (95% CI)	*P* value for the sex difference
Overall	1 (ref.)	2.42 (2.33–2.52)	2.02 (1.89–2.17)	$p < 0.001$
By age group	No CUD	Males with CUD adjusted incident rate ratio (95% CI)	Females with CUD adjusted incident rate ratio (95% CI)	
16–20 years	1 (ref.)	3.84 (3.43–4.29)	1.81 (1.53–2.15)	$p < 0.001$
21–25 years	1 (ref.)	2.58 (2.38–2.79)	1.91 (1.27–1.64)	$p = 0.02$
26–30 years	1 (ref.)	2.33 (2.12–2.57)	2.08 (1.72–2.52)	$p = 0.70$
31–40 years	1 (ref.)	2.13 (1.94–2.34)	2.31 (1.92–2.78)	$p = 0.91$
41+ years	1 (ref.)	2.18 (1.87–2.54)	2.98 (2.32–3.84)	$p = 0.16$

Adjusted for alcohol use disorder (AUD), other substance use disorder (SUD), other psychiatric disorders, parental history of CUD, AUD, SUD, schizophrenia, or other psychiatric disorders, and whether a person was Danish-born.

https://doi.org/10.1017/S0033291723000880 Published online by Cambridge University Press

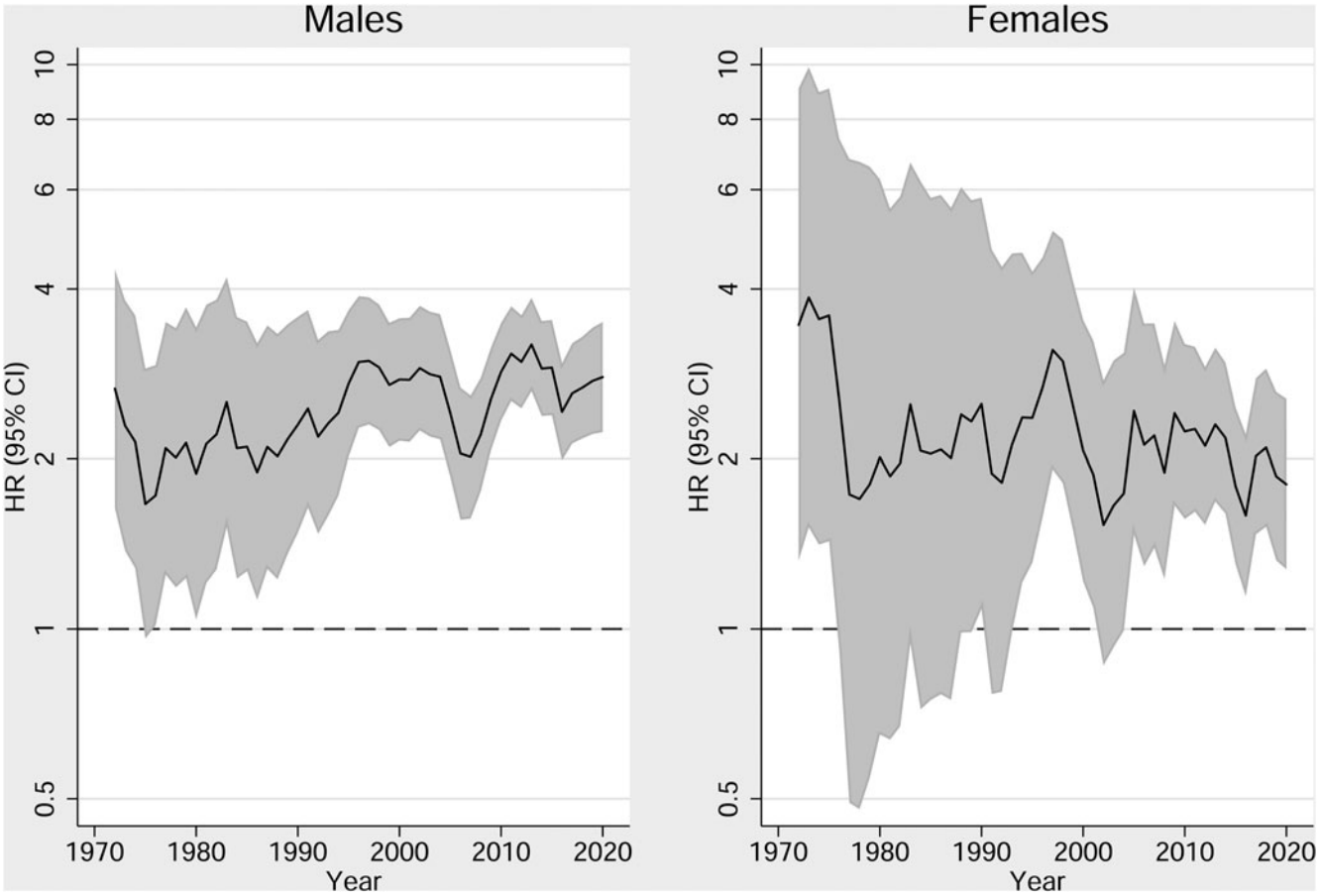


Figure 1. Rolling average of adjusted hazard ratios between cannabis use disorder and schizophrenia, by sex and calendar year.

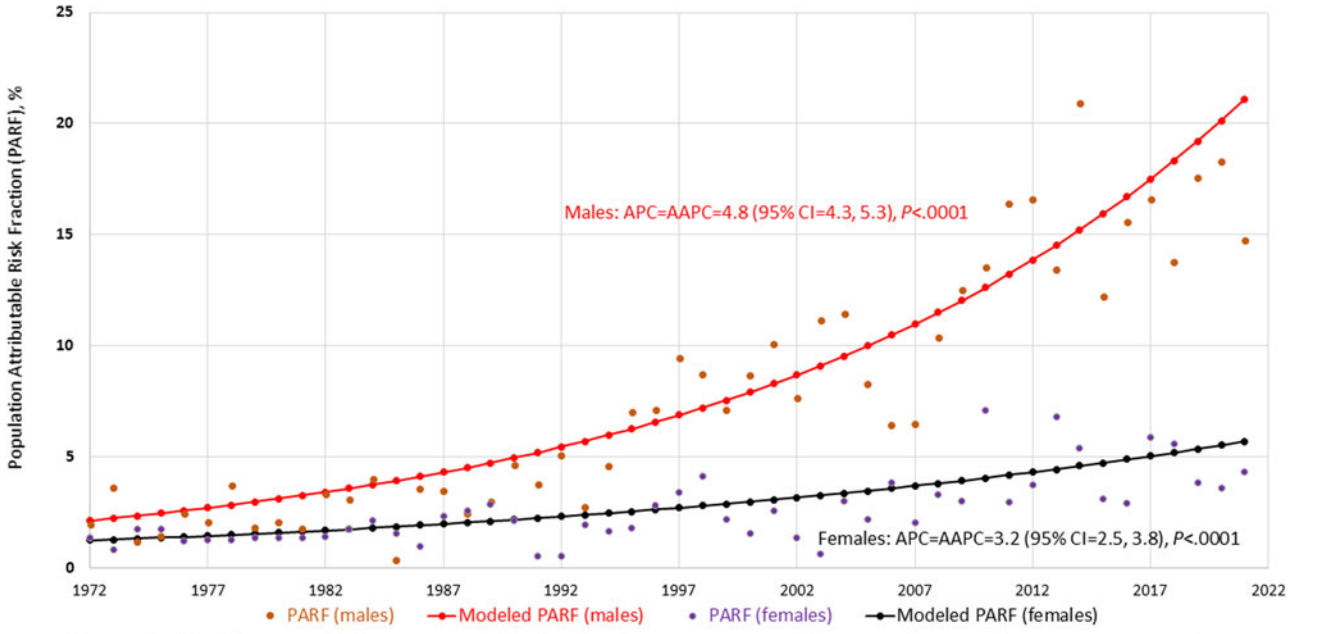


Figure 2. Trends in the proportion of schizophrenia attributable to cannabis use disorder in Denmark during 1972–2021, by sex.

https://doi.org/10.1017/S0033291723000880 Published online by Cambridge University Press

of schizophrenia would have been prevented if they did not have CUD.

In the fully adjusted Poisson regression model, a significant three-way interaction among CUD, age, and sex was identified ($p < 0.001$). Table 2 shows the incidence rate ratio (IRR) and 95% CI for each of the 10 age–sex combinations. For males, the highest adjusted incidence rate ratio (aIRR) of CUD on schizophrenia was observed in 16–20-year-olds (aIRR = 3.84, 95% CI 3.43–4.29), which was more than twice as high ($p < 0.001$) than their female similar-age peers (aIRR = 1.81, 95% CI 1.53–2.15). For males aged 21–25, adjusted IRR was 2.58 (95% CI 2.38–2.79), which was 1.4 times higher ($p = 0.02$) than their female similar-age peers [females aged 21–25: aIRR = 1.91 (95% CI 1.27–1.64)]. The association between CUD and schizophrenia was not statistically different between males and females for those aged ⩾26.

Trends in PARFs for the 10 age–sex combinations show very different patterns (Fig. 3). Among 16–20-year-old males, no clear pattern over time is observed, with PARFs generally fluctuating between 10% and 20%. In older males, the PARFs show a clearly increasing pattern, ending up around 20–30% until the age of 31–40 when PARFs fluctuate between <1% and nearly 20%. For females, the PARFs were not always estimable due to low numbers of exposed cases, but no clear association with time was observed, and with very few exceptions, PARFs for females were 10% or lower.

Discussion

In this nationwide, register-based cohort study, we found evidence of a stronger association between CUD and schizophrenia for males than for females, consistent with the results from a small clinical sample indicating that females experiencing CUD are at lower risk of developing psychosis than males (Arranz et al., 2015). Further, the stronger PARFs of CUD in schizophrenia for males than females consistently increased from 1972 to 2021. Under the assumption of causality, in 2021, approximately 15% of recent cases of schizophrenia among males would have been prevented in the absence of CUD, in contrast to 4% among females. For younger males, the proportion of preventable CUD-associated cases may be as high as 25% or even 30%. This increase in PARF is related to both increasing associations, likely caused by more potent cannabis, and increasing the prevalence of CUD with time.

The aHR for CUD on risk of schizophrenia were slightly higher for males than females, which might be misinterpreted to suggest an overall absence of strong sex-specific effects of cannabis, and instead indicate that the lower PARF for females is due to the fact that fewer females than males have CUD. Importantly, when subdividing the sample into specific age groups, a strong interaction effect among age, sex, and CUD on schizophrenia became evident. For 16–20-year-olds, the adjusted IRR for the association between CUD and schizophrenia was nearly twice as

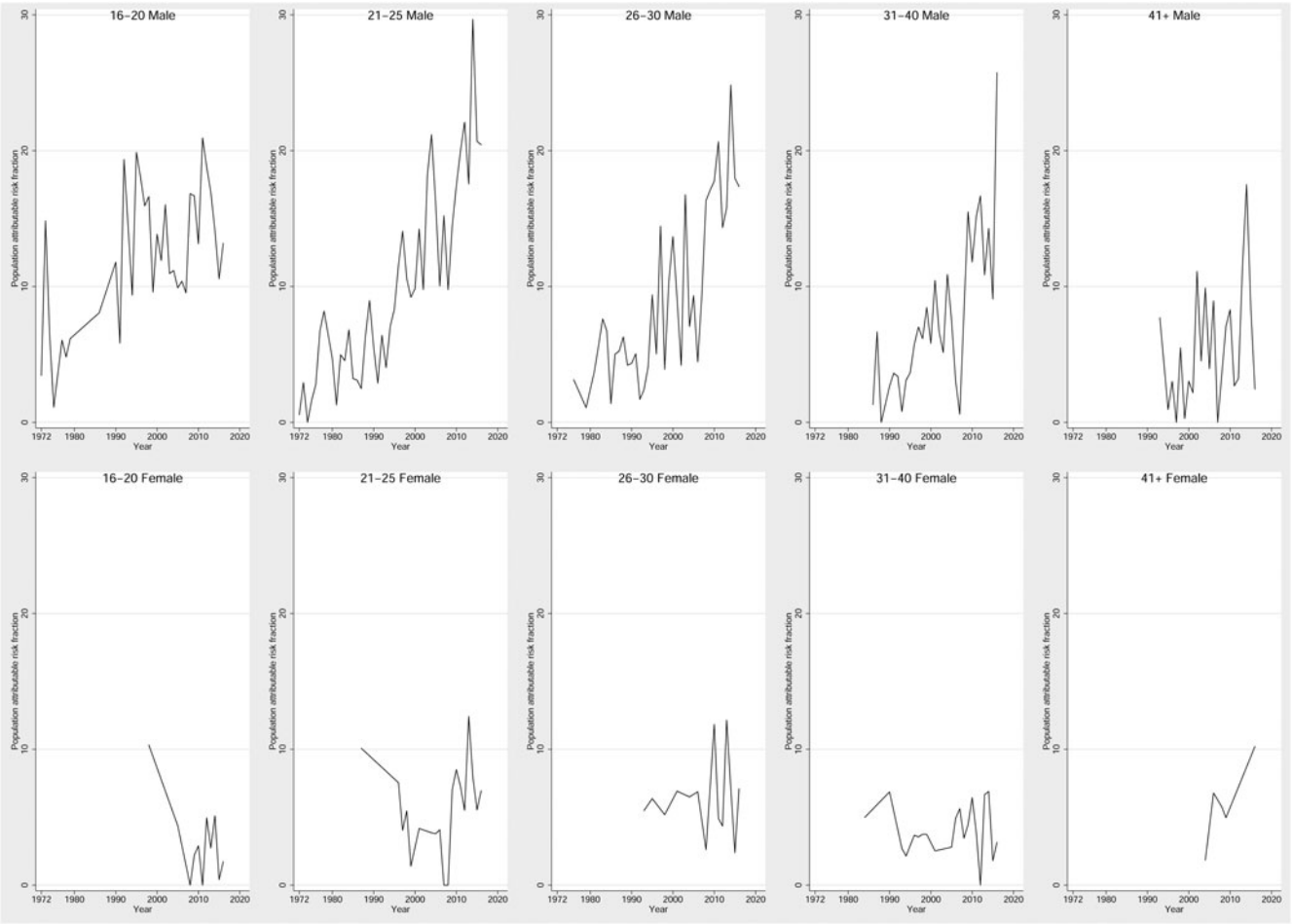


Figure 3. Trends in the proportion of schizophrenia attributable to cannabis use disorder in Denmark during 1972–2021, by sex and age.

https://doi.org/10.1017/S0033291723000880 Published online by Cambridge University Press

high for males than females; for 21–25-year-olds, the IRR was approximately 50% higher for males than females, whereas for those aged >26 years of age the IRRs was similar for males and females. Assuming causality, this suggests that young males compared to females of the same age might be more susceptible to the psychotogenic effects of cannabis on schizophrenia. Further research is needed to examine potential differences in THC concentration of exposures and frequency of cannabis consumption between young males and young females (Khan et al., 2013).

Previous studies have indicated that partial genetic confounding factors likely exist on the association between CUD and schizophrenia, i.e. genes shared between these conditions may account for some but not all of the association (Gillespie & Kendler, 2020). However, genetic confounding factors would be unlikely to explain the steeper increases in the PARFs of CUD on schizophrenia that we identified for males than females, as changes in the genetic risk-profile of an entire population would have to occur over generations. Both preclinical and clinical studies have provided evidence of significant differences between the sexes in response to the acute and long-term effects of cannabis (Cooper & Craft, 2018). We were able to adjust for alcohol and other specific drug use disorders in our study, but not for tobacco use or tobacco use disorder, which has also been linked to psychosis in some (Gurillo, Jauhar, Murray, & MacCabe, 2015) but not all studies (Fergusson, Hall, Boden, & Horwood, 2015). Thus, future research is needed to investigate the mechanisms underlying the higher vulnerability of young males to the effects of cannabis on schizophrenia than that of young females.

This study adds to the evidence suggesting a relationship between intense use of cannabis and risk of developing schizophrenia (Marconi et al., 2016; Urits et al., 2020; Volkow et al., 2016). At the individual level, this increased risk occurs in both sexes, but especially appears higher in young males. At the population level, this translates to CUD being a major modifiable risk factor for schizophrenia, particularly among males. Notably, an increasing proportion of cases with schizophrenia may be avertible by preventing CUD, and this increase is likely linked to the increase in THC concentration in cannabis as has been observed in confiscated samples in Denmark (Freeman et al., 2019; Thomsen et al., 2019). This apparent of schizophrenia conferred by CUD, in combination with observations that cannabis use among youth is associated with impaired cognition and reduced academic achievement (Lorenzetti, Hoch, & Hall, 2020), highlights the need to prevent cannabis use among youth and young adults. Interestingly, it has previously been shown that the association between cannabis and schizophrenia may be bidirectional (Ferdinand et al., 2005; Petersen, Toftdahl, Nordentoft, & Hjorthøj, 2019), and further investigation of the reverse association, schizophrenia being a risk factor for future cannabis use, by sex and over time warrants further study.

Our study has several strengths. The use of nationwide registers largely removes the risk of selection bias, since consent was not required for study sample participation. Furthermore, this data source reduces information bias to a certain degree, as a large range of information is available in the registers. Moreover, the registers are free from missing data in the traditional sense. Our results are likely highly generalizable to populations exposed to the same types of cannabis as are available on the Danish market. Finally, the national register-based nature of the longitudinal data over 5 decades allowed us to study nearly six million people, providing highly robust risk estimates.

This study also has certain limitations. The register-based nature of the study means that we only have information on both diagnosed CUD and diagnosed schizophrenia. This bias, however, is likely to be toward the null hypothesis, thus indicating that our results may be conservative, and PARFs of CUD on schizophrenia may be underestimated. Moreover, unmeasured and residual confounding factors likely exist, as the registers do not provide information on potentially important items such as frequency and amount of cannabis used, age of first use, or the THC content of cannabis products. Furthermore, we did not have access to genetic information, but as mentioned above, genetic confounding factors are unlikely to account for the observed differences. Finally, although the observational nature of this study does not directly allow for causal inference and we cannot be certain of the proportion of exposed individuals who might have developed schizophrenia even in the absence of CUD, it is unlikely that all of the associations between CUD and schizophrenia would be explained by confounding factors [e.g. tobacco use disorder (Fergusson et al., 2015; Gurillo et al., 2015)].

A further limitation is that the contents of the registers change over time. For instance, prior to 1995, outpatient psychiatric contacts were not included in the registers. Based on the results of our joinpoint regression analyses, there was no significant joinpoint identified in 1995 or years after 1995. It is thus unlikely to influence the increase in PARFs which only occurred later. We adjusted our models for other psychiatric disorders, which was the driver of the dramatic reduction in the magnitude of the HR compared to the unadjusted models. This is likely a case of over-adjustment, as some of these other psychiatric disorders might well be intermediate diagnoses between CUD and schizophrenia, and thus act as mediators rather than confounders. Consequently, our estimated aHR are likely highly conservative. Finally, we decided not to adjust for socioeconomic status, as this is more likely to be a product of CUD, other alcohol or substance use disorders, or schizophrenia, and thus not a potential confounder. By contrast, one of the salient factors is having a family history of schizophrenia. We controlled for the parental history of schizophrenia in our analyses. Moreover, we adjusted for age, sex, AUD, other substance use disorders, other psychiatric disorders, and parental history of CUD, AUD, and other psychiatric disorders, which are highly associated with schizophrenia.

In conclusion, this study finds strong evidence of an association between CUD and schizophrenia among both males and females, and the magnitude of this association appears to be consistently larger among males than females, especially among those aged 16–25. Importantly, 15% of cases of schizophrenia in males may be preventable if CUD was avoided. Although CUD is not responsible for most schizophrenia cases in Denmark, it appears to contribute to a non-negligible and steadily increasing proportion over the past five decades. In young males (21–30 years, possibly up to 40), the proportion may even be as high as 25–30%. There are global increases in legalization of nonmedical use of cannabis, increases in THC content of cannabis and in total THC doses consumed (Caulkins, Pardo, & Kilmer, 2020), increases in the prevalence of cannabis use and CUD, and decrease in public perception of harm from cannabis use (Chiu, Hall, Chan, Hides, & Leung, 2022). Alongside the increasing evidence that CUD is a modifiable risk factor for schizophrenia, our findings underscore the importance of evidence-based strategies to regulate cannabis use and to effectively prevent, screen for, and treat CUD as well as schizophrenia.

https://doi.org/10.1017/S0033291723000880 Published online by Cambridge University Press

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0033291723000880.

Financial support. This study received no funding.

Disclaimers. The findings and conclusions of this study are those of the authors and do not necessarily represent the official opinions of the National Institute on Drug Abuse, the National Institutes of Health, and the US Department of Health and Human Services.

References

Abel, K. M., Drake, R., & Goldstein, J. M. (2010). Sex differences in schizophrenia. *International Review of Psychiatry* (*Abingdon, England*), *22*(5), 417–428. https://doi.org/10.3109/09540261.2010.515205

Arranz, B., Safont, G., Corripio, I., Ramirez, N., Dueñas, R. M., Perez, V., … San, L. (2015). Substance use in patients with first-episode psychosis: Is gender relevant? *Journal of Dual Diagnosis*, *11*(3–4), 153–160. https://doi.org/10.1080/15504263.2015.1113761

Caulkins, J. P., Pardo, B., & Kilmer, B. (2020). Intensity of cannabis use: Findings from three online surveys. *The International Journal on Drug Policy*, *79*. https://doi.org/10.1016/J.DRUGPO.2020.102740

Chiu, V., Hall, W., Chan, G., Hides, L., & Leung, J. (2022). A systematic review of trends in US attitudes toward cannabis legalization. *57*(7), 1052–1061. https://doi.org/10.1080/10826084.2022.2063893

Compton, W. M., Han, B., Jones, C. M., Blanco, C., & Hughes, A. (2016). Marijuana use and use disorders in adults in the USA, 2002–14: Analysis of annual cross-sectional surveys. *The Lancet Psychiatry*, *3*(10), 954–964. https://doi.org/10.1016/S2215-0366(16)30208-5

Cooper, Z. D., & Craft, R. M. (2018). Sex-dependent effects of cannabis and cannabinoids: A translational perspective. *Neuropsychopharmacology*, *43*(1), 34–51. https://doi.org/10.1038/NPP.2017.140

Crane, N. A., Schuster, R. M., Mermelstein, R. J., & Gonzalez, R. (2015). Neuropsychological sex differences associated with age of initiated use among young adult cannabis users. *Journal of Clinical and Experimental Neuropsychology*, *37*(4), 389–401. https://doi.org/10.1080/13803395.2015.1020770

Crocker, C. E., & Tibbo, P. G. (2018). The interaction of gender and cannabis in early phase psychosis. *Schizophrenia Research*, *194*, 18–25. https://doi.org/10.1016/j.schres.2017.04.046

ElSohly, M. A., Chandra, S., Radwan, M., Majumdar, C. G., & Church, J. C. (2021). A comprehensive review of cannabis potency in the United States in the last decade. *Biological Psychiatry. Cognitive Neuroscience and Neuroimaging*, *6*(6), 603–606. https://doi.org/10.1016/J.BPSC.2020.12.016

Ferdinand, R. F., Sondeijker, F., van der Ende, J., Selten, J.-P., Huizink, A., & Verhulst, F. C. (2005). Cannabis use predicts future psychotic symptoms, and vice versa. *Addiction*, *100*(5), 612–618. https://doi.org/10.1111/j.1360-0443.2005.01070.x

Fergusson, D. M., Hall, W., Boden, J. M., & Horwood, L. J. (2015). Rethinking cigarette smoking, cannabis use, and psychosis. *The Lancet Psychiatry*, *2*(7), 581–582. https://doi.org/10.1016/S2215-0366(15)00208-4

Freeman, T. P., Groshkova, T., Cunningham, A., Sedefov, R., Griffiths, P., & Lynskey, M. T. (2019). Increasing potency and price of cannabis in Europe, 2006–16. *Addiction*, *114*(6), 1015–1023. https://doi.org/10.1111/add.14525

Gillespie, N. A., & Kendler, K. S. (2020). Use of genetically informed methods to clarify the nature of the association between cannabis use and risk for schizophrenia. In *JAMA Psychiatry*. American Medical Association. https://doi.org/10.1001/jamapsychiatry.2020.3564

Gurillo, P., Jauhar, S., Murray, R. M., & MacCabe, J. H. (2015). Does tobacco use cause psychosis? Systematic review and meta-analysis. *The Lancet Psychiatry*, *2*(8), 718–725. https://doi.org/10.1016/S2215-0366(15)00152-2

Haberstick, B. C., Young, S. E., Zeiger, J. S., Lessem, J. M., Hewitt, J. K., & Hopfer, C. J. (2014). Prevalence and correlates of alcohol and cannabis use disorders in the United States: Results from the national longitudinal study of adolescent health. *Drug and Alcohol Dependence*, *136*, 158–161. https://doi.org/10.1016/j.drugalcdep.2013.11.022

Han, B., Compton, W. M., Einstein, E. B., & Volkow, N. D. (2021). Associations of suicidality trends with cannabis use as a function of sex and depression status. *JAMA Network Open*, *4*(6), e2113025. https://doi.org/10.1001/jamanetworkopen.2021.13025

Hjorthøj, C., Posselt, C. M., & Nordentoft, M. (2021). Development over time of the population-attributable risk fraction for cannabis use disorder in schizophrenia in Denmark. *JAMA Psychiatry*, *78*(9), 1013–1019. https://doi.org/10.1001/JAMAPSYCHIATRY.2021.1471

Khan, S. S., Secades-Villa, R., Okuda, M., Wang, S., Pérez-Fuentes, G., Kerridge, B. T., & Blanco, C. (2013). Gender differences in cannabis use disorders: Results from the National Epidemiologic Survey of Alcohol and Related Conditions. *Drug and Alcohol Dependence*, *130*(1–3), 101–108. https://doi.org/10.1016/J.DRUGALCDEP.2012.10.015

Kühl, J. O. G., Laursen, T. M., Thorup, A., & Nordentoft, M. (2016). The incidence of schizophrenia and schizophrenia spectrum disorders in Denmark in the period 2000–2012. A register-based study. *Schizophrenia Research*, *176*(2–3), 533–539. https://doi.org/10.1016/j.schres.2016.06.023

Large, M., Sharma, S., Compton, M. T., Slade, T., & Nielssen, O. (2011). Cannabis use and earlier onset of psychosis: A systematic meta-analysis. *Archives of General Psychiatry*, *68*(6), 555–561. https://doi.org/10.1016/j.ypsy.2011.09.029

Lorenzetti, V., Hoch, E., & Hall, W. (2020). Adolescent cannabis use, cognition, brain health and educational outcomes: A review of the evidence. *European Neuropsychopharmacology*, *36*, 169–180. https://doi.org/10.1016/J.EURONEURO.2020.03.012

Lynge, E., Sandegaard, J. L., & Rebolj, M. (2011). The Danish national patient register. *Scandinavian Journal of Public Health*, *39*(7 Suppl), 30–33. https://doi.org/10.1177/1403494811401482

Marconi, A., Di Forti, M., Lewis, C. M., Murray, R. M., & Vassos, E. (2016). Meta-analysis of the association between the level of cannabis use and risk of psychosis. *Schizophrenia Bulletin*, *42*(5), 1262–1269. https://doi.org/10.1093/schbul/sbw003

Mors, O., Perto, G. P., & Mortensen, P. B. (2011). The Danish psychiatric central research register. *Scandinavian Journal of Public Health*, *39*(7 Suppl), 54–57. https://doi.org/10.1177/1403494810395825

Ochoa, S., Usall, J., Cobo, J., Labad, X., & Kulkarni, J. (2012). Gender differences in schizophrenia and first-episode psychosis: A comprehensive literature review. *Schizophrenia Research and Treatment*, *2012*, 916198. https://doi.org/10.1155/2012/916198

Pedersen, C. B. (2011). The Danish civil registration system. *Scandinavian Journal of Public Health*, *39*(7 suppl), 22–25. https://doi.org/10.1177/1403494810387965

Petersen, S. M., Toftdahl, N. G., Nordentoft, M., & Hjorthøj, C. (2019). Schizophrenia is associated with increased risk of subsequent substance abuse diagnosis: A nation-wide population-based register study. *Addiction*, *114*(12), 2217–2226. https://doi.org/10.1111/add.14746

Petrilli, K., Ofori, S., Hines, L., Taylor, G., Adams, S., & Freeman, T. P. (2022). Association of cannabis potency with mental ill health and addiction: A systematic review. *The Lancet. Psychiatry*, *9*(9), 736–750. https://doi.org/10.1016/S2215-0366(22)00161-4

SAMHSA. (2019). 2019 NSDUH Detailed Tables | CBHSQ Data. https://www.samhsa.gov/data/report/2019-nsduh-detailed-tables

Sommer, I. E., Tiihonen, J., van Mourik, A., Tanskanen, A., & Taipale, H. (2020). The clinical course of schizophrenia in women and men – a nation-wide cohort study. *Npj Schizophrenia*, *6*(1), 1–7. https://doi.org/10.1038/s41537-020-0102-z

Thomsen, K. R., Lindholst, C., Thylstrup, B., Kvamme, S., Reitzel, L. A., Worm-Leonhard, M., …Hesse, M. (2019). Changes in the composition of cannabis from 2000–2017 in Denmark: Analysis of confiscated samples of cannabis resin. *Experimental and Clinical Psychopharmacology*, *27*(4), 402–411. https://doi.org/10.1037/pha0000303

UNODC. (2021). World Drug Report 2021. https://www.unodc.org/res/wdr2021/field/WDR21_Booklet_1.pdf

Urits, I., Gress, K., Charipova, K., Li, N., Berger, A. A., Cornett, E. M., … Viswanath, O. (2020). Cannabis use and its association with psychological disorders. *Psychopharmacology Bulletin*, *50*(2), 56–67.

Veen, N. D., Selten, J.-P., van der Tweel, I., Feller, W. G., Hoek, H. W., & Kahn, R. S. (2004). Cannabis use and age at onset of schizophrenia. *The American Journal of Psychiatry*, *161*(3), 501–506. https://doi.org/10.1176/appi.ajp.161.3.501

Volkow, N. D., Swanson, J. M., Evins, A. E., DeLisi, L. E., Meier, M. H., Gonzalez, R., …Baler, R. (2016). Effects of cannabis use on human behavior, including cognition, motivation, and psychosis: A review. *JAMA Psychiatry*, *73*(3), 292–297. https://doi.org/10.1001/jamapsychiatry.2015.3278

Review

Autism spectrum disorder: Consensus guidelines on assessment, treatment and research from the British Association for Psychopharmacology

Journal of Psychopharmacology
2018, Vol. 32(1) 3–29

DOI: 10.1177/0269881117741766
journals.sagepub.com/home/jop

Oliver D Howes[1,2], Maria Rogdaki[1*], James L Findon[3*], Robert H Wichers[3*], Tony Charman[4], Bryan H King[5], Eva Loth[3], Gráinne M McAlonan[6,7], James T McCracken[8], Jeremy R Parr[9], Carol Povey[10], Paramala Santosh[11], Simon Wallace[12], Emily Simonoff[13] and Declan G Murphy[6,7]

Abstract

An expert review of the aetiology, assessment, and treatment of autism spectrum disorder, and recommendations for diagnosis, management and service provision was coordinated by the British Association for Psychopharmacology, and evidence graded. The aetiology of autism spectrum disorder involves genetic and environmental contributions, and implicates a number of brain systems, in particular the gamma-aminobutyric acid, serotonergic and glutamatergic systems. The presentation of autism spectrum disorder varies widely and co-occurring health problems (in particular epilepsy, sleep disorders, anxiety, depression, attention deficit/hyperactivity disorder and irritability) are common. We did not recommend the routine use of any pharmacological treatment for the core symptoms of autism spectrum disorder. In children, melatonin may be useful to treat sleep problems, dopamine blockers for irritability, and methylphenidate, atomoxetine and guanfacine for attention deficit/hyperactivity disorder. The evidence for use of medication in adults is limited and recommendations are largely based on extrapolations from studies in children and patients without autism spectrum disorder. We discuss the conditions for considering and evaluating a trial of medication treatment, when non-pharmacological interventions should be considered, and make recommendations on service delivery. Finally, we identify key gaps and limitations in the current evidence base and make recommendations for future research and the design of clinical trials.

Keywords

Autism, treatment, guidelines, neurodevelopmental, aetiology

Table of Contents

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder with an estimated lifetime prevalence of at least 1% (Baird et al., 2006; Brugha et al., 2011). Core symptoms include deficits in social communication and the presence of restricted and repetitive interests or activities and sensory anomalies, beginning in the early developmental period (American Psychiatric Association, 2013). The assessment and management of ASD is complex, due to its multifactorial aetiology, persistence into adulthood, presence of co-occurring mental and physical disorders and attendant disability. The total cost, including accommodation, treatment, loss of earnings and health care for individuals over their life span has been estimated to range between £0.92 m and £1.5 m for someone without or with an intellectual disability, respectively (Buescher et al., 2014). ASD-related difficulties are lifelong and often require on-going support. Many people with ASD are prescribed psychotropic medications at some point in their lives. According to a recent study in the UK, using a representative primary care database, psychotropic drugs are prescribed to 29% of people with ASD (Murray et al., 2014). In this study, the most commonly prescribed categories of drugs for ASD were sleep medications (9.7%), psychostimulants (7.9%) and antipsychotics (7.3%) (Murray et al., 2014). In particular, the use of psychostimulants and antipsychotics in ASD is much higher than in the general population (Murray et al., 2014). Similar rates of prescribing psychotropic drugs have also been reported in the USA and Canada. For example, a recent study of over 2800 children from the Autism Treatment Network in North America reported that 27% of children and adolescents with ASD are prescribed at least one psychotropic medication (Coury et al., 2012). Stimulants were most often prescribed (13%), followed by selective serotonin re-uptake inhibitors (SSRIs) (8%) and atypical antipsychotics (8%) (Coury et al., 2012). Evidence also indicates that prescription rates have increased, with a more than three-fold increase in antidepressant drug prescriptions for people with ASD in the USA between 1992–2001 (Aman et al., 2005b). Sixty per cent of adults with ASD have concerns about taking medications, particularly due to side-effects and lack of effectiveness (Wallace et al., 2013). This, and the commonplace use of drugs in ASD, suggests there is a need for comprehensive guidance on the assessment and management of ASD, which incorporates advice on the use of psychotropic medications. However, it should be recognised that some people with ASD do not want treatment for the core aspects of ASD. As such, discussion about treatment should take into consideration individual preferences.

In view of the uncertainties, the British Association for Psychopharmacology (BAP) coordinated the development of this consensus guideline to review and make recommendations for the assessment and management of ASD with a focus on drug treatments. We first review the aetiology of ASD to provide a

[1]MRC London Institute of Medical Sciences, London, UK
[2]Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, UK
[3]Sackler Institute for Translational Neurodevelopment, King's College London, London, UK
[4]Department of Psychology, King's College London, London UK
[5]Department of Psychiatry, University of California at San Francisco, San Francisco, USA
[6]The Sackler Centre and Forensic and Neurodevelopmental Science Behavioural and Developmental Psychiatry, Clinical Academic Group, South London and Maudsley NHS Foundation Trust, London, UK
[7]NIHR-BRC for Mental Health, South London and Maudsley NHS Foundation Trust, London, UK
[8]Department of Psychiatry and Biobehavioral Sciences, University of California at Los Angeles, Los Angeles, USA
[9]Institute of Neuroscience, Newcastle University, Newcastle, UK
[10]The National Autistic Society, London, UK
[11]Department of Child Psychiatry, King's College London, London, UK
[12]AT-Autism, London, UK
[13]Department of Child and Adolescent Psychiatry, King's College London, London, UK

*These authors contributed equally.

Corresponding author:
Oliver D Howes, MRC London Institute of Medical Sciences, London, UK and Institute of Psychiatry, Psychology & Neuroscience, King's College London, Box 67, Camberwell, London, SE5 8AF, UK.
Email: oliver.howes@lms.mrc.ac.uk

framework to understand the diagnostic assessment of ASD and treatment targets. Subsequently we address the management of core symptoms, the management of common co-occurring conditions, non-pharmacological treatments and the implications for service provision, before discussing future directions for clinical research.

Method

A consensus meeting was held with the support of the BAP involving a group of experts on ASD in children, adolescents and adults. The group consisted of psychiatrists, psychologists, researchers in the field and service user representatives. Members of the group gave presentations summarising each topic discussed in this paper, followed by discussion on the nature and quality of the evidence and its implications. Following the consensus meeting, a further literature review was conducted to support the consensus points. Drafts of the review and the recommendations were circulated to the expert group for comments, which were then revised by the expert group to derive the final version of the guidelines.

The evidence in each area was rated using the criteria by Shekelle et al. (1999) (Supplementary Table 1), which rank meta-analyses of randomised controlled trials (RCTs) and large, representative observational studies as the best evidence. Our recommendations were graded based on the strength of the evidence supporting them using the grading criteria described in previous BAP guidelines (Bolea-Alamañac et al., 2014). Thus, recommendations were rated A to D to reflect the evidence (see Supplementary Table 1), with grade A indicating the recommendation is supported by the highest quality evidence. Although, some of the recommendations were based on weaker evidence (B, C, D), this does not necessarily reflect their clinical importance. The category S corresponds to a standard for clinical care, which comprises a consensus on good clinical practice in the absence of other evidence. In summarising the pharmacological evidence, we have focused on the primary end-points of studies but, where this is not the case, we have indicated that the evidence is based on a secondary end-point. We have reported the doses used or, where the dose was variable, we have reported the mean dose used or range if the mean dose was not reported. We also summarise key aspects of the design of the study (whether the raters were blind to intervention, and whether the sample was randomised to a placebo or other comparator) and sample size (using the intention to treat sample) so that readers can gauge the strength of evidence.

Aetiology

Genetic risk factors

Genetic factors play a substantial role in the aetiology of ASD. Recent studies have shown about 80% heritability for ASD (Lichtenstein et al., 2010; Ronald and Hoekstra, 2011). However, within families, no one pattern of inheritance (e.g. autosomal dominant or recessive) is observed. With the exception of a small number of rare genetic variants (recently estimated at 71 variants; (Sanders et al., 2015)), the effect of more common individual risk variants so far identified is small. Monogenetic syndromes with high rates of overlapping disorders which often include ASD as part of their behavioural phenotype include Phelan–McDermid syndrome (PMS), fragile X syndrome (FXS) and tuberous sclerosis (Ghosh et al., 2013). These constitute about 10–15% of all cases of ASD.

In the majority of cases, the genetic risk for ASD is polygenic, involving multiple single nucleotide polymorphisms (SNPs), each of minor effect (Anney et al., 2012; Clarke et al., 2015; Gaugler et al., 2014; Klei et al., 2012). In addition to SNPs and monogenic disorders, a number of de novo suspected single gene loss of function mutations and copy number variants (CNVs; such as microdeletions or microduplications) spanning multiple genes have been reported to increase the risk of ASD and (often substantially), intellectual disability (de la Torre-Ubieta et al., 2016). Breakpoints consistently associated with ASD include the SHANK3 deletion, 1q21, 3q29, 7q11.23, 15q11.2-13.1, 15q12, 15q13, 16p11, 17q12, 22q11.2 and Xq (Vorstman et al., 2006).

The genetic risk variants for ASD implicate a number of key neurobiological pathways that are potential targets for drugs. Specific examples include the N-methyl-D-aspartate (NMDA) 2B glutamate ionotropic and gamma-aminobutyric acid (GABA) receptors (including GABARA3 and GABARB3), cell adhesion molecules, scaffolding proteins such as SHANK1, SHANK2, SHANK3 ankyrin repeat domain proteins (Bourgeron, 2015), and neuron-glia signalling and microglial activation (de la Torre-Ubieta et al., 2016). An improved understanding of the nature of the disruption in these pathways in ASD is needed to help develop targeted molecular therapies.

Environmental risk factors

A number of prenatal, perinatal and neonatal factors, including significant prematurity, perinatal hypoxia, maternal pre/perinatal infections, maternal vitamin D deficiency, higher paternal age, gestational valproate exposure, maternal obesity and very low birthweight (<1500 g), have been associated with an increased relative risk of ASD (for further details see reviews and meta-analyses: Eyles et al., 2013; Gardener et al., 2009, 2011; Mandy and Lai, 2016). Maternal use of SSRIs before or during pregnancy has also been identified in some studies, but significant questions have been raised about the causality of this association (Man et al., 2015). Interestingly, preclinical work shows that some of these environmental risk factors impact on the same pathways implicated by the genetic association studies (Basil et al., 2014; Richetto et al., 2014), suggesting risk factors may converge on common pathways at the molecular or higher-level brain circuit levels (Voineagu et al., 2011).

Biology of ASD

Brain structural differences in ASD have been identified early in life. The first studies examined head circumference and discovered that this proxy measure of brain size increased more in individuals with ASD than in controls and their unaffected siblings during the first years of their life (Constantino et al., 2010; Courchesne et al., 2003; Elder et al., 2008; Redcay and Courchesne, 2005). This is thought to be explained by a greater volume of both grey and white matter, with especially pronounced overgrowth in the frontal and temporal cortex (Schumann et al., 2010). Early brain overgrowth may include an increase in cortical thickness in ASD at ages 3–4 years old, but seems to be followed

by accelerated cortical thinning (Zielinski et al., 2014). Overall, the growth trajectory rate flattens in ASD such that, by the ages of 10–15 years old, average brain size in ASD is similar to typically developing children. Subsequently grey and white matter volumes may decrease in ASD in adulthood (Lange et al., 2015). However, it is noteworthy that not all head circumference or magnetic resonance imaging (MRI) volumetric studies support this model (Hansen et al., 2008; Raznahan et al., 2013a,b; Rogers, 2004). It is therefore possible that early brain overgrowth and subsequent growth trajectory flattening is present in only a subset of individuals with ASD (Lenroot and KaYeung, 2013).

Neurochemical alterations are also reported in ASD. One system which is repeatedly implicated is the serotonin system, which is thought to underpin some anatomical features of ASD. The serotonin system has a role in neurite outgrowth (Fricker et al., 2005), synaptogenesis (Faber and Haring, 1999; Mazer et al., 1997), differentiation and neurogenesis (Kesterson et al., 2002) and therefore its contribution to the early developmental aberrations reported in ASD is highly plausible. Serotonergic abnormalities in ASD include elevated serotonin levels in whole blood and platelets in upwards of 25% of affected individuals (Gabriele et al., 2014; Hanley et al., 1977) and alterations in the developmental trajectory of brain serotonin synthesis activity (Chugani et al., 1997). Together this evidence has provided a theoretical rationale for exploring the effect of serotonergic medications in ASD (Veenstra-VanderWeele et al., 2012) (see section 'serotonergic agents').

More recently, evidence for a pivotal role of the excitatory (E) glutamate and inhibitory (I) GABA systems in ASD has accumulated. An influential review by Rubenstein and Merzenich (2003) proposed that ASD is caused by an increased E/I ratio leading to pathological hyper-excitability within cortical circuits. Some preliminary support for the model comes from a positron-emission tomography (PET) study using a tracer that is relatively selective for the GABA-A alpha-5 receptor sub-type, showing elevated GABA receptor availability in ASD, potentially indicating reduced GABA transmitter levels (Mendez et al., 2013).

Criticisms of the model include that it may be overly simplistic and not specific to ASD, as E/I imbalance has been implied in epilepsy and schizophrenia. E/I balance is likely to differ depending on the brain region and cell-type studied (Nelson and Valakh, 2015; Rothman et al., 2011, 2012; Sibson et al., 1998). Furthermore, evidence from magnetic resonance spectroscopy (MRS) studies of glutamate and GABA in ASD has not been consistent. For example, a recent review of MRS studies reported that out of the 12 studies in frontal cortical regions, four studies failed to report any differences between individuals with ASD and healthy controls; four studies (three in childhood and one in adolescence) reported increased levels of glutamate and the combination of glutamate and glutamine (Glx), and one study reported reduced levels of Glx (Naaijen et al., 2015). In addition, three studies reported decreased levels of GABA in frontal regions (Naaijen et al., 2015). There are fewer studies in the thalamus, hippocampus and striatum, although the results are similarly inconsistent (Naaijen et al., 2015). However, although informative, MRS is limited as it is an overall measure of tissue glutamate and/or GABA which means intra-cellular levels may mask changes in synaptic levels. Thus, these inconsistencies may simply be a product of the current constraints of technology available to examine these neurotransmitters in the living human brain. However, they may also reflect the highly heterogeneous nature of ASD and/or pronounced differences between brain regions or developmental stages.

In contrast, preclinical studies have generated a much more consistent picture of E/I disruption in the perinatal period in rodent models relevant to ASD. In brief, animal models of ASD reveal an increase in spontaneous activity in sensory cortices in early life (Gonçalves et al., 2013; Gutierrez et al., 2009; Peixoto et al., 2016). This may, at least, be partly a consequence of a 'delay' in the switch in GABA responses in brain from excitatory, during prenatal life, to inhibitory, during postnatal life, in ASD and related conditions (Ben-Ari et al., 2012).

Animal and clinical studies have shown that the neuropeptide oxytocin regulates social bonding and recognition, suggesting alterations in the oxytocin system could contribute to manifestations of ASD or its treatment (Baumgartner et al., 2008; Domes et al., 2007; Ferguson et al., 2000; Insel and Shapiro, 1992). This has led to a number of clinical trials (see section on treatment of core symptoms). Furthermore, oxytocin modulates the switch from excitatory GABA to inhibitory function and is therefore an important regulator of E/I balance during the perinatal period (Tyzio et al., 2006, 2014). It is likely that oxytocin continues to have a modulatory effect on E/I later in postnatal life. Hence, oxytocin could serve as a target to modulate E/I balance in ASD.

Lastly, increasing evidence from animal and clinical studies has confirmed that a role for maternal and postnatal immune dysregulation in the aetiology of ASD cannot be overlooked. Taking into account the regulatory role that the immune system has on neuronal cells at every stage of brain development, it is plausible that immune dysfunction, caused by genetic mutations or environmental factors, could alter brain development and function (Estes and McAllister, 2015). Dysfunction of microglial cells, the resident brain immune cells, is one immune mechanism implicated in the pathogenesis of ASD. Data suggest that aberrant microglial function may lead to altered synaptic pruning, which may subsequently contribute to the pathogenesis of ASD (Koyama and Ikegaya, 2015). However, not all in vivo evidence finds support for immunologic dysregulation (Pardo et al., 2017).

Summary

In conclusion, ASD is a complex neurodevelopment disorder that, in the majority of cases, shows multifactorial, polygenic inheritance, although de novo mutations and CNVs are currently estimated to play an important role about 10–20% of patients. Environmental factors, in particular early insults (prenatal, perinatal and postnatal factors), also contribute to an increased risk of developing ASD. Brain development is disrupted from early childhood and shows alterations into adulthood. Key systems implicated in the pathophysiology of ASD are increasingly being identified and provide targets for clinical trials. These include (but are not limited to) the serotonin and oxytocin systems, the immune system and GABA/glutamate interactions.

Diagnostic criteria

Diagnostic and Statistical Manual, version 5 (DSM-5)

The DSM-5, published in 2013 (American Psychiatric Association, 2013), has the latest revision of the diagnostic

criteria for ASD, and a number of changes have been made to reflect recent research. The International Classification of Disease (ICD) version-10 (World Health Organization, 1992) is currently under review with a new revision, ICD-11, planned to be published in 2018. It is not yet clear to what degree ICD-11 will be aligned with DSM-5 – but it is likely there will be substantial agreement on core ASD characteristics.

The main features of the DSM-5 criteria for ASD are summarised in Supplementary Table 2. One key change is that the term 'autism spectrum disorder' is used rather than the term 'autism' and its related categories used in DSM-IV (1994) and ICD-10 (e.g. Asperger syndrome, pervasive developmental disorder – not otherwise specified (PDD-NOS), atypical autism). This shift acknowledges the lack of distinct neurobiological profiles between the different subtypes (Noterdaeme et al., 2010) and the inconsistency in their use (Lord et al., 2012a). The DSM-5 also includes a new category – social (pragmatic) communication disorder (SCD) – to describe individuals with deficits in social verbal and non-verbal communication, but who do not otherwise meet the criteria for a diagnosis for ASD because they do not show repetitive and restricted behaviours. As SCD was only recently introduced, its diagnostic reliability and validity, prognosis, and common features are still to be determined (Norbury, 2014).

The DSM-5 diagnostic criteria for ASD are broader than the DSM-IV criteria for autism. The previously separate domains of social interaction and communication under the DSM-IV classification have been unified as one domain in the DSM-5 (social communication). Hence, the DSM-5 classification of ASD covers two domains; social communication difficulties and repetitive and restricted behaviours (Supplementary Table 1), and include abnormal sensory responses as a cardinal symptom (restored from the DSM-III).

An important addition to the DSM-5 is the inclusion of severity specifiers to indicate the impact of symptoms on adaptive functioning (Supplementary Table 3). Adaptive functioning encompasses communication, occupation and daily living skills (Bal et al., 2015). As shown in Supplementary Table 3, there are three categories for each of the two core symptoms, indicating the level of support the affected individual requires, depending on his/her adaptive functioning. This addition is undoubtedly crucial, as the severity of core symptoms and deficits in adaptive functioning may vary considerably between individuals with the disorder (Constantino and Charman, 2015). Another modification in the DSM-5 is that the new diagnostic criteria offer the option to diagnose co-occurring psychiatric disorders.

Another important change is that the DSM-5 does not specify an age of onset, which has instead been revised to 'symptoms must be present in the early developmental period (but may not fully manifest until social demands exceed limited capacities, or may be masked by learned strategies in later life)' (American Psychiatric Association, 2013: 50-51). This recognises that ASD may not become fully apparent until later in life, and enables the diagnosis of ASD in adulthood (Howlin and Moss, 2012). In addition, the DSM-5 does not require all symptoms to be currently present but rather specifies that they should have occurred at some point in the lifetime. This takes account of the observation that some symptoms are more common at certain time points and also that they may be less evident when the individual is in an optimal environment.

A final change in the DSM-5 is the introduction of clinical specifiers to be noted alongside the ASD diagnosis. These include the presence or absence of the following components: (a) intellectual impairment, (b) language impairment, (c) known medical or genetic condition or environmental factor, (d) neurodevelopmental, mental or behavioural disorder, (e) catatonia (American Psychiatric Association, 2013).

Recent studies indicate that the overall prevalence of ASD may be lower under the DSM-5 compared to the prevalence of autism and related disorders based on the DSM-IV criteria (Maenner et al., 2014). This appears to be mostly due to ~20% of the individuals previously meeting the DSM-IV criteria for PDD-NOS not qualifying for a diagnosis of ASD according to the DSM-5 criteria (Maenner et al., 2014; Mazefsky et al., 2013; Wilson et al., 2013; World Health Organization, 1992).

In summary, the DSM-5 criteria may be particularly useful for diagnosis as they enable diagnosis of adults whose symptoms were not impairing in childhood, and it highlights the importance of co-occurring disorders and functional impact.

Consensus recommendations for diagnosis

- ASD is a complex neurodevelopmental disorder. Its diagnosis requires a neurodevelopmental history and, where possible, a multi-disciplinary approach. (B)
- It is recommended that established diagnostic criteria are used for diagnosis. (D).
- Consider using the DSM-5 criteria as they enable diagnosis in adults and the diagnosis of co-occurring disorders. (D)
- Further studies are required to evaluate the reliability and validity of the Social Communication Disorder diagnosis as a distinct disorder from ASD. (D)

Assessment and diagnosis

Making a diagnosis of ASD is a multi-stage process. It requires the multidisciplinary assessment of current symptoms, acquisition of a developmental history from a primary caregiver and the exclusion of alternative diagnoses by the clinician (National Institute for Health and Clinical Excellence, 2013) (evidence level IV).

Symptoms can manifest and be interpreted differently depending on the environmental context. Thus, evaluation of symptomatology in different environments (home, school, community, in addition to the clinic) should be an expectation. The clinician should consider whether impairment in adaptive function is exclusively due ASD or an additional psychiatric or medical disorder (Constantino and Charman, 2015). The high prevalence of co-occurring disorders in ASD indicates that medical and psychiatric disorders, including intellectual ability, should be routinely screened for in individuals presenting with ASD (Kielinen et al., 2004; Simonoff et al., 2008). In addition, cognitive, language and neuropsychological assessments may be considered, as they can provide valuable information about the individual's strengths and weaknesses and inform the management plan (Charman and Gotham, 2013; Ozonoff et al., 2005).

Reaching a diagnosis of ASD, particularly in adults, may be challenging for a variety of reasons. One of the key pillars of the diagnostic process is the acquisition of a developmental history, preferably from a primary caregiver. However, informants for

adults with a potential diagnosis of ASD may not be able to recall the developmental history in detail. In some instances, the primary caregivers may not be alive, so clinicians will need to rely on a history that they obtain from other informants, and additional sources of information (such as school reports). Careful consideration should be given to the accuracy of retrospective recall and specific examples of behaviour should be elicited. Where no informant is available, diagnosis should be based on the history and current circumstances (including the current assessment, and reports from employment or school) (Lai and Baron-Cohen, 2015). Similarly, the assessment of a non-verbal child with suspected ASD is also challenging with respect to accurate estimation of intellectual ability, social understanding, and co-occurring disorders.

Instruments and diagnostic tools

More than 20 screening and diagnostic tools for ASD have been developed over the last two decades (Charman and Gotham, 2013). The aim of screening tools is to identify individuals who are in need for further diagnostic assessment and evaluation. In the next section, we briefly summarise the most commonly used instruments used in childhood and adolescence (Supplementary Tables 4 and 5), and in adulthood (Supplementary Table 6).

Children. In Supplementary Table 4, we provide a list of the most frequently used screening instruments for children who may have ASD. Supplementary Table 5 summarises the structured diagnostic instruments for children who may have ASD based on a screening instrument or other information. The structured instruments vary from observational measures (e.g. Autism Diagnostic Observation Schedule (ADOS); Lord et al., 2012b) to caregiver interviews (e.g. Autism Diagnostic Interview Revised (ADI-R); Lord et al., 1994).

Adults. In Supplementary Table 6, we summarise the most common screening instruments for ASD in adults. Diagnostic tools for adults are summarised in Supplementary Table 5. The most robust observational measure for ASD diagnosis in adulthood is the ADOS (module 4) (Lord et al., 2000). However, it has limitations, including its relatively low sensitivity when used to diagnose higher functioning adults with ASD and low specificity in individuals with severe intellectual disability (when used without ADI-R) (Bastiaansen et al., 2011). Also, it may not fully capture repetitive behaviours and/or intense preoccupations (Kim and Lord, 2010).

The development of assessment tools for ASD has helped increase the identification of ASD and aided accuracy of diagnosis both in clinical and research settings (Johnson and Myers, 2007). Clinicians and researchers should be aware of the strengths and limitations of the instruments. For example, being screened positive on one of the screening instruments does not mean that the individual meets a diagnosis for ASD; in the same way, being screened negative does not exclude the diagnosis of ASD. Results of the instruments differ depending on the setting, the presence of other mental disorders, the sample characteristics and the purpose of the screening (Charman and Gotham, 2013) (evidence level II). Other limitations include the requirement for trained administrators and raters and the time taken to administer them; which can be several hours. One should also take into consideration that most of these tools have not been extensively validated in individuals with ASD and intellectual disability or non-western cultures (Rudra et al., 2014).

Despite the acknowledged limitations of individual instruments, the use of a structured diagnostic instrument is still highly recommended in the evaluation of an individual with suspected ASD to ensure a comprehensive and systematic assessment.

Diagnostic challenges

Individuals with ASD may present to services with co-occurring psychiatric disorders, and ASD may be overlooked if it is not considered during the assessment (Lai and Baron-Cohen, 2015). Adults with ASD may develop adaptive mechanisms to manage social situations, for example by mimicking the gestures and conversational style of others (Lai et al., 2011) (evidence level II). This can mask the presentation and make the diagnosis more challenging (Lai et al., 2011). Another complication is that some studies have shown that core symptoms may be manifested differently in females than in males (Van Wijngaarden-Cremers et al., 2014), which may delay diagnosis in females (Wilson et al., 2016). For example, females with ASD may have restricted interests that involve people (literature, pop bands) rather than objects, such as collection of stamps or trains, as seen in males with ASD. They also may have fewer stereotyped behaviours and have more socially accepted interests (Mandy et al., 2012; Van Wijngaarden-Cremers et al., 2014) (evidence level III). Females with ASD may be more likely to have developed coping strategies to manage social situations that mask the degree of their social isolation from peers (Dean et al., 2016). Females in particular may demonstrate overt shyness or bossiness and being perfectionist. These characteristics constitute the so-called 'female phenotype' (Lai and Baron-Cohen, 2015) (evidence level III), however they do not constitute core autistic symptoms and, importantly, may be present in people without ASD.

Consensus recommendations for the diagnostic process

- A multidisciplinary approach is recommended for the diagnosis of ASD. (D)
- The diagnostic process should involve a direct clinical assessment of the individual and, wherever possible, a detailed interview with the caregiver or other informants, reports from school and employment, and assessment of cognitive and language skills and a medical examination. (D)
- Screening instruments are useful in aiding diagnosis, but should not be used exclusively to make or exclude the diagnosis. (B)
- Diagnostic challenges are more pronounced when diagnosis is made in adulthood, and extra care should be made to rule a diagnosis out. (B)
- It is recommended that ASD is routinely considered in the differential diagnosis when an individual presents to mental health services with a psychiatric disorder. (D)
- Clinicians should be aware of the so-called female phenotype and a careful assessment in females is recommended. (C)

Prevalence of co-occurring mental health difficulties in ASD

Co-occurring psychiatric disorders are highly prevalent in ASD and are more common in ASD than in the general population (Croen et al., 2015). Between 69–79% of individuals with ASD experience at least one additional psychiatric condition during their lifetime (Buck et al., 2014; Lever and Geurts, 2016), compared to rates of lifetime psychiatric disorder of approximately 40% in the general population (Bijl et al., 1998).

Co-occurring difficulties in children with ASD

Irritability, self-injurious behaviour and temper tantrums are amongst the most common co-occurring symptoms in children with ASD, seen in around 85% of both high and lower functioning children with ASD (Mayes et al., 2011). Anxiety is also common, and anxiety disorders are seen in children with ASD with prevalence rates of between 42–55% (de Bruin et al., 2007; Simonoff et al., 2008). Of these, specific phobia (prevalence in ASD of between 9–44%) and social phobia (prevalence in ASD of between 8–29%) are the most common disorders (de Bruin et al., 2007; Leyfer et al., 2006; Simonoff et al., 2008). Attentional/hyperactive and behavioural difficulties are also common in ASD. Between 28–53% of children with ASD meet criteria for attention deficit/hyperactivity disorder (ADHD) and between 7–37% meet criteria for an oppositional defiant disorder or conduct disorder (de Bruin et al., 2007; Leyfer et al., 2006; Salazar et al., 2015; Simonoff et al., 2008; Sinzig et al., 2009). Bipolar disorder and psychotic disorders are less common in ASD, but occur at rates above those of comparison groups (Croen et al., 2015). Mood disorders also often occur in children with ASD (de Bruin et al., 2007; Leyfer et al., 2006; Simonoff et al., 2008). This constellation of problems might also contribute to the sleeping difficulties, which parents report are a concern in 50–80% of children (Richdale and Schreck, 2009). What it is not clear, however, is to what extent these are part and parcel of an ASD diagnosis, or a component of the other conditions that children with ASD may experience. Moreover, the underlying mechanisms may be different to those in people without ASD, which would have implications for using treatments developed for these difficulties in people without ASD (Maskey et al., 2013).

Co-occurring difficulties in adults with ASD

Mood and anxiety disorders are also common in adults with ASD (Wigham et al., 2017). Between 26–57% of adults with ASD experience a mood disorder at some point (Croen et al., 2015; Hofvander et al., 2009; Joshi et al., 2013; Lever and Geurts, 2016; Roy et al., 2015), and anxiety disorders, particularly social phobia, are similarly common (Croen et al., 2015; Hofvander et al., 2009; Joshi et al., 2013; Lever and Geurts, 2016; Roy et al., 2015). ADHD is also frequently reported in adults with ASD, with prevalence rates of between 11–43% (Croen et al., 2015; Hofvander et al., 2009; Joshi et al., 2013; Lever and Geurts, 2016). However, older adults with ASD have been reported to have lower rates of co-occurring psychiatric disorders than younger adults with ASD, and social phobia in particular appears to be significantly less common in this group (Lever and Geurts, 2016). Tic disorders and Tourette syndrome are also frequently reported co-occurring problems in adults with ASD. Between 20–22% of children and adults with ASD meet the criteria for a tic disorder (Canitano and Vivanti, 2007; Hofvander et al., 2009).

There is a lack of validated screening instruments for the detection of co-occurring conditions in ASD. However, a recent study demonstrated the validity of the Strengths and Difficulties Questionnaire (SDQ) in screening for emotional disorders and hyperactivity in adolescents and adults with ASD (Findon et al., 2016).

Together, these co-occurring disorders have a marked impact on functional impairment and caregiver burden, comparable to that reported by persons caring for individuals with a brain injury (Cadman et al., 2012). Thus, identifying and treating co-occurring disorders in adults with ASD is critical; and this has been identified as a priority by health services and agencies (such as the UK Department of Health and the US Agency on Healthcare Research and Quality).

Pharmacological treatment of core symptoms of ASD

In this section, we will initially summarise the results from studies of pharmacological treatments for the core symptoms of ASD (deficits in social communication and interaction, and restricted and repetitive interests or behaviours). We will then discuss the limitations of the evidence and, finally, proceed with recommendations.

Two main approaches have been taken to develop pharmacological agents for ASD. One is re-purposing treatments from other psychiatric disorders that have symptoms in common with ASD. The second approach is to target the putative neurobiological processes underlying ASD, in some instances by targeting potentially more homogenous sub-populations, such as those with rare genetic abnormalities who have ASD, for example individuals with FXS.

In the following sections, we summarise results from the pharmacological studies grouped by the system primarily targeted, in children first and then adults. In this and the section on co-occurring conditions and symptoms, studies with less than 20 participants in the intention to treat analysis are described as small, studies with 20–45 participants are described as medium, and those with more than 50 participants are described as large. An overview of study designs and findings is given in Supplementary Table 7.

Serotonergic agents

A number of trials of SSRIs have been undertaken but all bar one have involved small sample sizes and demonstrated mixed results (Supplementary Table 7).

A small study of fenfluramine was conducted in children with ASD (Barthelemy et al., 1989) (evidence Ib). Core symptoms were assessed using the Behavior Summarized Evaluation scale (Barthélémy, 1986). There was no significant difference from baseline or between the treatment and placebo groups. Reported side effects included withdrawal and sadness, and weight loss (Barthelemy et al., 1989). Fenfluramine is no longer marketed due to serious adverse effects and it is not recommended for use in ASD.

One large study of citalopram evaluated its effect on repetitive behaviours in children with ASD and failed to demonstrate any significant improvement on the Clinical Global Impression (CGI) Improvement subscale, or on any secondary outcomes including the Children's Yale-Brown Obsessive-Compulsive Scale (CY-BOCS) modified for pervasive developmental disorders (King et al., 2009) (evidence Ib). In addition, children on citalopram experienced significantly more adverse events than children given placebo, particularly increased energy levels, impulsiveness, decreased concentration, hyperactivity, increased stereotypy, diarrhoea, initial insomnia, dry skin and, in one case, a prolonged seizure (King et al., 2009). Citalopram is therefore not recommended for the treatment of core symptoms in children with ASD (evidence Ib). A medium-sized study of liquid fluoxetine was conducted in children and adolescents with ASD (Hollander et al., 2005). Using a low dose of fluoxetine, significant improvement was reported in repetitive behaviours as measured by the CY-BOCS, with an effect size of 0.76, but the overall reduction in repetitive behaviours was <10%. No significant differences between fluoxetine and placebo were reported for side effects (Hollander et al., 2005) (evidence Ib). However, another large completed but unpublished study registered on Clinicaltrials.gov of children and adolescents with ASD treated with fluoxetine was reportedly negative (evidence level Ib) (Neuropharm: clinicaltrails.gov, 2012).

In contrast with studies in children, the studies in adults have been more consistent. A small study of fluoxetine in six adults with ASD showed improvement in the CGI scale in three subjects (Buchsbaum et al., 2001) (evidence IIb double down-graded because of the small sample size). In addition, two small studies showed benefits for fluvoxamine and fluoxetine on measures of repetitive behaviours for adults (Hollander et al., 2012; McDougle et al., 1996). However, the benefits were small relative to placebo and the evidence is limited by the small sample sizes (evidence Ib). Therefore, there is not sufficient data to support the recommendation for fluvoxamine or fluoxetine.

A recent Cochrane review concluded that there is no evidence to support the use of serotonergic agents in children, whereas there are data to support their use in adults, particularly for repetitive behaviours (Williams et al., 2013) (evidence Ia). Fluoxetine seems the best tolerated of the serotonergic agents, but it should be noted that there have been no head-to-head comparisons of fluoxetine against other agents.

Glutamatergic agents

1. Metabotropic glutamate receptor 5 (mGLuR5) antagonists: animal models of fragile X syndrome (FXS) have shown that in the absence of fragile X mental retardation protein (FMRP), encoded by the *FMR* gene, there is an elevation of mGluR5 receptor levels (Dolen and Bear, 2008). Animal behaviours analogous to ASD symptoms are improved by mGluR5 antagonists (Silverman et al., 2014). Based on these findings, trials in FXS have focused on mGluR5 antagonists, such as AFQ056. The first small-sized study of AFQ056 did not find any statistically significant effect on the Aberrant Behaviour Checklist (ABC-C) (evidence level Ib), although a post-hoc analysis suggested an improvement in the ABC-Social Avoidance subscale for a subgroup of patients based upon level of *FMR1* methylation (Jacquemont et al., 2011) (evidence level IIb). Moreover, recent results from two large studies failed to demonstrate any efficacy in the primary endpoint, the ABC-C, (Berry-Kravis et al., 2016) (evidence level Ib) regardless of *FMR1* methylation status. Although the authors of these studies suggested that further trials in a younger population with longer treatment duration are needed in order to fully test the mGluR5 theory (Berry-Kravis et al., 2016), the current evidence does not support the use of AFQ056 and the value of targeting mGluR5 in ASD appears in doubt, at least for people with FXS.
2. Memantine is an uncompetitive NMDA antagonist that has been used in dementia (Reisberg et al., 2003). Preclinical studies have shown that when synaptic glutamate levels are high, memantine blocks NMDA receptors, whereas it has the opposite effect when synaptic glutamate levels are low (Parsons et al., 2007). A small study of memantine (Erickson et al., 2007) suggested promising effects, as measured with the CGI scale, however a subsequent large study (Aman et al., 2017) in children with ASD did not demonstrate any efficacy in either primary or secondary outcomes (evidence level Ib). Overall, the evidence does not support the routine use of memantine.
3. D-cycloserine is a partial agonist at the glycine$_b$ site on NMDA receptors. Several studies have indicated that it is beneficial for the treatment of the negative symptoms of schizophrenia (Tsai and Lin, 2010). Based on the overlap between negative symptoms in schizophrenia and social withdrawal in ASD, Posey et al. (2004) conducted a small study in children with pervasive developmental disorder (PDD). Results showed a significant improvement on the CGI and social withdrawal subscale of the ABC (Posey et al., 2004) (evidence level Ib). A recent medium-sized study in children with ASD reported that D-cycloserine with adjunctive social skills training was superior to placebo at reducing Social Responsiveness Scale scores at 22 weeks (Wink et al., 2017), but not 11 weeks (Minshawi et al., 2016) (evidence level Ib). Thus, while there are some promising results, the current evidence does not currently support the routine use of d-cycloserine.
4. CX516 is an allosteric positive modulator of AMPA receptors that has been trialled in FXS (Berry-Kravis et al., 2006). CX516 binds to the AMPA receptor complex, and is thought to slow down the rate of receptor closing to promote long-term potentiation (LTP) in the hippocampus (Arai et al., 1996). However, a medium-sized study in adults with FXS reported no significant effects on cognitive and behavioural outcome measures (Berry-Kravis et al., 2006) (evidence level Ib). Thus, the evidence does not currently support the use of CX516.

GABAergic agents

GABA$_b$ agonists. GABA$_b$ agonists such as arbaclofen activate GABA and preclinically inhibit the release of glutamate. Theoretically this should restore the balance of E/I neurotransmission in ASD. Three trials have been conducted with arbaclofen to date, one in individuals with FXS and two in individuals with ASD. The first was a medium-sized study in individuals with a

full mutation of the *FMR* gene (Berry-Kravis et al., 2012). Although there was no difference in the primary outcome (ABC-Irritability subscale) (evidence level Ib), post hoc analysis showed significant improvements with arbaclofen in the ABC-Social Avoidance subscale (evidence level IIb). The second study was a medium-sized study in children and young people with either ASD, PDD or PDD-NOS (Erickson et al., 2014). The results showed significant improvement on the primary outcome, ABC-Irritability scale, as well as in the Lethargy/Social Withdrawal subscale (Berry-Kravis et al., 2012; Erickson et al., 2014) (evidence level IIb). In a recent large study in children and young people with ASD (Veenstra-VanderWeele et al., 2016) (evidence level Ib), there was no significant change in the primary outcome, the ABC-Social Withdrawal Scale, but there was an improvement in secondary outcomes including on the CGI Severity and Improvement scale, and the Socialization and Communication subscales of the Vineland Adaptive Behavior Scale (VABS) (Veenstra-VanderWeele et al., 2016).

Overall, there is insufficient evidence to recommend the routine use of arbaclofen. Further studies are needed to evaluate this further and examine sub-groups (Brondino et al., 2016).

Pregnenolone is a neurosteroid that acts as a positive allosteric modulator of $GABA_A$ receptors to enhance $GABA_A$ receptor function (Hosie et al., 2006). A previous functional neuroimaging study in healthy volunteers reported that compared to placebo, administration of a single oral dose of 400 mg pregnenolone was associated with increased connectivity between the amygdala and medial prefrontal cortex, and reduced self-reported anxiety (Sripada et al., 2013). Subsequently a small study was conducted in adults with ASD (Fung et al., 2014). Among the secondary outcome measures, there was a significant improvement in the ABC-Lethargy/Social Withdrawal Scale (evidence level IIb). However, the study has a number of limitations, including the small sample size, open-label design and absence of a placebo group (Fung et al., 2014), which means it is premature to base recommendations on this study. Therefore, large scale, placebo-controlled randomised trials are necessary to test the benefit of pregnenolone further before it can be recommended in clinical practice. Until then, pregnenolone is not recommended.

Dopamine receptor blockers (antipsychotics)

1. Risperidone is a D2 dopamine receptor subtype antagonist and is one of the only two approved medications by the European Medicines Agency/Food and Drug Administration (FDA) for the treatment of irritability in ASD. Several trials have also measured core symptoms of ASD. Secondary analysis on participants selected for high irritability (McDougle et al., 2005) from the Research Units on Pediatric Psychopharmacology (RUPP) study ((McCracken et al., 2002) has shown significant decreases in repetitive behaviours with moderate effect sizes (Cohen's d=0.55), as measured with CY-BOCS scale, and paralleled by decreases in ABC-Stereotypy subscale scores with large effects (Cohen's d=0.8) (evidence level IIa (downgraded from Ib because it is based on secondary analyses). In a post hoc analysis of RUPP and RUPP2 (Aman et al., 2009; McCracken et al., 2002) risperidone studies, significantly greater decreases in ABC-Lethargy/Social Withdrawal and Hyperactivity subscale scores were observed in the risperidone- versus placebo-treated subjects (Scahill et al., 2013) (evidence level IIb). Common side effects reported in the studies included weight gain, elevated prolactin levels and sedation (seen in 37% of subjects), although sedation subsided after eight weeks of treatment (Aman et al., 2005a).
2. Aripiprazole is a D2 dopamine receptor subtype antagonist with some partial agonist properties (Kim et al., 2013). It is FDA-approved for irritability in ASD. It has been examined in ASD primarily to treat irritability, but secondary analyses have investigated effects for core symptoms. A recent Cochrane review of aripiprazole for ASD concluded that evidence from two RCTs suggested that it was effective as a short term medication for some behavioural aspects of ASD (Hirsch and Pringsheim, 2016). Significant improvements on the ABC-Stereotypy scale and CY-BOCS were reported from a large study in children and adolescents with ASD (Marcus et al., 2009) (evidence level IIa) (down-graded because this was not the primary outcome measure). In addition, a post hoc analysis that combined this study with another study (Aman et al., 2010; Owen et al., 2009) showed a significant improvement in the ABC stereotypic behaviour subscale scores, with greatest change on the item for repetitive hand, body or head movement (Aman et al., 2010) (evidence level IIa). The most common side effects reported were sedation (20%) and somnolence (10%) (Aman et al., 2010).

Taken together, the studies of dopamine receptor blockers provide evidence that these agents may be beneficial for the treatment of repetitive behaviours in ASD (evidence level IIa). However, there are some caveats: the outcome measures used do not differentiate between compulsions and stereotyped behaviours, the observed differences versus placebo were modest (20%), and the trials were short, hence there is no evidence to indicate if benefits are maintained. Furthermore, the level of clinical benefit due to reduction in these behaviours was not determined and the patient populations were all selected because of high levels of irritability, rather than high levels of repetitive behaviours. Overall, in view of the potential risk of adverse effects, it is not recommended that they are routinely used to treat repetitive behaviours. If they are used, a clear treatment goal should be agreed and a plan to measure this put in place; the risks and benefits should be carefully re-evaluated at regular intervals to ensure the balance continues to favour treatment. Furthermore, dosages should start small and build up over time.

Other approaches

Methylphenidate. Jahromi and colleagues conducted a four-week randomised, double-blind crossover sub-study of placebo in children with PDD and high levels of ADHD symptoms (Jahromi et al., 2009), as a part of a larger methylphenidate ASD trial (see below). Observational measures assessing social communication and self-regulation were recorded at each medication dose.

Sub-analysis of data from a larger methylphenidate ASD trial (Research Units on Pediatric Psychopharmacology (RUPP) Autism Network, 2005) in children with symptoms of ADHD reported dose-dependent improvements in joint attention and self-regulation (Jahromi et al., 2009). There was a moderate effect size

(Cohen's d=0.49) benefit for the best dose versus placebo for joint attention initiations and a moderate-large effect size (Cohen's d=0.61) for regulated affective state scores (Jahromi et al., 2009) (evidence level Ib). It is unclear whether these results are mediated by improvement in ADHD symptoms. Moreover, there is no theoretical reason to expect these findings generalise to individuals with ASD who do not have high levels of ADHD symptoms.

Oxytocin. Evidence from genetic and preclinical studies suggests that oxytocin plays a role in social recognition, attachment and stereotyped behaviours (Carter, 1998; Insel et al., 1999).

A small pilot study of intranasal oxytocin was conducted in adults with ASD (Anagnostou et al., 2012). This study reported no significant changes in the primary outcome measure (the Diagnostic Analysis of Nonverbal Accuracy and Repetitive Behaviour Scale Revised). However, there were significant changes in secondary outcomes, specifically the Reading the Mind in the Eyes Test, a measure related to social communication, and a quality of life questionnaire (Anagnostou et al., 2012). A further small study in adults with ASD reported a significant improvement in social reciprocity, as assessed by the ADOS. The effect size was large (Cohen's d=0.78), and improvement was correlated with increased functional connectivity between the anterior cingulate cortex and medial prefrontal cortex (Watanabe et al., 2015) (evidence level Ib). Results from studies in children and young people are also inconsistent. Guastella et al. (2015) conducted a medium-sized study in adolescent male individuals with ASD (evidence level Ib). Results did not suggest any clinical efficacy in primary outcomes including change in the Social Responsiveness Scale as rated by caregivers and the clinician rated CGI-Improvement scale. (Guastella et al., 2015) (evidence level Ib). A recent medium-sized study with intranasal oxytocin in young children showed significant improvement in the primary outcome of caregiver-rated social responsiveness (Yatawara et al., 2016) (evidence Ib).

In summary, the evidence for oxytocin shows some promise, but also inconsistencies, with the largest study failing to find clear benefits. Moreover, direct replications are lacking due to studies using different outcome measures. There are also few findings of improvements that directly relate to real world function. Additionally, little is known about the side effects of longer-term exposure to oxytocin (Okamoto et al., 2016). In sum, further studies are required to fully investigate oxytocin before it can be recommended for routine use.

Recommendations

Overall, the evidence is currently too limited to support the routine use of any of the agents discussed above for the core symptoms of ASD. Although risperidone and aripiprazole have both shown modest efficacy for the management of repetitive behaviours (evidence level IIa), these studies focussed on individuals with high levels of irritability and it is unclear whether the findings would generalise to the wider ASD population. Furthermore, side effects should be carefully considered. Oxytocin has shown some encouraging preliminary results for deficits in social cognition (evidence level Ib), although large scale randomised clinical trials for assessment of benefits for clinical outcomes and functioning and side effects of long-term exposure are warranted.

Clinical trials for ASD core symptoms in progress

There are at least 12 active studies of oxytocin currently underway and there is also one large phase 2 study of vasopressin in children with ASD (NCT01962870). Insulin growth factor-1 (IGF-1) is another promising target. Based on findings from preclinical studies, where it has been shown that IGF-1 ameliorates synaptic and behavioural deficits in SHANK3 deficient mice (Bozdagi et al., 2010, 2013), Kolevzon et al. conducted a small-scale feasibility study of IGF-1 treatment in nine children with PMS. Interestingly, this showed a statistically significant improvement in social impairment and restrictive behaviours (Kolevzon et al., 2014). Currently a large study on IGF-1 is taking place in children with ASD (NCT01970345), and the results of this, and further work on the potential long-term effects of IGF-1, are awaited.

Consensus recommendations of pharmacological management of core symptoms

- Evidence from clinical trials to date has not demonstrated clear efficacy for the use for any agent in the routine management of ASD core symptoms. (S)
- There is some evidence for the use of risperidone and aripiprazole in the management of repetitive behaviours but, in view of the potential adverse effects, routine use is not recommended for treating repetitive behaviours. If used, clinicians should weigh up the risks and benefits and re-evaluate these regularly. (B)

In the next sections, we summarise the results from studies of pharmacological treatments for co-occurring conditions and symptoms in ASD, discuss the limitations of the evidence, and finally, proceed with recommendations. As many co-occurring conditions and symptoms vary by age, we discuss findings in children separately (in the following section) from findings in adults (in the subsequent section). An overview of study designs and findings is given in Supplementary Table 8.

Pharmacological treatment of co-occurring conditions and symptoms in children with ASD

Treatment of depression in children with ASD.

SSRIs and other antidepressants are widely prescribed for people with ASD (Coury et al., 2012). However, there have been no rigorous studies that have investigated the role of SSRIs in treating mood disorders in children with ASD. Given the lack of direct evidence for SSRIs in ASD, the use of SSRIs to treat depression is therefore based on extrapolation from trials in patients without ASD (see BAP guidelines on major depression for recommendations; Cleare et al. (2015)). An additional consideration is the evidence of increased sensitivity to side effects of SSRIs (see chapter on pharmacological treatments of core symptoms) seen in children with ASD (King et al., 2009) (evidence level Ib). We therefore recommend that SSRIs should be used in low doses and titrated up gradually and monitored carefully for side-effects.

Treatment of anxiety and OCD in children with ASD

Although pharmacological treatment of anxiety disorders has not been studied specifically in ASD, symptoms of obsessive-compulsive disorder (OCD) and anxiety have been investigated in a number of trials.

Risperidone. A medium-sized trial in participants with ASD and high levels of irritability (but not necessarily anxiety) at two dose ranges (lower=0.125–0.175 mg/day; higher=1.25–1.75 mg/day) found improvement in OCD symptoms only in the high-dose group (Kent et al., 2013) (evidence level IIa). Similarly, a large study reported significant, albeit modest, improvements in OCD symptoms after risperidone treatment at 2 mg/day (McDougle et al., 2005).

In addition to improving symptoms of OCD, there is also evidence that risperidone may be effective at treating general symptoms of anxiety in ASD too. A medium-sized trial of risperidone in participants with ASD and high levels of irritability reported significant improvement relative to placebo on the insecure/anxious scale of the Nisonger Child Behavior Rating Form (N-CBRF) (parent version) (Shea et al., 2004) (evidence level IIa). However, it should be noted that a 16-week open-label study of 26 ASD child responders to risperidone reported increased anxiety in the mild-moderate range as a side-effect of treatment (Troost et al., 2005) (evidence level IIa).

Clomipramine. One small study that investigated clomipramine in children with ASD reported a significant improvement in OCD symptoms (Gordon et al., 1993) (evidence level IIa). However, cardiovascular side effects of clomipramine can be significant, and reports of treatment-emergent seizures have been noted (Alldredge, 1999; Pacher and Kecskemeti, 2004).

SSRIs. Two large studies have reported no effect of SSRIs (citalopram and fluoxetine) on obsessive-compulsive symptoms (Hollander et al., 2005; King et al., 2009). Neither drug was effective at reducing symptoms of OCD (evidence level IIa).

Overall, there is little or no evidence for treating anxiety or OCD symptoms with risperidone, clomipramine or an SSRI. The studies of risperidone are limited to participants with high levels of irritability and did not select participants on the basis of clinically significant anxiety or OCD symptoms. Hence it is unclear whether any positive effects are clinically meaningful or pertain to those with co-occurring anxiety/OCD. The same applies to studies of SSRIs, although here the combined evidence failed to identify an effect on anxiety or OCD symptoms. In view of the limited evidence, we recommend cautiously following the BAP guidelines for treating anxiety and OCD (Baldwin et al., 2014).

Treatment of sleep problems in children with ASD

Melatonin. A meta-analysis of five small studies supports the use of melatonin for sleep disorder in ASD (Rossignol and Frye, 2011) (evidence level Ia). Sleep duration was increased (the mean increase was 73 min versus baseline and 44 min versus placebo) and sleep onset latency decreased (mean decrease of 66 min compared with baseline, and in comparison with a 39 min decrease with placebo). However, there were no changes in night-time awakenings in children with ASD (Rossignol and Frye, 2011). The length of melatonin usage in these studies ranged from 14 days to over four years. Melatonin use was associated with minimal to no side effects. A further large study reported a small increase in total sleep time (by a mean of 22 min) and an improvement in sleep onset (with a mean improvement of 38 min), though waking times became earlier too (Gringras et al., 2012). There is also evidence that melatonin combined with cognitive-behavioural therapy (CBT) is superior to melatonin only, CBT only and placebo in reducing symptoms of insomnia (Cortesi et al., 2012). The combination group also had a greater proportion of treatment responders reaching clinically significant improvements and fewer dropouts after 12 weeks (Cortesi et al., 2012) (evidence level Ib). Thus, overall, melatonin has proven to be an effective and well-tolerated drug in treating sleeping problems in children with ASD. Adding a behavioural intervention may be of additional value, at least in the short-term.

Treatment of irritability in children with ASD

Common irritability symptoms seen in children with ASD include severe tantrums, aggression or self-injurious behaviours. It is important to note that irritability is more common in individuals with ASD and a co-occurring mood or anxiety disorder (Mayes et al., 2011). It is therefore important to consider and treat, if warranted, any co-occurring mood or anxiety disorder.

Risperidone was the first antipsychotic to receive approval by the USA FDA for irritability in ASD (Food and Drug Administration, 2006). It has been widely studied, with evidence from 10 RCTs reporting its efficacy (evidence level Ib). Several small studies have reported reductions in irritability with large effect sizes (0.7–1.03; Kent et al., 2013; Shea et al., 2004). Similarly, a large multi-site study reported a 57% reduction in irritability (effect size=1.2; McCracken et al., 2002). The most commonly reported side effects of risperidone were weight gain, increased appetite, fatigue, drowsiness and drooling. Long-term (six months) use of risperidone has been investigated by two small studies. Risperidone appears to be tolerated reasonably well but long-term (six months) use was associated with persistent side-effects, including increased appetite, weight gain, mild sedation, hypersalivation and hyperprolactinaemia (Luby et al., 2006; Nagaraj et al., 2006) (evidence level Ib). Hyperprolactinaemia, which is potentially caused by a blockade of dopamine receptors in the tubero-infundibular system (Howes et al., 2009), may normalise over the long-term (Findling and McNamara, 2004), however there is also evidence of increased prolactin after six months of treatment (Luby et al., 2006). In comparison to haloperidol, risperidone seems to be better tolerated with a smaller sedative effect and a lower risk of extrapyramidal symptoms (Miral et al., 2008).

Aripiprazole has also been approved by the FDA for the treatment of irritability in children with ASD (Waknine, 2010) and its effects studied in several RCTs. This evidence has been meta-analysed by Douglas-Hall et al. (2011) which reported a significant reduction in irritability relative to placebo with an effect size of 0.64 after eight weeks of treatment (evidence level Ib; downgraded because this study included only two RCTs (total n=316, dose range=2–15 mg/day). Similar to risperidone, side-effects of

aripiprazole included sedation, fatigue and increased appetite. Vomiting was also reported by some children. It is also noteworthy that no increase in serum prolactin was observed in the aripiprazole studies and reductions were seen in some children. This suggests that aripiprazole is preferable to risperidone in cases with concerns regarding hyperprolactinaemia. A line-item analysis of the ABC-I from the two RCTs revealed that aripiprazole had no effect on self-injurious behaviour, which was attributed to low baseline rates (Aman et al., 2010). Thus, although the construct of irritability includes self-injury, aripiprazole may not be helpful specifically for this symptom. A similar item analysis has not been performed for risperidone so it is unclear how this drug compares for self-injury.

A large long-term study of aripiprazole reported that the benefits of aripiprazole on irritability were maintained over the study period (Marcus et al., 2011). However, discontinuation due to side effects occurred in about 10%, with aggression and weight increase being the most commonly reported. No additional safety concerns were identified besides those evident in short-term exposure. Therefore, both risperidone and aripiprazole appear to retain most of their initial benefits on irritability seen in acute studies, and both agents are suitable for longer periods of treatment, with appropriate routine safety monitoring.

In conclusion, there is a reasonable body of evidence indicating that risperidone and aripiprazole are effective at treating irritability in ASD with moderate to large effect sizes. However, their potential benefits should be weighed against the risk of side effects. Behavioural and/or educational interventions should be considered prior to prescribing these drugs, given their side-effect profiles. It is recommended that if an antipsychotic is started, treatment targets should be set and progress against these regularly evaluated, and weighed against side-effects (including relevant medical assessments and laboratory tests) during treatment reviews. In view of the risk of persistent side-effects, we also recommend periodic attempts to reduce the daily dosage and discontinue to either confirm the necessity for on-going exposure, or establish that the need for the drug has resolved.

Other approaches to treating irritability in children with ASD

Minocycline has been investigated in an open-label add-on pilot study of individuals with FXS (Paribello et al., 2010). Minocycline significantly reduced irritability ratings and improved secondary outcome measures, including the CGI-I (average score 'mildly improved') and a visual analogue scale (VAS) for behaviour (Paribello et al., 2010) (evidence level IIb). The most common side effects were dizziness and diarrhoea. A medium-sized trial also in subjects with FSX reported a modest improvement (2.49 versus 2.97, minocycline versus placebo respectively) on CGI-I ratings, but not in any of the secondary outcomes including the ABC–C scale (Leigh et al., 2013) (evidence level Ib). Overall, minocycline's potential benefit for reducing irritability needs additional study before routine use can be recommended, particularly in non-FSX ASD populations.

Arbaclofen has been studied in children with ASD for irritability with inconsistent findings to date. One open-label study reported significant improvement on irritability ratings (Erickson et al., 2014) but two medium and large controlled trials reported no change on irritability ratings (Berry-Kravis et al., 2012; Veenstra-VanderWeele et al., 2016) (evidence level Ib).

Amantadine is a non-competitive NMDA antagonist. Despite encouraging case reports and small open-label studies, a small controlled trial by King et al. (2001) reported no effect of amantadine on responder rate or irritability ratings (evidence Ib). However, there were significant (albeit, modest) differences in clinician ratings for hyperactivity (amantadine reduction of –6.4 versus placebo reduction of –2.1) and inappropriate speech (amantadine reduction of –1.9 versus placebo reduction of 0.4) (King et al., 2001) (down-graded to evidence level IIa as a secondary analysis). No parent-reported measures were identified as being significantly different. Thus, current evidence does not support the use of amantadine for irritability.

In view of the limited data available for minocycline, randomised, double-blind controlled studies are required before recommendations can be made. The current evidence does not support the use of arbaclofen or amantadine for irritability.

Treatment of ADHD and hyperactivity symptoms in children with ASD

Methylphenidate has been reported as an effective treatment for ADHD in children with ASD by a meta-analysis of four studies (effect size=0.67) (evidence level Ia, but note this is based on only four studies) (Reichow et al., 2013). A variety of different ADHD outcome measures were used in these studies and the duration of exposure ranged between 1–4 weeks (see Supplementary Table 8). There is also evidence that the response rate to methylphenidate in individuals with ASD and ADHD is lower than in individuals with ADHD without ASD. For example, one medium-sized study reported a response rate of 50% in ASD subjects with symptoms of ADHD (Research Units on Pediatric Psychopharmacology Autism Network, 2005) compared to response rates of 70–80% in children with ADHD without ASD (Jensen, 1999). The severity of side-effects may also be greater in individuals with ASD and ADHD compared to individuals with ADHD without ASD. Discontinuation rates due to side effects were much higher in the ASD study (18%) compared to the non-ASD study (1.4%) (Jensen, 1999; Research Units on Pediatric Psychopharmacology Autism Network, 2005). The most commonly reported side effects in children with ASD were decreased appetite, sleeping difficulties, abdominal discomfort, social withdrawal, irritability and emotional outbursts, mostly similar to those seen in the treatment of ADHD for people without ASD. Taken together, these findings suggest that, although effective, methylphenidate may not be as effective in people with ASD as in people with ADHD and that individuals with ASD are more likely to experience side-effects.

Atomoxetine, a non-stimulant drug for ADHD is an alternative to methylphenidate. Evidence from one small and one medium study demonstrate improvement in symptoms of hyperactivity but not inattention (Hedge's g=0.83, effect size d=0.90) (Arnold et al., 2006; Harfterkamp et al., 2012) (evidence level Ib). The most common side effects were nausea, fatigue and sleeping difficulties (Arnold et al., 2006). A further large study investigated individual and combined-effectiveness of atomoxetine and parent training (PT). Atomoxetine, (both alone and combined with PT) significantly reduced ADHD symptoms (Handen et al., 2015). The authors conducted a 24-week extension study

demonstrating that atomoxetine combined with PT was superior at reducing ADHD symptoms than atomoxetine alone (Smith et al., 2016). The effect sizes reported in the atomoxetine studies (0.59–0.98) are similar to the effect size reported for methylphenidate in children with ASD (0.67) (Research Units on Pediatric Psychopharmacology Autism Network, 2005), suggesting equivalent efficacy.

The α2A receptor agonist antihypertensive-drugs clonidine, guanfacine and lofexidine have also been examined as treatments for ADHD in children with ASD.

Clonidine. Two small studies have reported improvements in symptoms ADHD – in particular, symptoms of hyperactivity (Fankhauser et al., 1992; Jaselskis et al., 1992) (evidence level IIa down-graded because of the small sample sizes). Reported side-effects included sedation, drowsiness, fatigue and decreased activity. Guanfacine appears to be less sedating than clonidine with promising evidence for its efficacy according to two studies (one small, one medium) (Scahill et al., 2006, 2015) (evidence level Ib). The study authors report a response rate of 50%, which is comparable to the group's earlier methylphenidate response rate of 48% (Research Units on Pediatric Psychopharmacology (RUPP) Autism Network, 2005). Notable side-effects included drowsiness, irritability, reduced blood pressure and bradycardia. There is some preliminary evidence for lofexidine based on one small, non-randomised study, which reported significant improvement in ADHD (in particular, hyperactivity) (Niederhofer et al., 2002), but this is insufficient evidence to support routine use (evidence level Ib).

In summary, there is good evidence that methylphenidate is an effective treatment for co-occurring ADHD in children with ASD. Atomoxetine should be considered as a good alternative to methylphenidate. There is also promising evidence for α2A receptor agonists, which should also be considered as alternatives amongst those who are not responsive or intolerant to this class of medication. Reports suggest that risperidone and aripiprazole significantly improve scores on the ABC-H relative to placebo, suggesting these drugs may also be useful. Further studies are required in samples of ASD+ADHD to confirm their effectiveness, since the samples were not selected for ADHD and the ABC-H was not the primary outcome measure. Treatment effect sizes are generally lower in ASD than typically developing populations, and, at least for stimulants, levels of adverse effects are higher. Close periodic monitoring of side effects (including relevant medical assessments) is thus of high importance where these treatments are used in ASD.

Pharmacological treatment of co-occurring conditions and symptoms in adults with ASD

Treatment of depression in adults with ASD

The evidence for treating mood disorders in adults with ASD is very limited. Only one SSRI (fluoxetine) has been studied in adults with ASD (Buchsbaum et al., 2001). This small study reported no change in depression relative to placebo (Buchsbaum et al., 2001) (evidence level IIa). Secondary analysis of risperidone in a trial for repetitive behaviours demonstrated significant reductions on a VAS for mood in a non-clinically depressed group (McDougle et al., 1998). The efficacy of risperidone for clinical depression in ASD remains to be tested.

Given the limited evidence-base of studies in ASD groups, we recommend following the BAP guidelines for treating depression in ASD (Cleare et al., 2015). These should be applied cautiously given the apparent increased propensity for behavioural activation associated with antidepressants in youth with ASD and that these guidelines are not specific to people with ASD (Vasa et al., 2014).

Treatment of anxiety and OCD in adults with ASD

Evidence for treating anxiety in adults with ASD is also limited and studies have been mainly focused on obsessive/compulsive symptoms.

1. Fluoxetine has been studied for anxiety in ASD in two small studies. One reported a significant improvement in obsessions but not compulsions (Buchsbaum et al., 2001) (evidence level IIa) and the other reported a significant reduction in self-reported compulsions (Hollander et al., 2012) (evidence level Ib). No change in compulsions was found in ratings by independent observers.
2. Fluvoxamine has been reported to reduce symptoms of both obsessions and compulsions by one small study at eight and 12 weeks (McDougle et al., 1996) (evidence level Ib). Apart from nausea and mild sedation in a few patients, fluvoxamine was well tolerated.
3. Risperidone was reported to reduce symptoms of anxiety/nervousness on a clinician-rated VAS and self-reported compulsions in a study of repetitive behaviours in ASD (McDougle et al., 1998) (evidence level Ib). The participants all scored above 10 on the Y-BOCS compulsion subscale at entry, indicating at least mild severity at baseline.

In summary, benefits have been reported in small studies using SSRIs as a treatment for anxiety disorders, predominantly OCD, in adults with ASD. Although SSRIs are generally well tolerated, the beneficial effects are modest, and the evidence is limited. There is currently insufficient evidence to recommend risperidone. In view of the limited specific evidence in ASD, we therefore recommend following the BAP guidelines for treating anxiety (Baldwin et al., 2014), but, as with the treatment of mood disorders, we would recommend proceeding cautiously.

Treatment of sleep problems in adults with ASD

Despite the evidence for its effectiveness in children with ASD, there are currently no published clinical trials of melatonin in adults with ASD. One small (n=6) retrospective study reported that melatonin was effective in reducing sleep onset latency and nocturnal awakenings and improved total sleep time (Galli-Carminati et al., 2009) (evidence level III). Effects remained after six months and no side effects were noted during the therapy.

Table 1. Consensus recommendations: Pharmacological treatment of co-occurring conditions and symptoms in children and adults with ASD.

	Children	Adults
Mood disorders	Decision on treatment needs to be made on a case-by-case basis. Follow the BAP guidelines for treating depression. (S)	Decision on treatment needs to be made on a case-by-case basis. Follow the BAP guidelines for treating depression. (S)
Anxiety disorders	Consider a cautious trial of an SSRIs followed by risperidone if poor response. Monitor for worsening of anxiety in some children. (B)	Decision on treatment needs to be made on a case-by-case basis. Follow the BAP guidelines for treating anxiety. (S)
Sleep disorders	Melatonin, if possible, in combination with a behavioural intervention. (A) Prolonged use of benzodiazepines and related GABA agonists is not recommended. (S)	Melatonin, if possible, in combination with behavioural intervention (extrapolation from findings in children). (S) Prolonged use of benzodiazepines and related GABA agonists is not recommended. (S)
Irritability	Risperidone or aripiprazole but only when behavioural or educational approaches have failed. (A)	Decision on treatment needs to be made on a case-by-case basis. Aripiprazole or risperidone or an SSRI should only be considered cautiously and after considering alternatives. (S)
ADHD	First line: methylphenidate. Second line: atomoxetine, or α2A receptor agonist. Children with ASD may experience more side-effects and show less response than non-ASD patients with ADHD. (A)	Decision on treatment needs to be made on a case-by-case basis. Follow the BAP guidelines for treating ADHD. (S)
Tic disorders and Tourette syndrome	Decision on treatment needs to be made on a case-by-case basis. (S)	Decision on treatment needs to be made on a case-by-case basis. (S)

ADHD: attention deficit/hyperactivity disorder; ASD: autism spectrum disorder; BAP: British Association for Psychopharmacology; GABA: gamma-aminobutyric acid; SSRI: selective serotonin re-uptake inhibitor.

Given the limited evidence, recommendations must be made by extrapolation from studies in children and adults without ASD. We recommend following the BAP guidelines on sleep disorders (Wilson et al., 2010) with the same general caveats discussed for mood and anxiety disorders. In addition, it is worth considering an early trial of melatonin, in view of the benefit in children, and its favourable side-effect profile. We do not recommend the prolonged use of benzodiazepines and related GABA-agonists due to the risk of tolerance and side-effects, in line with the BAP guidelines on sleep disorders.

Treatment of irritability in adults with ASD

Treating irritability has been less well studied in adults than it has been in children with ASD.

Risperidone was reported to significantly reduce symptoms of irritability and aggression in a small study after 12 weeks (McDougle et al., 1998) (evidence level IIa). The same group also investigated fluvoxamine in a small study that reported a reduction in aggression after 12 weeks of treatment (McDougle et al., 1996) (evidence level IIa). In both these studies, irritability and aggression were not the primary outcome measures. A small study of fluoxetine reported no effect on irritability, although this was not the primary outcome of this (Hollander et al., 2012) (evidence level IIa). A small study of pregnenolone reported significant improvement in irritability (Fung et al., 2014) (evidence level IIb).

In summary, there is limited evidence to guide the treatment of irritability in adults with ASD. A dopamine blocker such as risperidone or SSRI could be tried cautiously and side-effects should be carefully monitored (including relevant medical assessments and laboratory tests). Alternatives such as behavioural approaches should also be considered first (see section on non-pharmacological approaches below). Further studies on pregnenolone are warranted.

Treatment of ADHD in adults with ASD

There have not been any RCTs that have investigated the role of stimulant or non-stimulant medications in treating ADHD in adults with ASD. In view of this we recommend cautiously following the BAP guidelines for treating ADHD (Bolea-Alamañac et al., 2014), with the same general caveats discussed above for mood and anxiety disorders.

Treatment of tic and Tourette syndrome in ASD

No current studies are available for treating tic disorders in children or adults with ASD specifically. A recent review (Whittington et al., 2016) on tic disorders in the absence of ASD reported evidence favouring the use of the a2-adrenergic receptor agonists clonidine and guanfacine (standardised mean difference= −0.71; 95% confidence interval (CI) −1.03 to −0.40; evidence level Ia). This was based on four trials with a total n=164. As there are no studies on treating tic disorders in ASD we would recommend that the decision on using α2A receptor agonists with ASD is made on a case-by-case basis.

Summary

Most RCTs in ASD have centred on children and adolescents, and have overwhelmingly been focused on symptoms,

not co-occurring disorders. There is some limited evidence to suggest that both treatment response and side effects to pharmacological interventions differ from the general population, suggesting extrapolation from findings in non-ASD populations should be made cautiously (evidence level IIb). Currently the best studied medication classes include dopamine blockers to target irritability and drugs targeting ADHD symptoms (methylphenidate, atomoxetine, α2A receptor agonists). Secondary data analyses suggest the antipsychotics have modest benefits on repetitive behaviours. However, there remain very significant gaps in knowledge particularly with respect to some of the most common co-occurring conditions (e.g. anxiety and mood disorders) and some of the most widely prescribed drugs (e.g. antidepressants).

Consensus recommendations of pharmacological treatment of co-occurring conditions and symptoms in children and adults with ASD can be found in Table 1.

Non-pharmacological approaches for core symptoms of ASD in children

A full analysis of psychological interventions for ASD is beyond the scope of these guidelines. However, we summarise key elements of the evidence to provide context for the other aspects of management discussed, particularly drawing on recent National Institute for Health and Clinical Excellence (NICE) (National Institute for Health and Clinical Excellence, 2013) and US Agency for Healthcare Research and Quality (AHRQ) (Weitlauf et al., 2014) reviews of behavioural interventions.

Social-communication interventions

Individual focused interventions can be delivered to young children. They are commonly mediated by parents, teachers or peers and can be combined with joint-attention approaches and applied behaviour analysis (ABA). These interventions typically include developing patterns of communication that are directed by the child's interest in activities, repeating back or expanding on what the child says, sitting close to the child and making eye-contact and using mirroring/imitation of the child's actions. Parent-mediated interventions use parent-training programmes to improve parental sensitivity and responsiveness to child communication through techniques such as therapist-lead instruction and video feedback. Most programmes available for parents of children with ASD have been developed specifically for young preschool children with ASD. The effectiveness of such programmes has been assessed by NICE (National Institute for Clinical Excellence, 2012). This found there was evidence of efficacy for some of these programmes over treatment as usual (TAU) at reducing symptoms of social interaction impairment including communication acts, parent–child joint attention and joint engagement. However, these effects were small and often not clinically meaningful. For example, Green et al. (2010) compared the Preschool Autism Communication Trial (PACT) to TAU. ADOS-G scores were reduced by 3.9 points in the PACT group and 2.9 points in the TAU group, representing a between group effect size of –0.24. However, both parent synchrony and child initiations were improved in the short-term by the PACT treatment (Green et al., 2010) and recent long-term follow-up has reported reduced ASD symptoms as measured by the ADOS and sustained increases in child initiations six years after treatment ended (Pickles et al., 2016). Similarly, a RCT of Hanan's 'More Than Words' intervention versus TAU found no main effect of treatment on child outcomes immediately or at five months after treatment (Carter et al., 2011). However, there were gains on child communication at nine months which were moderated by baseline object interest (lower object interest=greater gains). Two further RCTs of parent-mediated interventions published after the NICE and AHRQ reviews indicate improvements in parent–child interactions (Kasari et al., 2014; Wetherby et al., 2014).

Peer-mediated social-communication interventions can be delivered to school-aged children. These typically involve free-play sessions between a child with ASD and typically-developing children who have undergone preparatory training. There is evidence (level Ib) of the effectiveness of such interventions on the core feature of reciprocal social communication and peer–child joint engagement from four RCTs and one non-randomised trial (see recent review by Chang and Locke (2016)). However, most of these studies were conducted with high functioning children with ASD. Hence there is a need for further research to establish the effectiveness of peer-mediated interventions in other age and functional groups with ASD.

There is also evidence (level Ib) of modest gains on the quality and frequency of social play after the parent-assisted 'Children's Friendship Training (CFT)' social skills group training programme (Frankel et al., 2010). This programme has also shown effectiveness on social skills in children with ADHD (Frankel et al., 1995, 1997) and children with foetal alcohol spectrum disorders (O'Connor et al., 2006). The CFT and other similar social skills interventions (Koenig et al., 2010; Lopata et al., 2010) typically involve mixed clinical groups with or without typically developing peers and the teaching of social skills through instruction, modelling, rehearsal, role-play, performance feedback and homework. The CFT programme has been adapted for adolescents and found to have beneficial effects on social skills among teens 13–17 years of age (Laugeson et al., 2009).

Behavioural interventions

Although not explicitly recommended in the NICE guidelines, the AHRQ review found evidence that a number of interventions based on high-intensity ABA applied over an extended time-frame had a positive effect on cognitive functioning and language skills (Weitlauf et al., 2014). These interventions include the Learning Experiences and Alternative Program for Preschoolers and their Parents (LEAP), the Lovaas Model and the Early Start Denver Model (ESDM). Of the 10 studies included in the AHRQ review, only two were RCTs and both were conducted in the USA (Dawson et al., 2010, 2012; Strain and Bovey, 2011), where health and education services may not readily generalisable to other settings. There is also evidence (level Ib) for combined joint attention training and ABA-based interventions. Two studies reviewed by NICE (Kasari et al., 2006; Landa et al., 2011) showed large effects (standardised mean difference [SMD]=1.11) of additional joint-attention training for the child responding to joint attention during child–examiner interactions, moderate to large effects

(SMD=0.55–0.69) on the duration of child-initiated joint attention during mother–child interaction and moderate effects (SMD=0.69) on pointing during examiner–child interaction. However, there is criticism that ABA does not generalise beyond the skills trained and thus should be combined with other interventions to promote the use of skills across settings (Smith, 2001). Hence, the effectiveness of ABA may be limited to the specific skills taught.

Alternative interventions

A number of alternative therapies, such as exclusion diets, secretin, chelation and hyperbaric oxygen therapy, have been tried for ASD. The evidence available indicates that exclusion diets such as gluten- or casein-free diets should not be routinely used for the management of core features of ASD (National Institute for Health and Clinical Excellence, 2013). Moreover, the available evidence indicates that secretin treatment is not effective (Sandler et al., 1999). Chelation and hyperbaric oxygen therapy are potentially harmful with little evidence of benefit and should not be used to manage ASD in any context (Davis et al., 2013; Goldfarb et al., 2016; National Institute for Clinical Excellence, 2012).

Recommendations

We recommend considering a specific social-communication intervention for the core features of ASD in children and adolescents that includes play-based strategies with parents, carers and teachers to increase joint attention, engagement and reciprocal communication. These interventions may also support the parents', carers', teachers' or peers' understanding of, and sensitivity and responsiveness to, the child or young person.

Consensus recommendations of non-pharmacological approaches for children and adolescents

1. A specific social-communication intervention should be offered to children and adolescents according to their developmental level. (A)
2. Social skills training should be offered to adolescents in either group or individual sessions. (A)
3. Exclusion diets and secretin, chelation and hyperbaric oxygen therapy should not be used for the management of ASD in children or adolescents. (D)

Psychological approaches to ASD in adults

A full analysis of psychological approaches for adults with ASD is beyond the scope of these BAP guidelines. In the following section, we summarise the key points to provide context for other aspects of management considered, drawing on the NICE guidelines and AHRQ review.

Social learning programmes

NICE guidelines recommend group- or individual social learning programmes for adults with ASD without a learning disability (LD) or with a mild to moderate LD, who have problems with social interaction. Social learning programmes to improve social interaction deficits apply behavioural therapy techniques within a social learning framework, such as using video modelling, peer/individual feedback, imitation and reinforcement to teach conventions of social engagement. There is evidence (level III) from observational studies in adults with ASD that suggest social skills groups may be effective at improving social interaction (Hillier et al., 2007; Howlin and Yates, 1999). However, the only RCT of social skills training found no positive treatment effect of emotion recognition training on general emotion recognition (Golan and Baron-Cohen, 2006), suggesting that social interaction programmes may only be effective when they include a more general social learning component.

Behavioural and life-skills interventions

Adults with ASD of all ranges of intellectual ability who need help with activities of daily living can be offered a structured, predictable training programme based on behavioural principles. However, the evidence (level Ib) of the effectiveness of these programmes is indirect and largely reliant on studies of adults with a learning disability (Matson et al., 1981).

In adults with ASD without LD or with mild to moderate LD who are socially isolated or have restricted social contact, interventions should focus on the acquisition of life skills based on the specific need of the individual. In recent years, there has been increased interest in providing structured leisure activities for people with ASD. There is evidence (level Ib) of the effectiveness of these programmes on overall quality of life and emotion recognition from two RCT's (see review by National Institute for Clinical Excellence, 2012).

Cognitive-behavioural interventions

CBT can help adults with ASD across a range of domains. Principally, CBT is effective at treating anxiety and OCD, and supporting adults who have difficulties with victimisation and obtaining or maintaining employment. Evidence (level II) from a systematic review demonstrates the effectiveness of CBT for anxiety in ASD (Lang et al., 2010). However, there is also evidence that anxiety management performs just as well as CBT in reducing symptoms of OCD in individuals with ASD (Russell et al., 2013).

Cognitive-behavioural interventions can be implemented to support individuals with ASD who are at risk of victimisation by teaching decision-making and problem-solving skills. Evidence (level Ib) from two RCTs suggests that CBT in adults with LD is effective at increasing skills to deal with victimisation (Khemka, 2000; Khemka et al., 2005). However, these studies are limited by including cases without ASD. Individual support programmes can be used to improve employment and job retention. Studies of supported employment programmes are consistently positive despite methodological concerns including lack of randomisation in one study.

Facilitated communication

Facilitated communication uses a facilitator to support the arm movement of an individual with ASD to point at letters on an alphabet board, keyboard or similar device. Positive reports of its effectiveness are almost exclusively based on anecdotal evidence (Biklen, 1990; Biklen et al., 1992, 1995; Biklen and

Schubert, 1991; Clarkson, 1994; Crossley and Remington-Gurney, 1992; Heckler, 1994; Janzen-Wilde et al., 1995; Olney, 1995; Sabin and Donnellan, 1993; Sheehan and Matuozzi, 1996; Weiss et al., 1996). There is no evidence of positive effects from any scientific study (Bebko et al., 1996; Beck and Pirovano, 1996; Bomba et al., 1996; Braman et al., 1995; Crews et al., 1995; Eberlin et al., 1993; Edelson et al., 1998; Hirshoren and Gregory, 1995; Hudson et al., 1993; Klewe, 1993; Konstantareas and Gravelle, 1998; Montee et al., 1995; Myles and Simpson, 1994; Myles et al., 1996; Oswald, 1994; Regal et al., 1994; Simon et al., 1996; Simpson and Myles, 1995a; Smith and Belcher, 1993; Smith et al., 1994; Szempruch and Jacobson, 1993; Vázquez, 1994; Wheeler et al., 1993). In addition to the lack of empirical support, there is evidence that facilitated communication can lead to significant harm. For example, there have been unsubstantiated claims of sexual abuse against family members made when using facilitated communication (Rimland, 1992; Simpson and Myles, 1995b). For these reasons, NICE strongly recommends that facilitated communication is not used (National Institute for Clinical Excellence, 2012).

Recommendations

It is recommended that adults with ASD are offered psychological interventions to optimise personal functioning, including developing the skills necessary for access to public transport, employment and leisure facilities. Interventions should focus on supporting access to community activities and increasing the individual's quality of life. Furthermore, psychological approaches can be used to help with acceptance of their difficulties, treat co-occurring conditions and to teach life skills specific to the needs of the individual.

Consensus recommendations of psychological approaches with adults

1. For adults with problems with social interaction, consider a group or individual social-learning programme. (A)
2. Do not provide facilitated communication for adults with ASD. (D)
3. For adults who need help with activities of daily living, consider offering training programmes based on behavioural principles. (A)

Service provision

The NICE guidelines published in 2011 recommend the development of multi-agency teams for people with ASD that include representatives from child health and mental health services, education, social care and the voluntary sector. However, service provision varies greatly and in many settings is significantly weighted towards diagnosis and children's services rather than treatment and support or adult care. In particular, there are unmet needs around common co-existing conditions including feeding problems, sleep problems, anxiety, hyperactivity and sensory problems (Maskey et al., 2013). Unfortunately, there has been very little health service research that has focused on ASD. In view of this, we review the available evidence below and make recommendations for service provision for children and adults, but it should be appreciated that these are largely based on the expert opinion of the working group.

Diagnostic services

Timely and valid diagnosis is important as early diagnosis and provision of appropriate management services is likely to improve long-term outcome (Magiati et al., 2014; Oono et al., 2013). Referral and diagnostic pathways for ASD vary, but in most cases, the initial concerns raised by parents or professionals will be directed to a general practitioner (GP), speech and language therapist or an educational psychologist. These will undertake an initial assessment and decide to refer the patient to children's health or mental health services for a full assessment. A full assessment should be made by professionals who are trained in the assessment, diagnosis and treatment of ASD using a combination of diagnostic tools, assessments and clinical experience (level IV). In general, diagnosis should not be formulated by one single professional and should involve a multi-disciplinary team (MDT) (Penner et al., 2017). This should ideally consist of a speech and language therapist, a clinical psychologist, paediatrician or child psychiatrist, and an occupational therapist. However, there is significant variation in service provision. For example, 47% of teams surveyed in the UK do not have access to a clinical psychologist (Palmer et al., 2010).

It is noteworthy that studies from some settings report a correlation between lower socioeconomic status and later diagnosis (Goin-Kochel et al., 2006), suggesting that there are social impediments to referral and diagnosis. However, studies in settings where there is universal free healthcare (such as the UK) do not show this relationship, or even show the opposite relationship (Brett et al., 2016). Other factors reportedly associated with earlier age of diagnosis in the UK include a 'core' ASD diagnosis (as opposed to broader ASD), language regression or delay, and greater degree of support needed (Brett et al., 2016). Furthermore, there is evidence of a sex bias in referral and diagnosis even in settings such as the UK, suggesting a delayed recognition of the disorder in young girls (Brett et al., 2016; Rutherford et al., 2016). Moreover, within the UK there is regional variation in the diagnostic services available (Gray et al., 2015; Palmer et al., 2010; Parr et al., 2013) (level IV).

Given these findings, there is a need for clear referral pathways that ensure adequate assessment is available to all who need it (Buckley, 2016). This needs to be coupled with increased training to raise awareness of ASD among GPs and healthcare visitors (level IV).

Management/treatment services

Given the complexity of ASD, its treatment and monitoring should be conducted by professionals who are trained and experienced in treating and monitoring ASD (S). For patients with severe symptoms, a structured multi-disciplinary approach that includes regular reviews of the overall care package, such as the care programme approach, is indicated (S). Ideally, treatment should be managed by a specialist MDT with experience in ASD and related disorders. Where this is not possible, services should consider a consultation-liaison model where recommendations are made by a specialist team but implemented by general health services such as general psychiatrists, paediatricians or GPs, with further liaison depending on response (S).

Individuals with ASD who are in child and adolescent services should be reassessed around 14 years of age to establish the need for continuing treatment into adulthood (S) (National Institute for Health and Clinical Excellence, 2013). Where ongoing treatment is required, arrangements should be made for a smooth transition into adult services and the individual kept informed about the treatment and services available to them (S) (National Institute for Health and Clinical Excellence, 2013). Information about adult services should be provided to the young person, and their parents or carers, including their right to a social care assessment at age 18 (S).

Finally, there is a clear need for health services research to evaluate diagnostic and treatment service models, both in terms of clinical outcomes and cost-effectiveness.

Improving services

Given the importance of timely diagnosis, early identification and referral is a priority. GP initiatives and specialist training for health visitors are recommended to improve early identification (S). Diagnostic and treatment services should be led by MDTs that consist of a minimum of a psychiatrist (or paediatrician where appropriate), a speech and language therapist, and a psychologist. Treatment recommendations should be made alongside diagnosis and followed up by a team experienced in treating ASD, using a structured care approach, such as the Care Programme Approach, particularly for people with severe and complex needs. There is a possibility that referral rates for ASD will increase given the greater public awareness and diagnostic service availability. This poses a challenge to services that are trying to provide a valid and timely diagnosis. It is therefore important to use screening tools in order to target service resources. Finally, it is important to consider costs when assessing current services or developing new ones. Services should be audited regularly to ensure quality and accessibility of care for patients.

Recommendations

There is large variation in services between settings and little research on the optimum service provision or cost-effectiveness. There is a need for well-designed studies to evaluate models of service provision, and RCTs developed to test these using patient experience, functional outcomes in addition to standard clinical measures (strength of recommendation: D). Finally, it is important to consider costs when assessing current services or developing new ones, and future studies of service provision should include cost-analysis (strength of recommendation: D). Services should be audited regularly to ensure quality and accessibility of care for patients (strength of recommendation: D).

Consensus recommendations for service provision

1. Reassess individuals with ASD in children's services during adolescence well in advance of the transition date to establish the need for continuing treatment. (S)
2. If continuing treatment is necessary, make arrangements for a smooth transition to adult services or GP and give information to the young person about the treatment and services they may need. (S)
3. For young people and adults whose needs are complex or severe, use the care programme approach or similar structured approach to coordinate their needs and to aid the transfer between services. (S)
4. Involve the individual with ASD in care planning and, where appropriate, their parents or carers. (S)
5. Provide information about adult services to the young person, and their parents or carers, including their right to a social care assessment at age 18 years. (S)

Future directions

The earlier sections have highlighted that there are a number of limitations and areas of uncertainty, particularly for core ASD symptoms. The following section discusses these and makes recommendations for future research.

Future design of clinical trials

Many studies are open-label and/or small scale, or lack an adequate placebo group. Hence, there is a general need for positive findings from these initial exploratory and proof-of-concept studies to be followed-up with large-scale randomised placebo-controlled trials. This presents a number of challenges for the field. First, as some potentially useful treatments are off-patent, the field will not be able to rely on the pharmaceutical industry to fund studies of these compounds. Thus, funding will need to be sought from other sources, including government funded agencies, and charities and foundations. Second, the field will need infrastructure for large-scale trials across many settings. This will also involve centres developing the capacity to screen and recruit people with ASD to clinical trials, and is likely to benefit from the involvement of support groups and charities (Warnell et al., 2015) (strength of recommendation: D).

One major limitation of the literature to date is that only a small number of clinical trials have included participants below the age of five years. Interventions that begin before the age of five years may have the most dramatic effect (Aman et al., 2015) as ASD symptoms start emerging and brain plasticity is at its peak during this period. However, there are important safety considerations regarding the use of pharmacological interventions in paediatric populations (Kearns et al., 2003). Consequently, it will be necessary to develop and trial interventions in this period both to prevent the onset of ASD and minimise its effects on brain and cognitive development (strength of recommendation: D). Another general limitation of studies to date is that individuals with ASD who have an intellectual disability are usually excluded, despite the fact that over a third of people with ASD have an intellectual disability (Baio, 2012). Thus, to be representative, future studies should include people with ASD and intellectual disability, and need to adopt designs that facilitate this (strength of recommendation: D). Clearly both these issues will involve overcoming the ethical and practical challenges of enrolling young children and people with intellectual disability into clinical trials. This may be helped by wider engagement with individuals and their families regarding the design of clinical trials. Another issue that needs addressing is the duration of trials, which have mostly been weeks or at most a few months to date. Given the long-standing and pervasive nature of core symptoms, it is highly unlikely that core

symptoms will improve in a few weeks or months and, where there is change, it is necessary to show that this is sustained (strength of recommendation: D).

Non-pharmacological interventions and service provision

There is a lack of large-scale, multicentre RCT's investigating the effectiveness of social-communication interventions, ABA, behaviourally-based life skills training and anti-victimisation CBT. Studies are required to assess the effectiveness of these interventions across a range of outcome measures including cost-effectiveness and quality of life (strength of recommendation: D). Similarly, models of service provision are under-tested and require empirical evaluation (strength of recommendation: D). These studies should use patient experience and functional outcomes in addition to standard clinical measures of improvement.

Future outcome measures

Another critical issue that needs to be addressed is the lack of agreement on the outcome measures to be used in trials to accurately capture changes in core ASD symptoms over time (Aman et al., 2004). An optimal tool needs to be reliable and suitable for repeated administration. Current functioning should also be a focus. Aman and colleagues provide a comprehensive review of potential instruments and also point out that other aspects such as language, intellectual level and adaptive behaviour should be incorporated in the outcome measures (Aman et al., 2004) (strength of recommendation: D). Another obstacle that needs to be considered in pharmacological studies is that in the absence of objective psychometric measures, evaluation of symptom change in children depends on the parent-report measures, which are prone to expectancy bias (Aman et al., 2015). Thus, blinding and placebo control is important, and findings from open-label studies should be treated with caution. Last, but not least, the perspective of individuals with ASD and their carers should be taken into account when deciding on outcome measures. A recent systematic review highlighted the disparity in the outcomes identified as important by parents and those identified by health professionals: parents highlighted the importance of social participation and emotional well–being, whereas health professionals concentrated on the content of the available instruments they have (McConachie et al., 2015). A tool that indexes the quality of life of individuals with ASD that should be included as an outcome measure in future clinical trials (strength of recommendation: D). Furthermore, monitoring of adverse effects should not be limited to studies of pharmacological interventions. Adverse effects should be monitored when studying psychological and other interventions too.

The challenge of biological heterogeneity

On top of these difficulties in designing clinical trials, another challenge is the genetic and neurobiological heterogeneity seen in ASD, which means that it is unlikely that any single drug will be effective for all patients. It is clear that we need better understanding of the neurobiology underlying ASD to identify key molecular and system pathways that are disrupted, and the determinants of heterogeneity. This will enable the development of treatments that target key components of the neurobiology. Coupled with this we need biomarkers to identify sub-types that will respond to particular approaches (Loth et al., 2016b). A considerable amount of work is currently on-going to develop imaging, genetic, proteomic and other biomarkers for this purpose (e.g. Loth et al., 2017). To date there is no independently validated biomarker for stratification of patients, and trials have rarely attempted to include biomarkers that would enable stratification. Thus, it is largely unknown if there are sub-groups that showed better or worse response in past trials.

Syndromic types of ASD with a defined genetic basis can be used in the absence of biomarkers. Pathophysiological changes are likely to more homogenous in syndromic ASD (e.g. FXS). As discussed earlier, open-label studies on FXS using lithium (Berry-Kravis et al., 2008) and minocycline (Paribello et al., 2010) have shown encouraging results, suggesting the potential of this approach, although studies need to be replicated in randomised, double-blind controlled studies. This highlights two over-riding issues; first of all, the level of complexity in the neurobiology of ASD (Ghosh et al., 2013) and secondly the importance of either conducting the studies in a clinically and biologically homogenous groups or including biomarkers to stratify heterogeneous groups.

So far, the identification of putative subgroups has been limited by small sample sizes, which limits the power of studies to test the influence of stratification by sub-groups on treatment response. Small discovery studies need to be followed by larger studies with the power to test the clinical utility of the potential biomarkers they identify (strength of recommendation: D). Therefore, in the future, large-scale, multicentre studies where patients are stratified according to their biological subtype are necessary in order to test whether stratification by particular biomarkers corresponds to improved response in a sub-group (strength of recommendation: D). In these studies, subgroups may be identified according to their genetic or molecular profile and then subsequently compared in terms of cognitive, neuroimaging and biochemical measures (Loth et al., 2016a). These advances will hopefully make it possible to identify biomarkers which, in the future, can be used to treat individuals with ASD more effectively and with a more personalised approach.

Consensus recommendations for future research directions

- Studies should include biomarkers to identify potential sub-groups in order to support stratification in future clinical trials where possible. (D)
- Clinical trials should ideally be multicentre, large-scale and include biomarkers where possible. This will ensure results are more generalisable and offer the opportunity to test whether a change in outcome measures is associated with change in biomarkers. (D)
- Clinical trials should include younger children and individuals with ASD and intellectual disability to ensure generalisability to the whole population of people with ASD. (D)
- Longer-term clinical trials lasting at least six months are required. (D)

- There is a need to develop objective outcome measures that can reliably capture changes of core symptoms over time. (D)
- Clinical trials should also include measures of quality of life of individuals with ASD. (D)
- Large-scale, multicentre, RCTs are needed to assess the effectiveness of social-communication interventions and applied behavioural analysis on a range of outcome measures. (D)
- All interventional studies, including those investigating psychological and social interventions, should include measures of adverse effects (D)
- Studies are needed to examine the effectiveness of behaviourally based daily life skills in adults with ASD. (D)
- Studies are needed to examine the effectiveness of anti-victimisation CBT in adults with ASD. (D)
- Studies are needed to evaluate models of service provision using patient experience and functional outcomes in addition to standard clinical measures (D).

Summary of guidelines and conclusions

These guidelines present recommendations based on the current literature and expert opinion for the diagnosis and management of ASD in children and adults. Our review of the evidence is not intended to be exhaustive, but to highlight key findings and also place them in a clinical context, drawing from the practical experience of the contributors. We hope that this balance will help the clinician who draws on the guidelines to place the evidence and our recommendations in the individual context of the person with ASD in front of them.

Current evidence does not support the routine use of any pharmacological treatment for the core symptoms of ASD. The evidence base is growing, particularly for co-occurring symptoms and disorders, yet much of the evidence is relatively nascent, particularly for core aspects of ASD. Aripiprazole and risperidone have shown some benefit for repetitive behaviours but are recommended only on a case-by-case basis in view of the risk of side-effects. There are a number of treatments for co-occurring conditions with a reasonable evidence base, although the evidence is still largely limited to symptomatology and mainly limited to children. In children, melatonin is recommended for sleep disorders, risperidone and aripiprazole may be cautiously used in the management of irritability if behavioural approaches are not possible, and methylphenidate is recommended for ADHD symptoms. There is very limited evidence for treatments for other co-occurring symptoms or disorders and in adults. Consequently, treatment is guided by extrapolation from studies in people without ASD. As treatment response and side-effect profiles in ASD may differ from the general population, treatment guided by extrapolation from studies in people without ASD must be cautious. Therefore, each treatment for an individual with ASD should be approached as an n=1 trial with careful evaluation of both benefits and side-effects.

In children and adolescents, social-communication interventions should be offered to increase joint-attention, engagement and reciprocal communication. In adults, psychological interventions should focus on the acquisition of life skills, access to community activities and quality of life.

ASD is a common and pervasive condition with a high health burden, and complex pathoaetiology involving a number of brain systems. The impact of ASD on the individual, their family and wider society is substantial, but may be reduced by timely diagnosis, the use of effective treatments, and avoiding inappropriate treatment. We recommend that service providers ensure people with ASD have timely access to diagnostic and treatment services with specialist expertise in ASD. Research into the genetics and neurobiology of ASD indicates that there is significant genetic and neurobiological heterogeneity. This is likely to lead to heterogeneity in the response to treatment and differential sensitivity to side-effects. It also highlights the need for biomarkers that can be used to guide the development of new treatments for core symptoms and co-occurring conditions, and help identify sub-groups who may respond better. Our analysis of the current evidence also highlights particular key gaps and limitations, and makes recommendations to address these. There are a number of studies of promising treatments being developed and we hope our recommendations will inform the development of studies. Finally, it is important to appreciate that we do not see these guidelines as set in stone. Indeed, we look forward to the evidence base growing, and anticipate revising these guidelines in the light of future developments.

Acknowledgements

The authors wish to thank the staff of the BAP, in particular Susan Chandler, and Lynne Harmer, for their help with organising the consensus meeting.

Declaration of conflicting interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Oliver D Howes: has received investigator-initiated research funding from and/or participated in advisory/speaker meetings organised by Astra-Zeneca, Autifony, BMS, Eli Lilly, Heptares, Jansenn, Lundbeck, Lyden-Delta, Otsuka, Servier, Sunovion, Rand and Roche. James T McCracken: Alcobra Pharmaceuticals (DSMB honorarium), Psyadon Pharmaceuticals (clinical trial), AstraZeneca (study medication). Paramala Santosh: Newron Pharmaceuticals (clinical trial). The remaining authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Supplementary material

Supplementary material is available for this article online.

References

Alldredge BK (1999) Seizure risk associated with psychotropic drugs: Clinical and pharmacokinetic considerations. *Neurology* 53: S68–S75.

Aman MG, Arnold LE and Hollway JA (2015) Assessing change in core autism symptoms: Challenges for pharmacological studies. *J Child Adolesc Psychopharmacol* 25: 282–285.

Aman MG, Arnold LE, McDougle CJ, et al. (2005a) Acute and long-term safety and tolerability of risperidone in children with autism. *J Child Adolesc Psychopharmacol* 15: 869–884.

Aman MG, Findling RL, Hardan AY, et al. (2017) Safety and efficacy of memantine in children with autism: Randomized, placebo-controlled study and open-label extension. *J Child Adolesc Psychopharmacol* 27: 403–412.

Aman MG, Kasper W, Manos G, et al. (2010) Line-item analysis of the Aberrant Behavior Checklist: Results from two studies of aripiprazole in the treatment of irritability associated with autistic disorder. *J Child Adolesc Psychopharmacol* 20: 415–422.

Aman MG, Lam KS and Van Bourgondien ME (2005b) Medication patterns in patients with autism: Temporal, regional, and demographic influences. *J Child Adolesc Psychopharmacol* 15: 116–126.

Aman MG, McDougle CJ, Scahill L, et al. (2009) Medication and parent training in children with pervasive developmental disorders and serious behavior problems: Results from a randomized clinical trial. *J Am Acad Child Adolesc Psychiatry* 48: 1143–1154.

Aman MG, Novotny S, Samango-Sprouse C, et al. (2004) Outcome measures for clinical drug trials in autism. *CNS Spectr* 9: 36–47.

American Psychiatric Association (2013) *Diagnostic and Statistical Manual of Mental Disorders (DSM-5)*. Arlington, VA: American Psychiatric Association.

Anagnostou E, Soorya L, Chaplin W, et al. (2012) Intranasal oxytocin versus placebo in the treatment of adults with autism spectrum disorders: A randomized controlled trial. *Mol Autism* 3: 16.

Anney R, Klei L, Pinto D, et al. (2012) Individual common variants exert weak effects on the risk for autism spectrum disorders. *Hum Mol Genet* 21: 4781–4792.

Arai A, Kessler M, Rogers G, et al. (1996) Effects of a memory-enhancing drug on DL-alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor currents and synaptic transmission in hippocampus. *J Pharmacol Exp Ther* 278: 627–638.

Arnold LE, Aman MG, Cook AM, et al. (2006) Atomoxetine for hyperactivity in autism spectrum disorders: Placebo-controlled crossover pilot trial. *J Am Acad Child Adolesc Psychiatry* 45: 1196–1205.

Baio J (2012) Prevalence of autism spectrum disorders: Autism and developmental disabilities monitoring network, 14 sites, United States, 2008. *MMWR Surveill Summ* 61: 1–19.

Baird G, Simonoff E, Pickles A, et al. (2006) Prevalence of disorders of the autism spectrum in a population cohort of children in South Thames: The Special Needs and Autism Project (SNAP). *Lancet* 368: 210–215.

Bal VH, Kim S-H, Cheong D, et al. (2015) Daily living skills in individuals with autism spectrum disorder from 2 to 21 years of age. *Autism* 19: 774–784.

Baldwin DS, Anderson IM, Nutt DJ, et al. (2014) Evidence-based pharmacological treatment of anxiety disorders, post-traumatic stress disorder and obsessive-compulsive disorder: A revision of the 2005 guidelines from the British Association for Psychopharmacology. *J Psychopharmacol* 28: 403–439.

Barthélémy C (1986) Evaluations cliniques quantitatives en pédopsychiatrie. *Neuropsychiatrie de l'Enfance et de l'Adolescence* 34: 63–91.

Barthelemy C, Bruneau N, Jouve J, et al. (1989) Urinary dopamine metabolites as indicators of the responsiveness to fenfluramine treatment in children with autistic behavior. *J Autism Dev Disord* 19: 241–254.

Basil P, Li Q, Dempster E, et al. (2014) Prenatal maternal immune activation causes epigenetic differences in adolescent mouse brain. *Transl Psychiatry* 4: e434.

Bastiaansen JA, Meffert H, Hein S, et al. (2011) Diagnosing autism spectrum disorders in adults: The use of Autism Diagnostic Observation Schedule (ADOS) module 4. *J Autism Dev Disord* 41: 1256–1266.

Baumgartner T, Heinrichs M, Vonlanthen A, et al. (2008) Oxytocin shapes the neural circuitry of trust and trust adaptation in humans. *Neuron* 58: 639–650.

Bebko JM, Perry A and Bryson S (1996) Multiple method validation study of facilitated communication: II. Individual differences and subgroup results. *J Autism Dev Disord* 26: 19–42.

Beck AR and Pirovano CM (1996) Facilitated communicators' performance on a task of receptive language. *J Autism Dev Disord* 26: 497–512.

Ben-Ari Y, Khalilov I, Kahle KT, et al. (2012) The GABA excitatory/inhibitory shift in brain maturation and neurological disorders. *Neuroscientist* 18: 467–486.

Berry-Kravis E, Des Portes V, Hagerman R, et al. (2016) Mavoglurant in fragile X syndrome: Results of two randomized, double-blind, placebo-controlled trials. *Sci Transl Med* 8: 321ra5.

Berry-Kravis E, Krause SE, Block SS, et al. (2006) Effect of CX516, an AMPA-modulating compound, on cognition and behavior in fragile X syndrome: A controlled trial. *J Child Adolesc Psychopharmacol* 16: 525–540.

Berry-Kravis E, Sumis A, Hervey C, et al. (2008) Open-label treatment trial of lithium to target the underlying defect in fragile X syndrome. *J Dev Behav Pediatrics* 29: 293–302.

Berry-Kravis EM, Hessl D, Rathmell B, et al. (2012) Effects of STX209 (arbaclofen) on neurobehavioral function in children and adults with fragile X syndrome: A randomized, controlled, phase 2 trial. *Sci Transl Med* 4:152ra127.

Bijl RV, Ravelli A and van Zessen G (1998) Prevalence of psychiatric disorder in the general population: Results of The Netherlands Mental Health Survey and Incidence Study (NEMESIS). *Soc Psychiatry Psychiatr Epidemiol* 33: 587–595.

Biklen D (1990) Communication unbound: Autism and praxis. *Harv Educ Rev* 60: 291–315.

Biklen D, Morton MW, Gold D, et al. (1992) Facilitated communication: Implications for individuals with autism. *Top Lang Disord* 12: 1–28.

Biklen D, Saha N and Kliewer C (1995) How teachers confirm the authorship of facilitated communication: A portfolio approach. *J Assoc Persons Severe Handicaps* 20: 45–56.

Biklen D and Schubert A (1991) New words: The communication of students with autism. *Rem Spec Educ* 12: 46–57.

Bolea-Alamañac B, Nutt DJ, Adamou M, et al. (2014) Evidence-based guidelines for the pharmacological management of attention deficit hyperactivity disorder: Update on recommendations from the British Association for Psychopharmacology. *J Psychopharmacol* 28: 179–203.

Bomba C, O'Donnell L, Markowitz C, et al. (1996) Evaluating the impact of facilitated communication on the communicative competence of fourteen students with autism. *J Autism Dev Disord* 26: 43–58.

Bourgeron T (2015) From the genetic architecture to synaptic plasticity in autism spectrum disorder. *Nat Rev Neurosci* 16: 551–563.

Bozdagi O, Sakurai T, Papapetrou D, et al. (2010) Haploinsufficiency of the autism-associated Shank3 gene leads to deficits in synaptic function, social interaction, and social communication. *Mol Autism* 1: 15.

Bozdagi O, Tavassoli T and Buxbaum JD (2013) Insulin-like growth factor-1 rescues synaptic and motor deficits in a mouse model of autism and developmental delay. *Mol Autism* 4: 9.

Braman BJ, Brady MP, Linehan SL, et al. (1995) Facilitated communication for children with autism: An examination of face validity. *Behav Disord* 21: 110–118.

Brett D, Warnell F, McConachie H, et al. (2016) Factors affecting age at ASD diagnosis in UK: No evidence that diagnosis age has decreased between 2004 and 2014. *J Autism Dev Disord* 46: 1974–1984.

Brondino N, Fusar-Poli L, Panisi C, et al. (2016) Pharmacological modulation of GABA function in autism spectrum disorders: A systematic review of human studies. *J Autism Dev Disord* 46: 825–839.

Brugha TS, McManus S, Bankart J, et al. (2011) Epidemiology of autism spectrum disorders in adults in the community in England. *Arch Gen Psychiatry* 68: 459–465.

Buchsbaum MS, Hollander E, Haznedar MM, et al. (2001) Effect of fluoxetine on regional cerebral metabolism in autistic spectrum disorders: A pilot study. *Int J Neuropsychopharmacol* 4: 119–125.

Buck TR, Viskochil J, Farley M, et al. (2014) Psychiatric comorbidity and medication use in adults with autism spectrum disorder. *J Autism Dev Disord* 44: 3063–3071.

Buckley C (2016) *RCGP position statement on autistic spectrum disorders*. Royal College of General Practitioners. Available at: http://www.rcgp.org.uk/clinical-and-research/clinical-resources/autistic-spectrum-disorder.aspx

Buescher AV, Cidav Z, Knapp M, et al. (2014) Costs of autism spectrum disorders in the United Kingdom and the United States. *JAMA Pediatr* 168: 721–728.

Cadman T, Eklund H, Howley D, et al. (2012) Caregiver burden as people with autism spectrum disorder and attention-deficit/hyperactivity disorder transition into adolescence and adulthood in the United Kingdom. *J Am Acad Child Adolesc Psychiatry* 51: 879–888.

Canitano R and Vivanti G (2007) Tics and Tourette syndrome in autism spectrum disorders. *Autism* 11: 19–28.

Carter AS, Messinger DS, Stone WL, et al. (2011) A randomized controlled trial of Hanen's 'More Than Words' in toddlers with early autism symptoms. *J Child Psychol Psychiatry* 52: 741–752.

Carter CS (1998) Neuroendocrine perspectives on social attachment and love. *Psychoneuroendocrinology* 23: 779–818.

Chang Y-C and Locke J (2016) A systematic review of peer-mediated interventions for children with autism spectrum disorder. *Res Autism Spectr Disord* 27: 1–10.

Charman T and Gotham K (2013) Measurement issues: Screening and diagnostic instruments for autism spectrum disorders – lessons from research and practice. *Child Adolesc Ment Health* 18: 52–63.

Chugani DC, Muzik O, Rothermel R, et al. (1997) Altered serotonin synthesis in the dentatothalamocortical pathway in autistic boys. *Ann Neurol* 42: 666–669.

Clarke TK, Lupton MK, Fernandez-Pujals AM, et al. (2015) Common polygenic risk for autism spectrum disorder (ASD) is associated with cognitive ability in the general population. *Mol Psychiatry* 21: 419–425.

Clarkson G (1994) Creative music therapy and facilitated communication: New ways of reaching students with autism. *Prev Sch Fail: Altern Educ Child Youth* 38: 31–33.

Cleare A, Pariante CM, Young AH, et al. (2015) Evidence-based guidelines for treating depressive disorders with antidepressants: A revision of the 2008 British Association for Psychopharmacology guidelines. *J Psychopharmacol* 29: 459–525.

Constantino JN and Charman T (2015) Diagnosis of autism spectrum disorder: Reconciling the syndrome, its diverse origins, and variation in expression. *Lancet Neurol* 15: 279–291.

Constantino JN, Majmudar P, Bottini A, et al. (2010) Infant head growth in male siblings of children with and without autism spectrum disorders. *J Neurodev Disord* 2: 39–46.

Cortesi F, Giannotti F, Sebastiani T, et al. (2012) Controlled-release melatonin, singly and combined with cognitive behavioural therapy, for persistent insomnia in children with autism spectrum disorders: A randomized placebo-controlled trial. *J Sleep Res* 21: 700–709.

Courchesne E, Carper R and Akshoomoff N (2003) Evidence of brain overgrowth in the first year of life in autism. *JAMA* 290: 337–344.

Coury DL, Anagnostou E, Manning-Courtney P, et al. (2012) Use of psychotropic medication in children and adolescents with autism spectrum disorders. *Pediatrics* 130(Suppl 2): S69–S76.

Crews WD, Sanders EC, Hensley LG, et al. (1995) An evaluation of facilitated communication in a group of nonverbal individuals with mental retardation. *J Autism Dev Disord* 25: 205–213.

Croen LA, Zerbo O, Qian Y, et al. (2015) The health status of adults on the autism spectrum. *Autism* 19: 814–823.

Crossley R and Remington-Gurney J (1992) Getting the words out: Facilitated communication training. *Top Lang Disord* 12: 29–45.

Davis TN, O'Reilly M, Kang S, et al. (2013) Chelation treatment for autism spectrum disorders: A systematic review. *Res Autism Spectr Disord* 7: 49–55.

Dawson G, Jones EJ, Merkle K, et al. (2012) Early behavioral intervention is associated with normalized brain activity in young children with autism. *J Am Acad Child Adolesc Psychiatry* 51: 1150–1159.

Dawson G, Rogers S, Munson J, et al. (2010) Randomized, controlled trial of an intervention for toddlers with autism: The Early Start Denver Model. *Pediatrics* 125: e17–e23.

Dean M, Harwood R and Kasari C (2016) The art of camouflage: Gender differences in the social behaviors of girls and boys with autism spectrum disorder. *Autism* 21: 678–689.

de Bruin EI, Ferdinand RF, Meester S, et al. (2007) High rates of psychiatric co-morbidity in PDD-NOS. *J Autism Dev Disord* 37: 877–886.

de la Torre-Ubieta L, Won H, Stein JL, et al. (2016) Advancing the understanding of autism disease mechanisms through genetics. *Nat Med* 22: 345–361.

Dolen G and Bear MF (2008) Role for metabotropic glutamate receptor 5 (mGluR5) in the pathogenesis of fragile X syndrome. *J Physiol* 586: 1503–1508.

Domes G, Heinrichs M, Michel A, et al. (2007) Oxytocin improves "mind-reading" in humans. *Biol Psychiatry* 61: 731–733.

Douglas-Hall P, Curran S, Bird V, et al. (2011) Aripiprazole: A review of its use in the treatment of irritability associated with autistic disorder patients aged 6-17. *J Cent Nerv Syst Dis* 3: 143–153.

Eberlin M, McConnachie G, Ibel S, et al. (1993) Facilitated communication: A failure to replicate the phenomenon. *J Autism Dev Disord* 23: 507–530.

Edelson SM, Rimland B, Berger CL, et al. (1998) Evaluation of a mechanical hand-support for facilitated communication. *J Autism Dev Disord* 28: 153–157.

Elder LM, Dawson G, Toth K, et al. (2008) Head circumference as an early predictor of autism symptoms in younger siblings of children with autism spectrum disorder. *J Autism Dev Disord* 38: 1104–1111.

Erickson CA, Posey DJ, Stigler KA, et al. (2007) A retrospective study of memantine in children and adolescents with pervasive developmental disorders. *Psychopharmacology (Berl)* 191: 141–147.

Erickson CA, Veenstra-Vanderweele JM, Melmed RD, et al. (2014) STX209 (arbaclofen) for autism spectrum disorders: An 8-week open-label study. *J Autism Dev Disord* 44: 958–964.

Estes ML and McAllister AK (2015) Immune mediators in the brain and peripheral tissues in autism spectrum disorder. *Nat Rev Neurosci* 16: 469–486.

Eyles DW, Burne TH and McGrath JJ (2013) Vitamin D, effects on brain development, adult brain function and the links between low levels of vitamin D and neuropsychiatric disease. *Front Neuroendocrinol* 34: 47–64.

Faber KM and Haring JH (1999) Synaptogenesis in the postnatal rat fascia dentata is influenced by 5-HT1a receptor activation. *Brain Res Dev Brain Res* 114: 245–252.

Fankhauser MP, Karumanchi VC, German ML, et al. (1992) A double-blind, placebo-controlled study of the efficacy of transdermal clonidine in autism. *J Clin Psychiatry* 53: 77–82.

Ferguson JN, Young LJ, Hearn EF, et al. (2000) Social amnesia in mice lacking the oxytocin gene. *Nat Genet* 25: 284–288.

Findling RL and McNamara NK (2004) Atypical antipsychotics in the treatment of children and adolescents: Clinical applications. *J Clin Psychiatry* 65(Suppl 6): 30–44.

Findon J, Cadman T, Stewart CS, et al. (2016) Screening for co-occurring conditions in adults with autism spectrum disorder using the strengths and difficulties questionnaire: A pilot study. *Autism Research* 9(12): 1353–1363.q

Food and Drug Administration (2006) FDA approves the first drug to treat irritability associated with autism, Risperdal. *FDA News*. Available at: http://www.fda.gov.

Frankel F, Myatt R and Cantwell DP (1995) Training outpatient boys to conform with social ecology of popular peers: Effects on parent and teacher ratings. *J Clin Child Psychol* 24: 300–310.

Frankel F, Myatt R, Cantwell DP, et al. (1997) Parent-assisted transfer of children's social skills training: Effects on children with and without attention-deficit hyperactivity disorder. *J Am Acad Child Adolesc Psychiatry* 36: 1056–1064.

Frankel F, Myatt R, Sugar C, et al. (2010) A randomized controlled study of parent-assisted children's friendship training with children having autism spectrum disorders. *J Autism Dev Disord* 40: 827–842.

Fricker AD, Rios C, Devi LA, et al. (2005) Serotonin receptor activation leads to neurite outgrowth and neuronal survival. *Brain Res Mol Brain Res* 138: 228–235.

Fung LK, Libove RA, Phillips J, et al. (2014) Brief Report: An open-label study of the neurosteroid pregnenolone in adults with autism spectrum disorder. *J Autism Dev Disord* 44: 2971–2977.

Gabriele S, Sacco R and Persico AM (2014) Blood serotonin levels in autism spectrum disorder: A systematic review and meta-analysis. *Eur Neuropsychopharmacol* 24: 919–929.

Galli-Carminati G, Deriaz N and Bertschy G (2009) Melatonin in treatment of chronic sleep disorders in adults with autism: A retrospective study. *Swiss Med Wkly* 139: 293–296.

Gardener H, Spiegelman D and Buka SL (2009) Prenatal risk factors for autism: Comprehensive meta-analysis. *Br J Psychiatry* 195: 7–14.

Gardener H, Spiegelman D and Buka SL (2011) Perinatal and neonatal risk factors for autism: A comprehensive meta-analysis. *Pediatrics* 128: 344–355.

Gaugler T, Klei L, Sanders SJ, et al. (2014) Most genetic risk for autism resides with common variation. *Nat Genet* 46: 881–885.

Ghosh A, Michalon A, Lindemann L, et al. (2013) Drug discovery for autism spectrum disorder: Challenges and opportunities. *Nat Rev Drug Discov* 12: 777–790.

Goin-Kochel RP, Mackintosh VH and Myers BJ (2006) How many doctors does it take to make an autism spectrum diagnosis? *Autism* 10: 439–451.

Golan O and Baron-Cohen S (2006) Systemizing empathy: Teaching adults with Asperger syndrome or high-functioning autism to recognize complex emotions using interactive multimedia. *Dev Psychopathol* 18: 591–617.

Goldfarb C, Genore L, Hunt C, et al. (2016) Hyperbaric oxygen therapy for the treatment of children and youth with Autism Spectrum Disorders: An evidence-based systematic review. *Res Autism Spectr Disord* 29: 1–7.

Gonçalves JT, Anstey JE, Golshani P, et al. (2013) Circuit level defects in the developing neocortex of Fragile X mice. *Nat Neurosci* 16: 903–909.

Gordon CT, Nelson JE, Hamburger SD, et al. (1993) A double-blind comparison of clomipramine, desipramine, and placebo in the treatment of autistic disorder. *Arch Gen Psychiatry* 50: 441–447.

Gray L, Gibbs J, Jolleff N, et al. (2015) Variable implementation of good practice recommendations for the assessment and management of UK children with neurodisability. *Child Care Health Dev* 41: 938–946.

Green J, Charman T, McConachie H, et al. (2010) Parent-mediated communication-focused treatment in children with autism (PACT): A randomised controlled trial. *Lancet* 375: 2152–2160.

Gringras P, Gamble C, Jones A, et al. (2012) Melatonin for sleep problems in children with neurodevelopmental disorders: Randomised double masked placebo controlled trial. *BMJ* 345: e6664.

Guastella AJ, Gray KM, Rinehart NJ, et al. (2015) The effects of a course of intranasal oxytocin on social behaviors in youth diagnosed with autism spectrum disorders: A randomized controlled trial. *J Child Psychol Psychiatry* 56: 444–452.

Gutierrez R, Hung J, Zhang Y, et al. (2009) Altered synchrony and connectivity in neuronal networks expressing an autism-related mutation of neuroligin 3. *Neuroscience* 162: 208–221.

Handen BL, Aman MG, Arnold LE, et al. (2015) Atomoxetine, parent training, and their combination in children with autism spectrum disorder and attention-deficit/hyperactivity disorder. *J Am Acad Child Adolesc Psychiatry* 54: 905–915.

Hanley HG, Stahl SM and Freedman DX (1977) Hyperserotonemia and amine metabolites in autistic and retarded children. *Arch Gen Psychiatry* 34: 521–531.

Hansen RL, Ozonoff S, Krakowiak P, et al. (2008) Regression in autism: Prevalence and associated factors in the CHARGE Study. *Ambul Pediatr* 8: 25–31.

Harfterkamp M, van de Loo-Neus G, Minderaa RB, et al. (2012) A randomized double-blind study of atomoxetine versus placebo for attention-deficit/hyperactivity disorder symptoms in children with autism spectrum disorder. *J Am Acad Child Adolesc Psychiatry* 51: 733–741.

Heckler S (1994) Facilitated communication: A response by child protection. *Child Abuse Negl* 18: 495–503.

Hillier A, Fish T, Cloppert P, et al. (2007) Outcomes of a social and vocational skills support group for adolescents and young adults on the autism spectrum. *Focus Autism Other Dev Disabl* 22: 107–115.

Hirsch LE and Pringsheim T (2016) Aripiprazole for autism spectrum disorders (ASD). *Cochrane Database Syst Rev* 6: CD009043.

Hirshoren A and Gregory J (1995) Further negative findings on facilitated communication. *Psychol Sch* 32: 109–113.

Hofvander B, Delorme R, Chaste P, et al. (2009) Psychiatric and psychosocial problems in adults with normal-intelligence autism spectrum disorders. *BMC Psychiatry* 9: 35.

Hollander E, Phillips A, Chaplin W, et al. (2005) A placebo controlled crossover trial of liquid fluoxetine on repetitive behaviors in childhood and adolescent autism. *Neuropsychopharmacology* 30: 582–589.

Hollander E, Soorya L, Chaplin W, et al. (2012) A double-blind placebo-controlled trial of fluoxetine for repetitive behaviors and global severity in adult autism spectrum disorders. *Am J Psychiatry* 169: 292–299.

Hosie AM, Wilkins ME, da Silva HM, et al. (2006) Endogenous neurosteroids regulate GABAA receptors through two discrete transmembrane sites. *Nature* 444: 486–489.

Howes O, Egerton A, Allan V, et al. (2009) Mechanisms underlying psychosis and antipsychotic treatment response in schizophrenia: Insights from PET and SPECT imaging. *Curr Pharm Des* 15: 2550–2559.

Howlin P and Moss P (2012) Adults with autism spectrum disorders. *Can J Psychiatry* 57: 275–283.

Howlin P and Yates P (1999) The potential effectiveness of social skills groups for adults with autism. *Autism* 3: 299–307.

Hudson A, Melita B and Arnold N (1993) Brief report: A case study assessing the validity of facilitated communication. *J Autism Dev Disord* 23: 165–173.

Insel TR and Shapiro LE (1992) Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. *Proc Natl Acad Sci* 89: 5981–5985.

Insel TR, O'Brien DJ and Leckman JF (1999) Oxytocin, vasopressin, and autism: Is there a connection? *Biol Psychiatry* 45: 145–157.

Jacquemont S, Curie A, Des Portes V, et al. (2011) Epigenetic modification of the FMR1 gene in fragile X syndrome is associated with differential response to the mGluR5 antagonist AFQ056. *Sci Transl Med* 3: 64ra61.

Jahromi LB, Kasari CL, McCracken JT, et al. (2009) Positive effects of methylphenidate on social communication and self-regulation in children with pervasive developmental disorders and hyperactivity. *J Autism Dev Disord* 39: 395–404.

Janzen-Wilde ML, Duchan JF and Higginbotham DJ (1995) Successful use of facilitated communication with an oral child. *J Speech Hear Res* 38: 658–676.

Jaselskis CA, Cook EH Jr, Fletcher KE, et al. (1992) Clonidine treatment of hyperactive and impulsive children with autistic disorder. *J Clin Psychopharmacol* 12: 322–327.

Jensen PS (1999) A 14-month randomized clinical trial of treatment strategies for attention-deficit/hyperactivity disorder. *Arch Gen Psychiatry* 56: 1073–1086.

Johnson CP and Myers SM (2007) Identification and evaluation of children with autism spectrum disorders. *Pediatrics* 120: 1183–1215.

Joshi G, Wozniak J, Petty C, et al. (2013) Psychiatric comorbidity and functioning in a clinically referred population of adults with autism spectrum disorders: A comparative study. *J Autism Dev Disord* 43: 1314–1325.

Kasari C, Freeman S and Paparella T (2006) Joint attention and symbolic play in young children with autism: A randomized controlled intervention study. *J Child Psychol Psychiatry* 47: 611–620.

Kasari C, Lawton K, Shih W, et al. (2014) Caregiver-mediated intervention for low-resourced preschoolers with autism: An RCT. *Pediatrics* 134: e72–e79.

Kearns GL, Abdel-Rahman SM, Alander SW, et al. (2003) Developmental pharmacology—drug disposition, action, and therapy in infants and children. *N Engl J Med* 349: 1157–1167.

Kent JM, Kushner S, Ning X, et al. (2013) Risperidone dosing in children and adolescents with autistic disorder: A double-blind, placebo-controlled study. *J Autism Dev Disord* 43: 1773–1783.

Kesterson KL, Lane RD and Rhoades RW (2002) Effects of elevated serotonin levels on patterns of GAP-43 expression during barrel development in rat somatosensory cortex. *Brain Res Dev Brain Res* 139: 167–174.

Khemka I (2000) Increasing independent decision-making skills of women with mental retardation in simulated interpersonal situations of abuse. *Am J Ment Retard* 105: 387–401.

Khemka I, Hickson L, Reynolds G, et al. (2005) Evaluation of a decision-making curriculum designed to empower women with mental retardation to resist abuse. *Am J Ment Retard* 110: 193–204.

Kielinen M, Rantala H, Timonen E, et al. (2004) Associated medical disorders and disabilities in children with autistic disorder a population-based study. *Autism* 8: 49–60.

Kim E, Howes OD, Turkheimer FE, et al. (2013) The relationship between antipsychotic D2 occupancy and change in frontal metabolism and working memory. *Psychopharmacology (Berl)* 227: 221–229.

Kim SH and Lord C (2010) Restricted and repetitive behaviors in toddlers and preschoolers with autism spectrum disorders based on the Autism Diagnostic Observation Schedule (ADOS). *Autism Res* 3: 162–173.

King BH, Hollander E, Sikich L, et al. (2009) Lack of efficacy of citalopram in children with autism spectrum disorders and high levels of repetitive behavior: Citalopram ineffective in children with autism. *Arch Gen Psychiatry* 66: 583–590.

King BH, Wright DM, Handen BL, et al. (2001) Double-blind, placebo-controlled study of amantadine hydrochloride in the treatment of children with autistic disorder. *J Am Acad Child Adolesc Psychiatry* 40: 658–665.

Klei L, Sanders SJ, Murtha MT, et al. (2012) Common genetic variants, acting additively, are a major source of risk for autism. *Mol Autism* 3: 9.

Klewe L (1993) Brief report: An empirical evaluation of spelling boards as a means of communication for the multihandicapped. *J Autism Dev Disord* 23: 559–566.

Koenig K, White SW, Pachler M, et al. (2010) Promoting social skill development in children with pervasive developmental disorders: A feasibility and efficacy study. *J Autism Dev Disord* 40: 1209–1218.

Kolevzon A, Bush L, Wang AT, et al. (2014) A pilot controlled trial of insulin-like growth factor-1 in children with Phelan-McDermid syndrome. *Mol Autism* 5: 54.

Konstantareas MM and Gravelle G (1998) Facilitated communication: The contribution of physical, emotional and mental support. *Autism* 2: 389–414.

Koyama R and Ikegaya Y (2015) Microglia in the pathogenesis of autism spectrum disorders. *Neurosci Res* 100: 1–5.

Lai M-C and Baron-Cohen S (2015) Identifying the lost generation of adults with autism spectrum conditions. *Lancet Psychiatry* 2: 1013–1027.

Lai MC, Lombardo MV, Pasco G, et al. (2011) A behavioral comparison of male and female adults with high functioning autism spectrum conditions. *PLoS One* 6: e20835.

Landa RJ, Holman KC, O'Neill AH, et al. (2011) Intervention targeting development of socially synchronous engagement in toddlers with autism spectrum disorder: A randomized controlled trial. *J Child Psychol Psychiatry* 52: 13–21.

Lang R, Regester A, Lauderdale S, et al. (2010) Treatment of anxiety in autism spectrum disorders using cognitive behaviour therapy: A systematic review. *Dev Neurorehabil* 13: 53–63.

Lange N, Travers BG, Bigler ED, et al. (2015) Longitudinal volumetric brain changes in autism spectrum disorder ages 6-35 years. *Autism Res* 8: 82–93.

Laugeson EA, Frankel F, Mogil C, et al. (2009) Parent-assisted social skills training to improve friendships in teens with autism spectrum disorders. *J Autism Dev Disord* 39: 596–606.

Leigh MJ, Nguyen DV, Mu Y, et al. (2013) A randomized double-blind, placebo-controlled trial of minocycline in children and adolescents with fragile X syndrome. *J Dev Behav Pediatr* 34: 147–155.

Lenroot RK and KaYeung P (2013) Heterogeneity within autism spectrum disorders: What have we learned from neuroimaging studies? *Front Hum Neurosci* 7: 733.

Lever AG and Geurts HM (2016) Psychiatric co-occurring symptoms and disorders in young, middle-aged, and older adults with autism spectrum disorder. *J Autism Dev Disord* 46: 1916–1930.

Leyfer OT, Folstein SE, Bacalman S, et al. (2006) Comorbid psychiatric disorders in children with autism: Interview development and rates of disorders. *J Autism Dev Disord* 36: 849–861.

Lichtenstein P, Carlstrom E, Rastam M, et al. (2010) The genetics of autism spectrum disorders and related neuropsychiatric disorders in childhood. *Am J Psychiatry* 167: 1357–1363.

Lopata C, Thomeer ML, Volker MA, et al. (2010) RCT of a manualized social treatment for high-functioning autism spectrum disorders. *J Autism Dev Disord* 40: 1297–1310.

Lord C, Petkova E, Hus V, et al. (2012a) A multisite study of the clinical diagnosis of different autism spectrum disorders. *Arch Gen Psychiatry* 69: 306–313.

Lord C, Risi S, Lambrecht L, et al. (2000) The autism diagnostic observation schedule—Generic: A standard measure of social and communication deficits associated with the spectrum of autism. *J Autism Dev Disord* 30: 205–223.

Lord C, Rutter M, DiLavore P, et al. (2012b) *Autism Diagnostic Observation Schedule–(ADOS-2)*, 2nd edn. Los Angeles, CA: Western Psychological Corporation.

Lord C, Rutter M and Le Couteur A (1994) Autism diagnostic interview-revised: A revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *J Autism Dev Disord* 24: 659–685.

Loth E, Charman T, Mason L, et al. (2017) The EU-AIMS Longitudinal European Autism Project (LEAP): Design and methodologies to identify and validate stratification biomarkers for autism spectrum disorders. *Mol Autism* 8: 24.

Loth E, Murphy DG and Spooren W (2016a) Defining precision medicine approaches to autism spectrum disorders: Concepts and challenges. *Front Psychiatry* 7.

Loth E, Spooren W, Ham LM, et al. (2016b) Identification and validation of biomarkers for autism spectrum disorders. *Nat Rev Drug Discov* 15: 70–73.

Luby J, Mrakotsky C, Stalets MM, et al. (2006) Risperidone in preschool children with autistic spectrum disorders: An investigation of safety and efficacy. *J Child Adolesc Psychopharmacol* 16: 575–587.

McConachie H, Parr JR, Glod M, et al. (2015) Systematic review of tools to measure outcomes for young children with autism spectrum disorder. *Health Technol Assess* 19: 1–506.

McCracken JT, McGough J, Shah B, et al. (2002) Risperidone in children with autism and serious behavioral problems. *N Engl J Med* 347: 314–321.

McDougle CJ, Holmes JP, Carlson DC, et al. (1998) A double-blind, placebo-controlled study of risperidone in adults with autistic disorder and other pervasive developmental disorders. *Arch Gen Psychiatry* 55: 633–641.

McDougle CJ, Naylor ST, Cohen DJ, et al. (1996) A double-blind, placebo-controlled study of fluvoxamine in adults with autistic disorder. *Arch Gen Psychiatry* 53: 1001–1008.

McDougle CJ, Scahill L, Aman MG, et al. (2005) Risperidone for the core symptom domains of autism: Results from the study by the autism network of the research units on pediatric psychopharmacology. *Am J Psychiatry* 162: 1142–1148.

Maenner MJ, Rice CE, Arneson CL, et al. (2014) Potential impact of DSM-5 criteria on autism spectrum disorder prevalence estimates. *JAMA Psychiatry* 71: 292–300.

Magiati I, Tay XW and Howlin P (2014) Cognitive, language, social and behavioural outcomes in adults with autism spectrum disorders: A systematic review of longitudinal follow-up studies in adulthood. *Clin Psychol Rev* 34: 73–86.

Man KK, Chan EW, Coghill DR, et al. (2015) Prenatal antidepressant exposure and the risk of autism spectrum disorder and attention-deficit hyperactivity disorder. In: *31st International Conference on Pharmacoepidemiology & Therapeutic Risk Management, ICPE 2015, Boston, MA, 23–26 August 2015*. Bethesda, MD: International Society for Pharmacoepidemiology (ISPE).

Mandy W, Chilvers R, Chowdhury U, et al. (2012) Sex differences in autism spectrum disorder: Evidence from a large sample of children and adolescents. *J Autism Dev Disord* 42: 1304–1313.

Mandy W and Lai MC (2016) Annual Research Review: The role of the environment in the developmental psychopathology of autism spectrum condition. *J Child Psychol Psychiatry* 57: 271–292.

Marcus RN, Owen R, Kamen L, et al. (2009) A placebo-controlled, fixed-dose study of aripiprazole in children and adolescents with irritability associated with autistic disorder. *J Am Acad Child Adolesc Psychiatry* 48: 1110–1119.

Marcus RN, Owen R, Manos G, et al. (2011) Safety and tolerability of aripiprazole for irritability in pediatric patients with autistic disorder: A 52-week, open-label, multicenter study. *J Clin Psychiatry* 72: 1270–1276.

Maskey M, Warnell F, Parr JR, et al. (2013) Emotional and behavioural problems in children with autism spectrum disorder. *J Autism Dev Disord* 43: 851–859.

Matson JL, DiLorenzo TM and Esveldt-Dawson K (1981) Independence training as a method of enhancing self-help skills acquisition of the mentally retarded. *Behav Res Ther* 19: 399–405.

Mayes SD, Calhoun SL, Murray MJ, et al. (2011) Anxiety, depression, and irritability in children with autism relative to other neuropsychiatric disorders and typical development. *Res Autism Spectr Disord* 5: 474–485.

Mazefsky C, McPartland J, Gastgeb H, et al. (2013) Brief report: Comparability of DSM-IV and DSM-5 ASD research samples. *J Autism Dev Disord* 43: 1236–1242.

Mazer C, Muneyyirci J, Taheny K, et al. (1997) Serotonin depletion during synaptogenesis leads to decreased synaptic density and learning deficits in the adult rat: A possible model of neurodevelopmental disorders with cognitive deficits. *Brain Res* 760: 68–73.

Mendez MA, Horder J, Myers J, et al. (2013) The brain GABA-benzodiazepine receptor alpha-5 subtype in autism spectrum disorder: A pilot [11 C] Ro15-4513 positron emission tomography study. *Neuropharmacology* 68: 195–201.

Minshawi NF, Wink LK, Shaffer R, et al. (2016) A randomized, placebo-controlled trial of D-cycloserine for the enhancement of social skills training in autism spectrum disorders. *Mol Autism* 7: 2.

Miral S, Gencer O, Inal-Emiroglu FN, et al. (2008) Risperidone versus haloperidol in children and adolescents with AD - A randomized, controlled, double-blind trial. *Eur Child Adolesc Psychiatry* 17: 1–8.

Montee BB, Miltenberger RG and Wittrock D (1995) An experimental analysis of facilitated communication. *J Appl Behav Anal* 28: 189–200.

Murray ML, Hsia Y, Glaser K, et al. (2014) Pharmacological treatments prescribed to people with autism spectrum disorder (ASD) in primary health care. *Psychopharmacology (Berl)* 231: 1011–1021.

Myles BS and Simpson RL (1994) Facilitated communication with children diagnosed as autistic in public school settings. *Psychol Sch* 31: 208–220.

Myles BS, Simpson RL and Smith SM (1996) Collateral behavioral and social effects of using facilitated communication with individuals with autism. *Focus Autism Other Devel Disabl* 11: 163–169.

Naaijen J, Lythgoe DJ, Amiri H, et al. (2015) Fronto-striatal glutamatergic compounds in compulsive and impulsive syndromes: A review of magnetic resonance spectroscopy studies. *Neurosci Biobehav Rev* 52: 74–88.

Nagaraj R, Singhi P and Malhi P (2006) Risperidone in children with autism: Randomized, placebo-controlled, double-blind study. *J Child Neurol* 21: 450–455.

National Institute for Clinical Excellence (2012) Autism: Recognition, referral, diagnosis and management of adults on the autism spectrum. *Natl Inst Health Care Excell* 142: 18–19.

National Institute for Health and Clinical Excellence (2013) *Autism spectrum disorder in under 19s: Support and management*. NICE Guidline (CG170). London: National Institute for Health and Clinical Excellence. Available at: https://www.nice.org.uk/guidance/cg170.

Nelson SB and Valakh V (2015) Excitatory/Inhibitory balance and circuit homeostasis in autism spectrum disorders. *Neuron* 87: 684–698.

Neuropharm: Clinicaltrails.gov. (2012) *Study of Fluoxetine in Autism (SOFIA)*. Available at: https://clinicaltrials.gov/ct2/show/NCT00515320.

Niederhofer H, Staffen W and Mair A (2002) Lofexidine in hyperactive and impulsive children with autistic disorder. *J Am Acad Child Adolesc Psychiatry* 41: 1396–1397.

Norbury CF (2014) Practitioner review: Social (pragmatic) communication disorder conceptualization, evidence and clinical implications. *J Child Psychol Psychiatry* 55: 204–216.

Noterdaeme M, Wriedt E and Hohne C (2010) Asperger's syndrome and high-functioning autism: Language, motor and cognitive profiles. *Eur Child Adolesc Psychiatry* 19: 475–481.

O'Connor MJ, Frankel F, Paley B, et al. (2006) A controlled social skills training for children with fetal alcohol spectrum disorders. *J Consult Clin Psychol* 74: 639.

Okamoto Y, Ishitobi M, Wada Y, et al. (2016) The potential of nasal oxytocin administration for remediation of autism spectrum disorders. *CNS Neurol Disord Drug Targets* 15: 564–577.

Olney M (1995) Reading between the lines: A case study on facilitated communication. *J Assoc Persons Severe Handicaps* 20: 57–65.

Oono IP, Honey EJ and McConachie H (2013) Parent-mediated early intervention for young children with autism spectrum disorders (ASD). *Cochrane Database Syst Rev* 8: 2380–2479.

Oswald DP (1994) Facilitator influence in facilitated communication. *J Behav Educ* 4: 191–199.

Owen R, Sikich L, Marcus RN, et al. (2009) Aripiprazole in the treatment of irritability in children and adolescents with autistic disorder. *Pediatrics* 124: 1533–1540.

Ozonoff S, Goodlin-Jones BL and Solomon M (2005) Evidence-based assessment of autism spectrum disorders in children and adolescents. *J Clin Child Adolesc Psychol* 34: 523–540.

Pacher P and Kecskemeti V (2004) Cardiovascular side effects of new antidepressants and antipsychotics: New drugs, old concerns? *Curr Pharm Des* 10: 2463–2475.

Palmer E, Ketteridge C, Parr J, et al. (2010) Autism spectrum disorder diagnostic assessments: Improvements since publication of the National Autism Plan for Children. *Arch Dis Child* 96: 473–475.

Paribello C, Tao L, Folino A, et al. (2010) Open-label add-on treatment trial of minocycline in fragile X syndrome. *BMC Neurol* 10: 91.

Pardo CA, Farmer CA, Thurm A, et al. (2017) Serum and cerebrospinal fluid immune mediators in children with autistic disorder: A longitudinal study. *Mol Autism* 8: 1.

Parr J, Jolleff N, Gray L, et al. (2013) Twenty years of research shows UK child development team provision still varies widely for children with disability. *Child Care Health Dev* 39: 903–907.

Parsons CG, Stoffler A and Danysz W (2007) Memantine: A NMDA receptor antagonist that improves memory by restoration of homeostasis in the glutamatergic system–too little activation is bad, too much is even worse. *Neuropharmacology* 53: 699–723.

Peixoto RT, Wang W, Croney DM, et al. (2016) Early hyperactivity and precocious maturation of corticostriatal circuits in Shank3B-/-mice. *Nature Neurosci* 19: 716–724.

Penner M, Anagnostou E, Andoni LY, et al. (2017) Systematic review of clinical guidance documents for autism spectrum disorder diagnostic assessment in select regions. *Autism*. Epub ahead of print 1 May 2017. DOI: 10.1177/1362361316685879.

Pickles A, Le Couteur A, Leadbitter K, et al. (2016) Parent-mediated social communication therapy for young children with autism (PACT): Long-term follow-up of a randomised controlled trial. *Lancet* 388: 2501–2509.

Posey DJ, Kem DL, Swiezy NB, et al. (2004) A pilot study of D-cycloserine in subjects with autistic disorder. *Am J Psychiatry* 161: 2115–2117.

Raznahan A, Lenroot R, Thurm A, et al. (2013a) Mapping cortical anatomy in preschool aged children with autism using surface-based morphometry. *NeuroImage Clin* 2: 111–119.

Raznahan A, Wallace GL, Antezana L, et al. (2013b) Compared to what? Early brain overgrowth in autism and the perils of population norms. *Biol Psychiatry* 74: 563–575.

Redcay E and Courchesne E (2005) When is the brain enlarged in autism? A meta-analysis of all brain size reports. *Biol Psychiatry* 58: 1–9.

Regal RA, Rooney JR and Wandas T (1994) Facilitated communication: An experimental evaluation. *J Autism Dev Disord* 24: 345–355.

Reichow B, Volkmar FR and Bloch MH (2013) Systematic review and meta-analysis of pharmacological treatment of the symptoms of attention-deficit/hyperactivity disorder in children with pervasive developmental disorders. *J Autism Dev Disord* 43: 2435–2441.

Reisberg B, Doody R, Stoffler A, et al. (2003) Memantine in moderate-to-severe Alzheimer's disease. *N Engl J Med* 348: 1333–1341.

Research Units on Pediatric Psychopharmacology (RUPP) Autism Network (2005) Randomized, controlled, crossover trial of methylphenidate in pervasive developmental disorders with hyperactivity. *Arch Gen Psychiatry* 62: 1266–1274.

Richdale AL and Schreck KA (2009) Sleep problems in autism spectrum disorders: Prevalence, nature, & possible biopsychosocial aetiologies. *Sleep Med Rev* 13: 403–411.

Richetto J, Calabrese F, Riva MA, et al. (2014) Prenatal immune activation induces maturation-dependent alterations in the prefrontal GABAergic transcriptome. *Schizophren Bull* 40: 351–361.

Rimland B (1992) Facilitated communication: Problems, puzzles and paradoxes: Six challenges for researchers. *Autism Res Rev* 5: 3.

Rogers SJ (2004) Developmental regression in autism spectrum disorders. *Mental Retard Dev Disabl Res Rev* 10: 139–143.

Ronald A and Hoekstra RA (2011) Autism spectrum disorders and autistic traits: A decade of new twin studies. *Am J Med Gen B Neuropsychiatr Genet* 156: 255–274.

Rossignol DA and Frye RE (2011) Melatonin in autism spectrum disorders: A systematic review and meta-analysis. *Dev Med Child Neurol* 53: 783–792.

Rothman DL, De Feyter HM, Graaf RA, et al. (2011) 13C MRS studies of neuroenergetics and neurotransmitter cycling in humans. *NMR Biomed* 24: 943–957.

Rothman DL, De Feyter HM, Maciejewski PK, et al. (2012) Is there in vivo evidence for amino acid shuttles carrying ammonia from neurons to astrocytes? *Neurochem Res* 37: 2597–2612.

Roy M, Prox-Vagedes V, Ohlmeier MD, et al. (2015) Beyond childhood: Psychiatric comorbidities and social background of adults with Asperger syndrome. *Psychiatr Danub* 27: 50–59.

Rubenstein J and Merzenich MM (2003) Model of autism: Increased ratio of excitation/inhibition in key neural systems. *Genes Brain Behavior* 2: 255–267.

Rudra A, Banerjee S, Singhal N, et al. (2014) Translation and usability of autism screening and diagnostic tools for autism spectrum conditions in India. *Autism Res* 7: 598–607.

Russell AJ, Jassi A, Fullana MA, et al. (2013) Cognitive behavior therapy for comorbid obsessive-compulsive disorder in high functioning autism spectrum disorders: A randomized controlled trial. *Depress Anxiety* 30: 697–708.

Rutherford M, McKenzie K, Johnson T, et al. (2016) Gender ratio in a clinical population sample, age of diagnosis and duration of assessment in children and adults with autism spectrum disorder. *Autism* 20: 628–634.

Sabin LA and Donnellan AM (1993) A qualitative study of the process of facilitated communication. *J Assoc Pers Severe Handicaps* 18: 200–211.

Salazar F, Baird G, Chandler S, et al. (2015) Co-occurring psychiatric disorders in preschool and elementary school-aged children with autism spectrum disorder. *J Autism Dev Disord* 45: 2283–2294.

Sanders SJ, He X, Willsey AJ, et al. (2015) Insights into autism spectrum disorder genomic architecture and biology from 71 risk loci. *Neuron* 87: 1215–1233.

Sandler AD, Sutton KA, DeWeese J, et al. (1999) Lack of benefit of a single dose of synthetic human secretin in the treatment of autism and pervasive developmental disorder. *N Engl J Med* 341: 1801–1806.

Scahill L, Aman MG, McDougle CJ, et al. (2006) A prospective open trial of guanfacine in children with pervasive developmental disorders. *J Child Adolesc Psychopharmacol* 16: 589–598.

Scahill L, Hallett V, Aman MG, et al. (2013) Brief report: Social disability in autism spectrum disorder: Results from Research Units on Pediatric Psychopharmacology (RUPP) Autism Network trials. *J Autism Dev Disord* 43: 739–746.

Scahill L, McCracken JT, King BH, et al. (2015) Extended-release guanfacine for hyperactivity in children with autism spectrum disorder. *Am J Psychiatry* 172: 1197–1206.

Schumann CM, Bloss CS, Barnes CC, et al. (2010) Longitudinal magnetic resonance imaging study of cortical development through early childhood in autism. *J Neurosci* 30: 4419–4427.

Shea S, Turgay A, Carroll A, et al. (2004) Risperidone in the treatment of disruptive behavioral symptoms in children with autistic and other pervasive developmental disorders. *Pediatrics* 114: E634–E641.

Sheehan CM and Matuozzi RT (1996) Investigation of the validity of facilitated communication through the disclosure of unknown information. *Mental Retard* 34: 94.

Shekelle PG, Woolf SH, Eccles M, et al. (1999) Clinical guidelines: Developing guidelines. *BMJ* 318: 593.

Sibson NR, Dhankhar A, Mason GF, et al. (1998) Stoichiometric coupling of brain glucose metabolism and glutamatergic neuronal activity. *Proc Natl Acad Sci* 95: 316–321.

Silverman L, Hollway JA, Smith T, et al. (2014) A multisite trial of atomoxetine and parent training in children with autism spectrum disorders: Rationale and design challenges. *Res Autism Spectr Disord* 8: 899–907.

Simon EW, Whitehair PM and Toll DM (1996) A case study: Follow-up assessment of facilitated communication. *J Autism Dev Disord* 26: 9–18.

Simonoff E, Pickles A, Charman T, et al. (2008) Psychiatric disorders in children with autism spectrum disorders: Prevalence, comorbidity, and associated factors in a population-derived sample. *J Am Acad Child Adolesc Psychiatry* 47: 921–929.

Simpson RL and Myles BS (1995a) Effectiveness of facilitated communication with children and youth with autism. *J Spec Educ* 28: 424–439.

Simpson RL and Myles BS (1995b) Facilitated communication and children with disabilities: An enigma in search of a perspective. *Focus Except Child* 27: 1–16.

Sinzig J, Walter D and Doepfner M (2009) Attention deficit/hyperactivity disorder in children and adolescents with autism spectrum disorder: Symptom or syndrome? *J Atten Disord* 13: 117–126.

Smith MD and Belcher RG (1993) Brief report: Facilitated communication with adults with autism. *J Autism Dev Disord* 23: 175–183.

Smith MD, Haas PJ and Belcher RG (1994) Facilitated communication: The effects of facilitator knowledge and level of assistance on output. *J Autism Dev Disord* 24: 357–367.

Smith T (2001) Discrete trial training in the treatment of autism. *Focus Autism Other Dev Disabl* 16: 86–92.

Smith T, Aman MG, Arnold LE, et al. (2016) Atomoxetine and parent training for children with autism and attention-deficit/hyperactivity disorder: A 24-week extension study. *J Am Acad Child Adolesc Psychiatry* 55: 868–876. e862.

Sripada RK, Marx CE, King AP, et al. (2013) Allopregnanolone elevations following pregnenolone administration are associated with enhanced activation of emotion regulation neurocircuits. *Biol Psychiatry* 73: 1045–1053.

Strain PS and Bovey EH (2011) Randomized, controlled trial of the LEAP model of early intervention for young children with autism spectrum disorders. *Top Early Child Spec Educ* 31: 133–154.

Szempruch J and Jacobson JW (1993) Evaluating facilitated communications of people with developmental disabilities. *Res Dev Disabl* 14: 253–264.

Troost PW, Lahuis BE, Steenhuis M-P, et al. (2005) Long-term effects of risperidone in children with autism spectrum disorders: A placebo discontinuation study. *J Am Acad Child Adolesc Psychiatry* 44: 1137–1144.

Tsai GE and Lin P-Y (2010) Strategies to enhance N-methyl-D-aspartate receptor-mediated neurotransmission in schizophrenia, a critical review and meta-analysis. *Curr Pharm Des* 16: 522–537.

Tyzio R, Cossart R, Khalilov I, et al. (2006) Maternal oxytocin triggers a transient inhibitory switch in GABA signaling in the fetal brain during delivery. *Science* 314: 1788–1792.

Tyzio R, Nardou R, Ferrari DC, et al. (2014) Oxytocin-mediated GABA inhibition during delivery attenuates autism pathogenesis in rodent offspring. *Science* 343: 675–679.

Van Wijngaarden-Cremers PJ, van Eeten E, Groen WB, et al. (2014) Gender and age differences in the core triad of impairments in autism spectrum disorders: A systematic review and meta-analysis. *J Autism Dev Disord* 44: 627–635.

Vasa RA, Carroll LM, Nozzolillo AA, et al. (2014) A systematic review of treatments for anxiety in youth with autism spectrum disorders. *J Autism Dev Disord* 44: 3215–3229.

Vázquez CA (1994) Brief report: A multitask controlled evaluation of facilitated communication. *J Autism Dev Disord* 24: 369–379.

Veenstra-VanderWeele J, Cook EH, King BH, et al. (2016) Arbaclofen in children and adolescents with autism spectrum disorder: A randomized, controlled, phase 2 Trial. *Neuropsychopharmacology* 42: 1390–1398.

Veenstra-VanderWeele J, Muller CL, Iwamoto H, et al. (2012) Autism gene variant causes hyperserotonemia, serotonin receptor hypersensitivity, social impairment and repetitive behavior. *Proc Natl Acad Sci U S A* 109: 5469–5474.

Voineagu I, Wang X, Johnston P, et al. (2011) Transcriptomic analysis of autistic brain reveals convergent molecular pathology. *Nature* 474: 380–384.

Vorstman J, Staal W, Van Daalen E, et al. (2006) Identification of novel autism candidate regions through analysis of reported cytogenetic abnormalities associated with autism. *Mol Psychiatry* 11: 1, 18–28.

Waknine Y (2010) *FDA approves aripiprazole to treat irritability in autistic children*. Available at: https://www.medscape.com/viewarticle/713006 (accessed 23 February).

Wallace S, Parr J and Hardy A (2013) One in a hundred: Putting families at the heart of autism research. *Autistica*. Available at: https://www.rcpsych.ac.uk/pdf/One%20in%20a%20Hundred%20-%20Autistica's%20Report.pdf (accessed April 2015).

Warnell F, George B, McConachie H, et al. (2015) Designing and recruiting to UK autism spectrum disorder research databases: Do they include representative children with valid ASD diagnoses? *BMJ Open* 5: e008625.

Watanabe T, Kuroda M, Kuwabara H, et al. (2015) Clinical and neural effects of six-week administration of oxytocin on core symptoms of autism. *Brain* 138: 3400–3412.

Weiss MJS, Wagner SH and Bauman ML (1996) A validated case study of facilitated communication. *Mental Retard* 34: 220.

Weitlauf AS, McPheeters ML, Peters B, et al. (2014) *Therapies for children with autism spectrum disorder: Behavioral Interventions Update*. Comparative Effectiveness Review No. 137. (Prepared by the Vanderbilt Evidence-based Practice Center under Contract No. 290-2012-00009-I.) AHRQ Publication No. 14-EHC036-EF. Rockville, MD: Agency for Healthcare Research and Quality. Available at: https://effectivehealthcare.ahrq.gov/topics/autism-update/research (accessed 22 November 2017).

Wetherby AM, Guthrie W, Woods J, et al. (2014) Parent-implemented social intervention for toddlers with autism: An RCT. *Pediatrics* 134: 1084–1093.

Wheeler DL, Jacobson JW, Paglieri RA, et al. (1993) An experimental assessment of facilitated communication. *Mental Retard* 31: 49.

Whittington C, Pennant M, Kendall T, et al. (2016) Practitioner Review: Treatments for Tourette syndrome in children and young people–a systematic review. *J Child Psychol Psychiatry* 57: 988–1004.

Wigham S, Barton S, Parr JR, et al. (2017) A systematic review of the rates of depression in children and adults with high-functioning autism spectrum disorder. *J Mental Health Res Intell Disabl*: 1–21.

Williams K, Brignell A, Randall M, et al. (2013) Selective serotonin reuptake inhibitors (SSRIs) for autism spectrum disorders (ASD). *Cochrane Database Syst Rev* 8: CD004677.

Wilson CE, Gillan N, Spain D, et al. (2013) Comparison of ICD-10R, DSM-IV-TR and DSM-5 in an adult autism spectrum disorder diagnostic clinic. *J Autism Dev Disord* 43: 2515–2525.

Wilson CE, Murphy CM, McAlonan G, et al. (2016) Does sex influence the diagnostic evaluation of autism spectrum disorder in adults? *Autism* 20: 808–819.

Wilson SJ, Nutt D, Alford C, et al. (2010) British Association for Psychopharmacology consensus statement on evidence-based treatment of insomnia, parasomnias and circadian rhythm disorders. *J Psychopharmacol* 24: 1577–1601.

Wink LK, Minshawi NF, Shaffer RC, et al. (2017) D-Cycloserine enhances durability of social skills training in autism spectrum disorder. *Mol Autism* 8: 2.

World Health Organization (1992) *International Classification of Mental and Behavioural Disorders (ICD-10)*. Geneva, IL: WHO.

Yatawara CJ, Einfeld SL, Hickie IB, et al. (2016) The effect of oxytocin nasal spray on social interaction deficits observed in young children with autism: A randomized clinical crossover trial. *Mol Psychiatry* 21: 1225–1231.

Zielinski BA, Prigge MB, Nielsen JA, et al. (2014) Longitudinal changes in cortical thickness in autism and typical development. *Brain* 137: 1799–1812.

JAMA | Review

Autism Spectrum Disorder
A Review

Tomoya Hirota, MD; Bryan H. King, MD, MBA

IMPORTANCE Autism spectrum disorder (ASD), characterized by deficits in social communication and the presence of restricted, repetitive behaviors or interests, is a neurodevelopmental disorder affecting approximately 2.3% children aged 8 years in the US and approximately 2.2% of adults. This review summarizes evidence on the diagnosis and treatment of ASD.

OBSERVATIONS The estimated prevalence of ASD has been increasing in the US, from 1.1% in 2008 to 2.3% in 2018, which is likely associated with changes in diagnostic criteria, improved performance of screening and diagnostic tools, and increased public awareness. No biomarkers specific to the diagnosis of ASD have been identified. Common early signs and symptoms of ASD in a child's first 2 years of life include no response to name when called, no or limited use of gestures in communication, and lack of imaginative play. The criterion standard for the diagnosis of ASD is a comprehensive evaluation with a multidisciplinary team of clinicians and is based on semistructured direct observation of the child's behavior and semistructured caregiver interview focused on the individual's development and behaviors using standardized measures, such as the Autism Diagnostic Observation Schedule-Second Edition and the Autism Diagnostic Interview. These diagnostic measures have sensitivity of 91% and 80% and specificity of 76% and 72%, respectively. Compared with people without ASD, individuals with ASD have higher rates of depression (20% vs 7%), anxiety (11% vs 5%), sleep difficulties (13% vs 5%), and epilepsy (21% with co-occurring intellectual disability vs 0.8%). Intensive behavioral interventions, such as the Early Start Denver Model, are beneficial in children 5 years or younger for improvement in language, play, and social communication (small to medium effect size based on standardized mean difference). Pharmacotherapy is indicated for co-occurring psychiatric conditions, such as emotion dysregulation or attention-deficit/hyperactivity disorder. Risperidone and aripiprazole can improve irritability and aggression (standardized mean difference of 1.1, consistent with a large effect size) compared with placebo. Psychostimulants are effective for attention-deficit/hyperactivity disorder (standardized mean difference of 0.6, consistent with a moderate effect size) compared with placebo. These medications are associated with adverse effects including, most commonly, changes in appetite, weight, and sleep.

CONCLUSIONS AND RELEVANCE ASD affects approximately 2.3% of children aged 8 years and approximately 2.2% of adults in the US. First-line therapy consists of behavioral interventions, while co-occurring psychiatric conditions, such as anxiety or aggression, may be treated with specific behavioral therapy or medication.

JAMA. 2023;329(2):157-168. doi:10.1001/jama.2022.23661

Author Affiliations: Department of Psychiatry and Behavioral Sciences, UCSF Weill Institute for Neurosciences, University of California, San Francisco.

Corresponding Author: Bryan H. King, MD, MBA, Department of Psychiatry and Behavioral Sciences, UCSF Weill Institute for Neurosciences, 401 Parnassus Ave, Ste LP 358, San Francisco, CA 94143-0984 (bryan.king@ucsf.edu).

Section Editor: Mary McGrae McDermott, MD, Deputy Editor.

Autism spectrum disorder (ASD) is a neurodevelopmental disorder defined by social communication impairments and restricted, repetitive behaviors (**Box 1**).[1,2] ASD consists of a spectrum of symptoms reflecting impaired social communication and restricted, repetitive behaviors and ranges in severity from mildly impairing to severe (**Table 1**). It is recognized as a collection of related disorders of different etiologies. Manifestations of ASD are heterogeneous and can include individuals with intellectual disability and limited language ability and those with significantly above-average intellectual and language function who have difficulty with social communication. These difficulties manifest in the pragmatics or social norms associated with communication, such as speaking with appropriate volume, interacting at appropriate physical distance, and detecting and adapting communication in response to gestures and facial expression. Rigidity, manifested by requiring others to speak or behave in specific ways or needing to adhere to prescribed schedules or activities, is common. The complexity and heterogeneity of ASD are related to both developmental factors, such as age and IQ, and environmental factors, such as availability of support including individualized educational services and speech, language, and behavioral interventions. Intellectual disability, language disorder and medical and psychiatric conditions, including

Box 1. Diagnostic Criteria for Autism Spectrum Disorder (ASD) Based on *Diagnostic and Statistical Manual of Mental Disorders, 5th Edition*, Text Revision[a]

To meet diagnostic criteria for ASD according to DSM-5, a child must have persistent deficits in each of 3 areas of social communication and interaction (see A.1. through A.3. below) plus at least 2 of 4 types of restricted, repetitive behaviors (see B.1. through B.4. below).

A. Persistent deficits in social communication and social interaction across multiple contexts, as manifested by the following, currently or by history (examples are illustrative, not exhaustive; see below):
   1. Deficits in social-emotional reciprocity, ranging, for example, from abnormal social approach and failure of normal back-and-forth conversation; to reduced sharing of interests, emotions, or affect; to failure to initiate or respond to social interactions.
   2. Deficits in nonverbal communicative behaviors used for social interaction, ranging, for example, from poorly integrated verbal and nonverbal communication; to abnormalities in eye contact and body language or deficits in understanding and use of gestures; to a total lack of facial expressions and nonverbal communication.
   3. Deficits in developing, maintaining, and understanding relationships, ranging, for example, from difficulties adjusting behavior to suit various social contexts; to difficulties in sharing imaginative play or in making friends; to absence of interest in peers.

Specify current severity: Severity is based on social communication impairments and restricted repetitive patterns of behavior. (See below.)

A. Restricted, repetitive patterns of behavior, interests, or activities, as manifested by at least two of the following, currently or by history (examples are illustrative, not exhaustive; see text):
   1. Stereotyped or repetitive motor movements, use of objects, or speech (eg, simple motor stereotypies, lining up toys or flipping objects, echolalia, idiosyncratic phrases).
   2. Insistence on sameness, inflexible adherence to routines, or ritualized patterns or verbal nonverbal behavior (eg, extreme distress at small changes, difficulties with transitions, rigid thinking patterns, greeting rituals, need to take same route or eat food every day).
   3. Highly restricted, fixated interests that are abnormal in intensity or focus (eg, strong attachment to or preoccupation with unusual objects, excessively circumscribed or perseverative interest).
   4. Hyper- or hyporeactivity to sensory input or unusual interests in sensory aspects of the environment (eg, apparent indifference to pain/temperature, adverse response to specific sounds or textures, excessive smelling or touching of objects, visual fascination with lights or movement).

Specify current severity: Severity is based on social communication impairments and restricted, repetitive patterns of behavior. (See below.)

A. Symptoms must be present in the early developmental period (but may not become fully manifest until social demands exceed limited capacities or may be masked by learned strategies in later life).
B. Symptoms cause clinically significant impairment in social, occupational, or other important areas of current functioning.
C. These disturbances are not better explained by intellectual disability (intellectual developmental disorder) or global developmental delay. Intellectual disability and autism spectrum disorder frequently co-occur; to make comorbid diagnoses of autism spectrum disorder and intellectual disability, social communication should be below that expected for general developmental level.

Note: Individuals with a well-established *DSM-IV* diagnosis of autistic disorder, Asperger disorder, or pervasive developmental disorder not otherwise specified should be given the diagnosis of ASD. Individuals who have marked deficits in social communication, but whose symptoms do not otherwise meet criteria for ASD, should be evaluated for social (pragmatic) communication disorder.

Specify if:

- **With or without accompanying intellectual impairment**
- **With or without accompanying language impairment** (Coding note: use additional code to identify the associated medical or genetic condition.)
- **Associated with another neurodevelopmental, mental, or behavioral disorder** (Coding note: use additional code[s] to identify the associated neurodevelopmental, mental, or behavioral disorder[s].)
- **With catatonia**
- **Associated with a known medical or genetic condition or environmental factor**

[a] Reproduced with permission from the *Diagnostic and Statistical Manual of Mental Disorders, Text Revision*, pp 56-57. © 2022, American Psychiatric Association.

epilepsy, sleep problems, anxiety, and depression, are common.[3,4] This review summarizes current evidence regarding the epidemiology, pathophysiology, diagnosis, and clinical management of ASD.

Methods

We searched PubMed for English-language studies of the epidemiology, etiology, pathogenesis, diagnosis (screening and assessment), and treatment of ASD published from January 1, 2010, to October 31, 2022. We manually searched the references of selected publications for additional relevant articles. When selecting papers to include, randomized clinical trials, systematic reviews, meta-analyses, clinical practice guidelines, and articles relevant to a general medical readership were prioritized. Of 591 publications identified, 46 articles were included, consisting of 11 randomized clinical trials, 6 cohort studies, 5 systematic reviews, and 24 meta-analyses. The effect sizes reported in this review consist of standardized mean difference (SMD), also known as Cohen *d*, unless otherwise specified. An SMD of 0.2 to 0.5 indicates a small effect, 0.5 to 0.8 indicates a medium effect, and greater than 0.8 indicates a large effect.

Discussion

Epidemiology

Among children aged 8 years in the US, the estimated prevalence of ASD has increased from approximately 1.1% in 2008 to 2.3% in 2018.[5] Studies that use administrative databases (eg, special

Table 1. Severity Levels for Autism Spectrum Disorder[a]

Severity level	Social communication	Restricted, repetitive behaviors
Level 3: requiring very substantial support	Severe deficits in verbal and nonverbal social communication skills cause severe impairments in functioning, very limited initiation of social interactions, and minimal response to social overtures from others. For example, a person with few words of intelligible speech who rarely initiates interaction and, when they do, makes unusual approaches to meet needs only and responds to only very direct social approaches.	Inflexibility of behavior, extreme difficulty coping with change, or other restricted/repetitive behaviors markedly interfere with functioning in all spheres. Great distress/difficulty changing focus or action.
Level 2: requiring substantial support	Marked deficits in verbal and nonverbal social communication skills; social impairments apparent even with supports in place; limited initiation of social interactions; and reduced or abnormal responses to social overtures from others. For example, a person who speaks simple sentences, whose interaction is limited to narrow special interests, and who has markedly odd nonverbal communication.	Inflexibility of behavior, difficulty coping with change, or other restricted/repetitive behaviors appear frequently enough to be obvious to the casual observer and interfere with functioning in a variety of contexts. Distress and/or difficulty changing focus or action.
Level 1: requiring support	Without supports in place, deficits in social communication cause noticeable impairments. Difficulty initiating social interactions and clear examples of atypical or unsuccessful response to social overtures of others. May appear to have decreased interest in social interactions. For example, a person who is able to speak in full sentences and engages in communication but whose to-and-fro conversation with others fails and whose attempts to make friends are odd and typically unsuccessful.	Inflexibility of behavior causes significant interference with functioning in one or more contexts. Difficulty switching between activities. Problems of organization and planning hamper independence.

[a] Reprinted with permission from the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition*, (Copyright 2013). American Psychiatric Association.

education data, health records) tend to underestimate the prevalence of ASD compared with studies that use more rigorous methods for case ascertainment, such as a total population sampling and standardized screening and diagnostic measures.[6,7]

Changes in diagnostic criteria, increased awareness of ASD, improved ascertainment, and greater access to services, such as early behavioral intervention and education with individualized programs designed for children with ASD, have likely contributed to the higher prevalence of ASD.[8,9] In addition, the definition of autism now includes a broader spectrum, which may partially explain the increase in prevalence over earlier estimates.

The estimated prevalence of ASD is higher in males than in females (3.7% [95% CI, 3.5-3.8] in boys and 0.9% [95% CI, 0.8-0.9] in girls aged 8 years in the surveillance conducted by the Autism and Developmental Disabilities Monitoring Network in 11 states in the US).[5] Studies that used rigorous methods for case ascertainment reported lower male-to-female ratios of the estimated prevalence of ASD: 3.7% (95% CI, 2.6-4.9) in boys and 1.5% (95% CI, 0.6-2.4) in girls.[9] Girls and women are more likely or better able to minimize ASD symptoms, including social communication difficulties (clinically referred to as "camouflaging").[10] It is also possible that current diagnostic procedures are less sensitive to the presence of ASD among females.[11] A study using a large database of 641 860 adults residing in the community reported higher ASD rates in transgender and gender-diverse individuals compared with cisgender individuals.[12] Children with ASD who are Black tend to present at older ages than those who are White and more often present with intellectual disability,[5] suggesting racial disparities in access to care.[13]

In a study of 664 children with ASD in which younger siblings were followed up prospectively from birth, 19% of these younger siblings were diagnosed with ASD by the age of 36 months.[14] Rates of diagnosis of ASD were 14% in male siblings compared with 5% in female siblings in an observational study of 39 460 children with ASD using an administrative database.[15]

Pathogenesis and Pathophysiology

In population-based data sets from 3 Nordic countries that collectively included 22 156 people with ASD and studied mean estimated heritability, the variation in ASD traits attributed to genetic factors was 81% (95% CI, 74%-85%).[16] Environmental factors were associated with 14% to 22% of the risk of ASD in the same study. A relatively small number of rare genetic variants in approximately 100 genes (eg, *KMT2A*, *NRXN1*, *SHANK3*) have been identified that were associated with significant risk,[17] whereas a larger number, perhaps thousands, of common variants were associated with smaller risk but, in combination, accounted for the majority of cases.[18,19] Genetic risk factors for ASD overlap with other diverse developmental and psychiatric disorders.[16,20,21] A variety of genetic and environmental factors have been associated with ASD, but none are absolutely specific for the development of ASD.

Many of the autism risk genes affect gene expression regulation, neurogenesis, chromatin modification, and synaptic function. Additional support for the role of genetic factors was reported by Willsey et al.[22] This study used an in vivo *Xenopus* model and examined 10 genes with the strongest statistical association with ASD that all were expressed in the telencephalon (the forebrain that is primarily composed of the cerebral hemispheres) at time points corresponding to human mid-prenatal prefrontal cortex development.[22] Estrogen mitigates the effects of ASD risk gene disruption, and this may help explain the sex differences in prevalence.

A meta-analysis of studies identified that maternal factors, such as gestational hypertension (odds ratio, 1.4 [95% CI, 1.2-1.5]), overweight before or during pregnancy (relative risk [RR], 1.3 [95% CI, 1.2-1.4]), preeclampsia (RR, 1.3 [95% CI, 1.2-1.5]), and maternal age of 35 years or older (RR, 1.3 [95% CI, 1.2-1.5]) were associated with higher rates of ASD in offspring (absolute rates not provided).[23] In addition, cohort and case-control studies reported that advanced paternal age (21% increase in ASD diagnosis in offspring for every 10-year increase in paternal age),[24] medication use in pregnancy, and

Box 2. Early Behavior Signs of Possible Autism Spectrum Disorder

Absence of Developmentally Expected Milestone Attainment

Avoids or does not maintain eye contact.

Does not respond to name by 9 months of age.

Does not show facial expressions of emotions by 9 months of age.

Rarely shares enjoyment with caregivers.

No simple interactive games (eg, pat-a-cake) by 12 months of age.

Uses no or few gestures (eg, does not wave goodbye).

Does not share interests with others.

Does little or no imitation of other people or does not pretend.

No pointing (to show caregivers something interesting) by 18 months of age.

Emergence of Aberrant Behaviors

Lines up toys in a particular order and gets upset when the order is changed.

Uses repetitive words and phrases.

Moves their fingers, hands, or body in an unusual way (finger flicking, hand flapping, body rocking, spinning self in circles, for example).

Shows excessive interest in particular objects.

Has obsessive interests in certain objects and attachment to unusual objects.

Has unusual reactions to sensory stimuli (eg, getting upset about a clothing tag, avoiding eating food with certain textures).

Has strong interest in and seeks unusual sensory experiences (eg, squinting or flapping hands to certain lights, excessively rubbing certain textures, licking or smelling objects.).

both short (<12 months) and long (≥72 months) periods between pregnancies[25] were associated with an increased risk for the diagnosis of ASD in offspring. Regarding medication use during pregnancy and risk of ASD, the absolute risk for ASD for offspring was 4.4% (95% CI, 2.6%-7.5%) with exposures to valproic acid during pregnancy compared with the risk of 1.5% (95% CI, 1.5%-1.6%) for ASD without exposure to valproic acid in a population-based study of 655 615 children in Denmark.[26] Although selective serotonin reuptake inhibitor (SSRI) use during pregnancy has been associated with an increased risk for ASD in cohort studies, a meta-analysis underscored that the association was confounded by other factors, especially the indication for SSRI use and the genetic association between maternal depression and risk for ASD.[27] Therefore, evidence does not preclude the use of SSRIs for treating depression during pregnancy when indicated. A large body of research refutes claims of linkage between vaccines and ASD.[28-30]

Clinical Presentation

The presenting symptoms of ASD depend on age, language levels (from nonverbal to fully fluent), cognitive abilities, and sex. In the first 2 years of life, common features include poor acquisition of or declines in language skills and communicative gestures or failure to learn or adopt these skills. ASD is also characterized by diminished responsiveness in social interactions and presence of repetitive behaviors, such as no response to name when called, hand flapping, and lining up toys in a particular way (**Box 2**).[31-33]

Behavioral or cognitive rigidity (eg, insisting that routines are precisely followed or that others adhere to specific verbal scripts), lack of interest in socializing, restricted interests, and lack of imaginative play typically become more apparent as a child develops. Children with visual and/or hearing impairment may have delays in attaining developmental milestones (eg, deficits in nonverbal communication due to blindness) compared with those without sensory impairment and exhibit behaviors that overlap with ASD symptoms (eg, stereotyped, repetitive motor movements),[34] requiring careful assessment to determine whether behaviors these children exhibit are part of the symptoms of ASD. The estimated prevalence of ASD is higher in individuals with special health needs. For example, the estimated prevalence of ASD is 19% in people with visual impairment, 9% in people with hearing impairment,[35] 18% in people with intellectual disability,[36] 16% in people with Down syndrome,[37] and 30% in people with fragile X syndrome.[37]

Children who have ASD that is not associated with delays in acquiring language or other developmental milestones may experience delays in diagnosis of ASD. These individuals may first come to receive medical attention because of behavioral problems associated with ASD, such as disruptive behaviors, difficulties following instructions due to intense interest in preferred activities, or co-occurring neurodevelopmental and psychiatric disorders. Individuals without moderate or severe intellectual or learning disability may seek professional evaluation in adulthood if they encounter challenges obtaining or sustaining education or employment and have characteristics of ASD.[38]

Co-occurring developmental and psychiatric conditions are common in people with ASD. Other neurodevelopmental disorders, such as attention-deficit/hyperactivity disorder (ADHD) and intellectual disability, are more likely to co-occur in people with ASD compared with those without ASD (28% vs 7% for ADHD and 23% vs 0.7% for intellectual disability).[3,39,40] Anxiety and depressive disorders also co-occur more frequently in people with ASD than those without ASD (20% vs 7% for anxiety disorder and 11% vs 5% for depressive disorders).[3] In older children, adolescents, and adults, coexistent mood disorders and related behaviors (eg, depression and suicidality) may greatly contribute to reduced quality of life and increased mortality.[41] Severe behaviors, such as aggression and self-injury, may occur with ASD.[42] People with ASD are at increased risk of specific medical conditions, such as epilepsy (21% in people with ASD and intellectual disability and 8% in those without intellectual disability vs 0.8% in a general population sample),[43,44] feeding problems (eg, focus on specific foods, sensitivity to textures),[45] motor coordination difficulties such as trouble coordinating movements between the left and right side of the body or problems maintaining their posture (37% vs 5%),[46,47] gastrointestinal conditions (eg, constipation [22%]),[48] and sleep difficulties (13% vs 5%)[3] compared with those without ASD. These conditions may bring children to medical attention and lead to a diagnosis of ASD.[4]

Savant skills, defined as special skills that exceed what conventionally seems humanly possible, most commonly manifest in memory, art, music, mental arithmetic, and calendar calculation (eg, the ability to provide the day of the week for any given date going back hundreds of years)[49] are more common in people with ASD. People with extreme savant skills may capture media attention, but may provide stereotyped portrayals of ASD. Savant skills may occur in as many as approximately 29% of affected individuals.[50] Some

features of ASD, such as restricted interests and repetitive behaviors, may predispose to intense focus and relentless practice of skills that eventually become superior.[49,51]

Diagnosis of ASD

Screening

The American Academy of Pediatrics recommends that all children be screened at 18 and 24 months of age for ASD.[52] In contrast, in 2016 the US Preventive Services Task Force concluded that evidence was insufficient to recommend screening for ASD in young children for whom no concerns were raised by their parents because of a lack of randomized clinical trials that addressed the question of whether early identification of ASD in young children through screening could improve core symptoms of ASD.[53] The US Preventive Services Task Force called for additional research on whether earlier identification through universal screening is associated with improved outcomes in children with ASD to update the statement issued in 2016.[54]

The Modified-Checklist for Autism in Toddlers, Revised (M-CHAT-R), a 20-item screening questionnaire, is one of the frequently used autism-specific screening tools in primary care settings designed to identify children aged 16 to 30 months who are at risk for ASD from a general population.[55] Children whose M-CHAT-R total score is higher than 2 are considered at risk for ASD and require follow-up questions by health care professionals for additional information about the items the child did not pass on the M-CHAT, which increase its specificity (sensitivity of 85% and specificity of 99% for the M-CHAT-R with follow-up questions). Guidelines generally do not recommend one autism-specific screening tool over others. Children who have a positive screening test result for ASD should undergo a comprehensive evaluation and referral for developmental services, consisting of early behavioral intervention and family guidance. There is no evidence to support screening for ASD in asymptomatic adults, and few screening tools for ASD exist for adults.[55] The National Institute for Health and Care Excellence in the UK recommends that clinicians consider using the Autism Spectrum Quotient, a 10-item screening tool, for adults with suspected ASD who do not have moderate or severe intellectual disability to determine whether a referral for a comprehensive assessment is indicated.[38]

Diagnostic Assessment

The criterion standard for an ASD diagnosis is the best-estimate clinical consensus, defined as agreement within a multidisciplinary professional team based on a detailed developmental history and observation of the individual's behaviors using standardized diagnostic tools. The most widely used of these standardized diagnostic tools for ASD include the Autism Diagnostic Interview, Revised (ADI-R), a semi-structured interview with the parent(s), and the Autism Diagnostic Observation Schedule, Second Edition (ADOS-2), a semi-structured direct observation of a child's behavior. The diagnostic conclusion incorporates definitions from the *Diagnostic and Statistical Manual of Mental Disorders* and *International Classification of Diseases* as well as the diagnostic impression and opinions from multidisciplinary professionals involved in the assessment.[42] The ADI-R and the ADOS-2 have sensitivity of 80% (95% CI, 79%-82%) and 91% (95% CI, 90%-92%), respectively. Specificity is 72% (95% CI, 70%-74%) for the ADI-R and 76% (95% CI, 74%-78%) for the ADOS-2.[56] Nevertheless, these diagnostic tools should inform and not supersede clinical judgement. Cognitive and adaptive function testing, such as the Differential Ability Scales, Wechsler Intelligence Scale for Children, and Vineland Adaptive Behavior Scales, and assessment of speech and language to evaluate quantitative and qualitative speech abilities and communication skills are important for establishing the diagnosis of ASD as well as developing therapy plans. Sensory and motor assessments can provide useful supplementary information related to functional fine and gross motor skills and sensory processing differences (eg, sensory hypersensitivity and hyposensitivity).[57] Multidisciplinary assessment can assist clinicians in differentiating between ASD and other disorders (eg, intellectual disability, pragmatic language disorder, ADHD). ASD diagnostic tools are not standardized in individuals with visual impairment and hearing loss. Co-occurring emotional and behavioral problems may also affect performance on diagnostic measures for ASD.[58]

The American Academy of Pediatrics and the American College of Medical Genetics and Genomics recommend genetic testing for individuals who are diagnosed with ASD.[42,52,59] Ascertaining a genetic etiology of ASD through genetic testing can provide patients, families, and clinicians with information about recurrence risk and prognosis and help navigate patients and families to support and resources specific to genetic conditions. In particular, chromosomal microarray is recommended to scan the genome for copy number variants. Fragile X testing is recommended for all individuals diagnosed with ASD, and females with developmental regression should be tested for Rett syndrome (MECP2 gene sequencing). Clinicians should consider referring the patient to a geneticist if ASD associated with a genetic syndrome is suspected based on family history and congenital anomalies, such as craniofacial anomalies or macrocephaly, on physical examination. The detection of potentially causal genetic abnormalities can inform family planning discussions and subsequent medical surveillance. However, the probability of clinically actionable findings from testing must be balanced by the potential financial or physical burden of testing on patients.

Individuals with established ASD benefit from thorough physical examinations given high rates of gastrointestinal problems, such as constipation and abdominal discomfort; dermatological conditions, such as atopic dermatitis; and neurological manifestations in certain genetic disorders, such as tuberous sclerosis complex. An electroencephalogram is not recommended as part of the evaluation for ASD unless there are concerns about epilepsy or specific developmental disorders associated with abnormal encephalographic findings (eg, Landau-Kleffner syndrome, which is characterized by aphasia and agnosia).[42]

Management

The goal of therapy is to improve an individual's function and well-being.[60] Behavioral interventions are well-supported by evidence (eg, see the National Standards Project by the National Autism Center[61]). No medications have demonstrated efficacy for the core diagnostic symptoms of ASD.[62,63] Pharmacologic interventions, such as aripiprazole and risperidone, can mitigate behavioral and emotional dysregulation that co-occur in individuals with ASD. Current evidence is summarized for psychosocial interventions in **Table 2** and for pharmacological interventions in **Table 3**. **Box 3** highlights some commonly asked questions regarding clinical care for individuals with ASD.

Table 2. Therapeutic Interventions for Individuals With Autism Spectrum Disorder (ASD)

Therapy type	Appropriate age range for therapy	Target condition(s)	Description of therapy	Summary of evidence
Behavioral approaches (eg, early intensive behavioral intervention [EIBI], Discrete Trial Training)	Young children (aged <5 y)	Adaptive skills, cognition, language, motor skills, social communication, and emotional and behavioral disorders	Intensive, individualized behavior analytic approaches, where antecedents of (environments leading to) behaviors and functions of behaviors are analyzed through behavior observation to build new repertoires and reduce interfering behaviors.	The pooled effect size of intensive behavioral therapy (approximately 25 h/w) in a meta-analysis of 21 studies, including both RCTs and quasi-experimental studies was small to medium: 0.24 (95% CI, 0.01-0.47) for language, 0.29 (95% CI, 0.05-0.54) for cognitive ability, 0.38 (95% CI, 0.19-0.56) for adaptive behavior, and 0.40 (95% CI, 0.18-0.61) for social communication.[64]
Developmental approaches (eg, Developmental, Individual-Differences, Relationship-Based/ Floortime model, Preschool Autism Communication Trial)			Social-pragmatic approaches intended to promote social communication and interactions. In this model, development is considered to be the result of children's active exploration of their physical and social surroundings.	A meta-analysis of 11 RCTs demonstrated that developmental approaches were associated with improved social communication (effect size, 0.27 [95% CI, 0.05-0.48]), but not language effect size, 0.06 (95% CI, −0.08 to 0.21).[64]
Naturalistic Developmental Behavioral Intervention (NDBI) (eg, ESDM, pivotal response training, JASPER, Project ImPACT)			An approach integrating both behavioral and developmental principles: the NDBI approaches emphasize the developmental systems approach and are diverse from the EIBI in a way that instructions and teaching are delivered in a physical environment that looks similar to typical daily experiences.	A meta-analysis of 17 RCTs of people with ASD showed that, compared with control/behavioral interventions commonly available in the community, the NDBIs were associated with better developmental outcomes, including language (effect size, 0.21 [95% CI, 0.01-0.41]), play (effect size, 0.33 [95% CI, 0.13-0.54]), and social communication (effect size, 0.42 [95% CI, 0.23-0.62]) domains.[64] In a randomized clinical trial of 87 participants, the effect of 2 different treatment intensities (15 h/wk for 12 mo vs 25 h/wk for 12 mo) of the ESDM, one of the NDBIs did not significantly differ on the intervention outcomes, including autism severity, expressive communication, receptive language, and nonverbal development.[65]
Treatment and Education of Autistic and Related Communication Children (TEACCH)	Children, adolescents, and adults	ADL, language, communication, social skills, executive functioning, and engagement	Emphasizes a close working relationship between parents and practitioners, adapts the intervention to the particular characteristics of the individual client, and makes use of structured teaching experiences. TEACCH is one of the most widely used approaches in school settings.	A meta-analysis of 6 studies (4 quasi-experimental studies and 2 RCTs) of 202 participants showed no significant association of TEACCH with improvement in social communication outcomes (effect size, −0.11 [95% CI, −0.93 to 0.71]).[65] However, the lack of benefit was likely due to a noncluster randomized design used in the RCTs and the lack of studies with large sample sizes.
Psychotherapy (cognitive behavioral therapy [CBT])	School-age children, adolescents	Anxiety	CBT is based on the cognitive model, in which people's behaviors and emotions are influenced by their perceptions of events. In CBT for anxiety, imaginal and in-vivo exposure tasks are essential, in addition to identifying cognition in anxiety-provoking situations and developing cognitive reappraisal. The focus of CBT is problem-oriented, with an emphasis on the present.	In a meta-analysis of 45 RCTs and 6 quasi RCTs of 2485 participants with ASD, the effect size of CBT on social-emotional problems in individuals with ASD was statistically significant, but was associated with a modest effect (effect size, 0.57 [95% CI, 0.24-0.90]).[66] In a 16-wk RCT of 167 children aged 6-13 y, adapted CBT for ASD (90-min weekly with parental involvement) was significantly better for reducing anxiety symptoms compared with generic CBT (effect size, 0.63 [95% CI, 0.27-0.99]) and treatment as usual/non-CBT psychotherapy commonly available in the community (effect size, 1.69 [95% CI, 1.10-2.26]).[67] Studies targeting adults with ASD are scarce but emerging.[68]
Group social skills interventions (GSSIs)	Adolescents, young adults	Social skills	Group interventions with a manual providing strategies to foster social competence, including direct instruction with written or visual materials, modeling, role-play, and group sessions.	In a meta-analysis of 9 RCTs with 362 participants with ASD, UCLA PEERS, the most widely used manual-based GSSI for ASD, consisting of 12 90-min sessions delivered once a week, was associated with an increase in self-reported social knowledge (effect size, 2.15 [95% CI, 1.54-2.77]) and parent-reported social functioning at week 12 (effect size, 0.71 [95% CI, 026-1.15]) compared with the delayed-treatment control group.[69] In the RCTs included in the meta-analysis, the delayed-treatment control group received UCLA PEERS intervention after the postintervention assessment was conducted at wk 12.

Abbreviations: ESDM, Early Start Denver Model; JASPER, Joint Attention, Symbolic Play, Engagement, and Regulation; PEERS, Program for Excellence in Education and Research in the Sciences; Project ImPACT, Project Improving Parents as Communication Teachers; RCT, randomized clinical trial.

Table 3. Pharmacological Interventions for Individuals With Autism Spectrum Disorder (ASD)

Medication by condition	Category of medication and mechanism of action	Strength of evidence[a]	Common adverse events[b]
Irritability, aggression, emotional dysregulation			
Aripiprazole	Atypical antipsychotics; partial agonist at D_2 dopamine receptor and serotonin (5-HT)1A receptors and an antagonist at serotonin 5-HT2A receptor.	In a meta-analysis of 5 RCTs and 808 children and adolescents with ASD, aripiprazole was associated with a reduction of emotional dysregulation, including irritability and aggression compared to placebo (SMD, 1.18; 95% CI, 0.84 to 1.52).[70]	Drowsiness ,10.4% (vs 3.96% in the placebo group); vomiting, 13.6% (vs 5.94%); increased appetite, 9.43% (vs 6.93%); and extrapyramidal symptoms 8.96% (vs 3.96%)
Risperidone	Atypical antipsychotics; Antagonist at D_2 dopamine receptor and 5-HT2A receptors but has a higher affinity for 5-HT2A receptors than for D_2 receptors. Adrenergic and histaminergic receptors are also involved in its mechanism of action.	In a meta-analysis of 6 RCTs and 372 participants with ASD, risperidone was associated with improvement in irritability and emotional dysregulation compared with placebo (SMD, 1.07 [95% CI, 0.82-1.33]).[70]	Drowsiness, 40.0% (vs 8.0% in the placebo group); vomiting, 17.3% (vs 16.0%); constipation, 14.0% (vs 6.40%); increased appetite, 40.7% (vs 16.8%); and extrapyramidal symptoms 16.0% (vs 8.0%)
ADHD			
Methylphenidate (MPH)	Psychostimulant medication; blocks the reuptake of dopamine and noradrenaline through the blockade of dopamine and noradrenaline transporters.	In a meta-analysis of 4 placebo-controlled RCTs and 117 children with ASD, MPH was associated with a reduction of ADHD symptoms (SMD, 0.60 [95% CI, 0.23-0.96] for parent-rated overall ADHD symptoms).[71] All 4 studies used a crossover study design, and study duration was short (1-2 wk).	Appetite decrease, 29.8% (vs 9.65% in the placebo group); sleep problems, 27.2% (vs 2.91%); irritability, 21.1% (vs 17.5%); headache, 6.14% (vs 1.75%); and stomach discomfort, 9.65% (vs 1.75%)
Atomoxetine	Nonpsychostimulant ADHD medication; inhibits the presynaptic noradrenaline transporter and prevents the reuptake of noradrenaline.	Atomoxetine, compared with placebo, was associated with improvement in ADHD symptoms (SMD, 0.44 [95% CI, 0.06-0.93]) in a meta-analysis of 4 RCTs with 237 children with ASD.[71]	Appetite decrease, 43.0% (vs 22.5% in the placebo group); irritability, 33.6% (vs 34.9%); sleep problems, 30.5% (vs 17.8%); and vomiting, 25% (vs 14.7%)
Extended-release guanfacine	Nonpsychostimulant ADHD medication; stimulates postsynaptic α_{2A}-adrenergic receptors to enhance noradrenaline neurotransmission.	The effectiveness of extended-release guanfacine is supported by evidence from an 8-week placebo-controlled RCT (n = 62) for improvement in the investigator-rated total ADHD symptoms (SMD, 1.20 [95% CI, 0.66-1.75).[72]	Drowsiness, 86.7% (9.4% in the placebo group); decreased appetite, 43.3% (vs 6.25%); emotional/tearful, 40% (vs 3.1%); and dry mouth 40% (vs 3.1%)
Restricted, repetitive behaviors			
SSRIs (citalopram, escitalopram, fluoxetine, fluvoxamine, sertraline)	Antidepressants; inhibit the serotonin transporter at the presynaptic axon terminal and inhibit the reuptake of serotonin, thereby increasing the availability of serotonin in the synapse.	In a meta-analysis of 7 RCTs and 631 participants, SSRIs overall were not associated with improvement in restricted, repetitive behaviors in ASD (effect size, 0.09 [95% CI, −0.21 to 0.39]).[73]	Gastrointestinal problems, 16.1% (vs 11.3% in the placebo group); mood disturbance, 28.7% (vs 23.7%); energy increase, 30.5% (vs 16.5%); insomnia, 31.1% (vs 25.3%); and vivid dreams 8.42% (vs 0%)
Anxiety and depression		No RCTs conducted specifically to examine the efficacy of SSRIs for mood and anxiety symptoms in individuals with ASD.	
Sleep problems			
Melatonin	Other; activates melatonin receptors with a high affinity for the melatonin 1 receptor, thereby regulating the sleep/wake cycle.	In a 13-wk RCT comparing prolonged-release melatonin and placebo in 125 children (96.8% had ASD; 3.2% had Smith-Magenis syndrome), melatonin increased total sleep time by 51.2 min vs 18.7 min in the placebo group (P = .03; Cohen d = 0.4) and decreased sleep latency by 37.9 min vs 12.6 min in the placebo group (P = .01; Cohen d = 0.5).[74]	Somnolence, 28.3% (vs 10.8% in the placebo group), headache 13.3% (vs 6.2%)
Hyperactivity, irritability			
N-Acetylcysteine (NAC)	Other; functions as an antioxidant through its contribution to the production of glutathione, a major intracellular antioxidant within the central nervous system.	In a meta-analysis of RCTs (5 trials, 225 individuals with ASD), NAC, compared with placebo, was associated with a significant reduction in hyperactivity (mean difference, 4.80 [95% CI, 1.20-8.40]) and irritability (mean difference, 4.07 [95% CI, 1.13-7.01]), but there were no differences between these 2 groups in overall changes in social communication and stereotyped behavior.[75]	GI symptoms, 32.3% (vs 20.6% in the placebo group) and drowsiness, 12.9% (vs 6.5%)
Social communication challenges			
Oxytocin	Other; neuropeptide potentially plays an important role in modulating social-communicative behaviors.	Despite initial positive findings in small sample-sized trials, oxytocin failed to demonstrate efficacy in improving social communication compared with placebo (least square mean change, −3.7 [95% CI, −4.8 to −2.8] vs −3.5; [95% CI, −4.4 to −2.6]; P = .61) in the largest sample-sized RCT with 290 children and adolescents with ASD.[76]	Increased appetite, 16% (vs 10% in the placebo group); increased energy, 10% (vs 3%); and restlessness 8% (vs 2%)

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; RCT, randomized clinical trial; SSRI, selective serotonin reuptake inhibitor.

[a] A standardized mean difference (SMD) of 0.2 to 0.5 indicates a small effect, 0.5 to 0.8 indicates a medium effect, and greater than 0.8 indicates a large effect.

[b] Adverse events listed here can occur in a general population and are not specific to individuals with ASD.

Box 3. Commonly Asked Questions About Autism Spectrum Disorder (ASD)

Among Adults Presenting for the First Time With Possible ASD, What Other Conditions Should Be Considered in the Differential Diagnoses?

Individuals who present in adulthood with a neurodevelopmental disorder typically have symptoms that are mild, have developed strategies to minimize the effects of social communication difficulties, and do not have obvious repetitive behaviors. The differential diagnosis generally includes anxiety disorders (eg, social anxiety), obsessive compulsive disorder (restricted or repetitive behaviors), social pragmatic disorder (awkward social communication), and perhaps attention-deficit/hyperactivity disorder (inattention and impulsivity leading to poor social relationships). All of these diagnoses may coexist with ASD, which adds to the diagnostic complexity for adults presenting for the first time with possible ASD.

Are There Special Considerations in General Medical Care for Individuals With ASD?

People with ASD who have limited communication ability and coexisting medical conditions (eg, infections, constipation, pain) may present with behavioral problems, including aggression or self-injurious behaviors (head-banging, self-hitting). Additionally, adverse effects from medications prescribed for physical problems, including anticonvulsants for epilepsy, can exacerbate behavioral problems. Abrupt changes in behavior may indicate underlying disease.

Are There Accommodations That Can Improve Interactions With Health Care Clinicians During Outpatient Medical Appointments With Patients Who Have ASD?

It can be helpful to discuss specific strategies to achieve improved outcomes with family or caregivers in advance of an appointment (eg, managing sensory sensitivities, transitions, or specific fears). Scheduling patients with ASD at the beginning or end of the day can reduce time in the waiting room, which can be particularly stressful for patients with ASD. It may be helpful for some individuals to have a preclinic visit to see the setting and meet staff prior to presenting for examination.

What Are Evidence-Based Treatment Options for Individuals With ASD?

Behavioral interventions early in life are effective for improving social communication and interaction and reducing problem behaviors, which can be used across the lifespan. Emotional and behavioral problems, such as anxiety, aggression, or attention-deficit/hyperactivity disorder associated with ASD can be mitigated by cognitive behavioral therapy and pharmacotherapy.

Behavioral Interventions

Early intervention based on well-established behavioral analytic principles has focused primarily on young children, but can be used in people of any age to help them acquire specific skills and address problem behaviors across the lifespan. These principles focus on attaining behavior change based on understanding and manipulating predisposing environmental conditions or events that may reinforce specific behaviors after they occur. In general, therapies are more effective for improving symptoms associated with ASD, such as using language effectively, than for features of ASD such as impairment in social communication and repetitive, restricted patterns of behaviors. The Naturalistic Developmental Behavioral Interventions involve the use of applied behavior analytic principles of learning with a focus on teaching children developmentally appropriate skills in natural settings (eg, play, routine activities).[77] The Naturalistic Developmental Behavioral Interventions were associated with an improvement in children's language, play, and social communication in a meta-analysis that included 16, 7, and 17 randomized clinical trials, with effect sizes of 0.2 (95% CI, 0.1-0.4), 0.3 (95% CI, 0.1-0.5), and 0.4 (95% CI, 0.2-0.6), respectively.[64] At least 25 hours per week of these interventions is recommended to achieve optimal developmental outcomes,[78] but a 2021 randomized clinical trial of 87 participants did not demonstrate differences in the composite scores of the intervention outcomes, including autism severity (P = .80), expressive communication (P = .36), receptive language (P = .96), and nonverbal abilities such as fine motor skills and daily living skills (P = .54), between therapies with different intensities (15 hours/week for 12 months vs 25 hours/week for 12 months).[65] Parent-mediated intervention, consisting of joint attention therapy (therapy focusing on improving skills to share focus on an object or area with another person, such as finger pointing to look at something and making eye contact with someone when sharing an experience), social communication therapy, and behavioral therapy, delivered by trained parents is currently under evaluation as a potential treatment.[79] In a meta-analysis of 19 randomized clinical trials, parent-delivered interventions were associated with significant, but relatively small, improvement in ASD symptom severity (effect size, 0.2 [95% CI, 0.03-0.4]), socialization (effect size, 0.2 [95% CI, 0.09-0.4]), and cognition (effect size, 0.2 [95% CI, 0.03-0.5]).[80]

School-age children diagnosed with ASD generally have access to behavioral, speech, occupational, and physical therapies in the school setting. The inclusion of children in general education classrooms with visual support strategies (visual cues, such as a picture of a child placing a sweater on a coat hook at the entrance to a classroom, or social scripts, such as a short text that outlines what a child can expect during a visit to the doctor) to prompt and reinforce positive social behaviors can promote adaptive behaviors.[81] Treatment and Education of Autistic and Related Communication-Handicapped Children is one of the well-established educational programs characterized by highly structured work routines and visual presentation of information to facilitate acquisition of learning goals from individualized schedules that are based on each person's learning characteristics, skills, and strengths.[82]

Approximately 20% of people with ASD have anxiety and approximately 11% have depression. Anxiety and depression can interfere with adaptive functioning and well-being.[83] Cognitive behavioral therapy (CBT) is a first-line treatment for these conditions in individuals with ASD.[84] In a 16-week randomized clinical trial for 167 children with ASD aged 6 to 13 years, CBT adapted for ASD (90 minutes weekly with parental involvement) was superior to treatment as usual (ie, non-CBT services) in reducing anxiety symptoms (Cohen d, 1.7 [95% CI, 1.1-2.3]).[67] However, few randomized clinical trials have studied CBT for depression in people with ASD.[68]

In children and adolescents with ASD who have no or mild cognitive impairment, social deficits and poor friendship quality typically worsen during adolescence when social skills may not be developed enough to meet social demands.[85] Providing people with ASD with social skills training delivered in a group format is associated with modest effects on social competence.[86] Effective therapies

are needed to help people with ASD succeed in postsecondary education and the work environment.[87,88]

Pharmacological and Other Biomedical Interventions

Risperidone and aripiprazole, which have dopaminergic and serotonergic antagonistic effects, are currently approved by the US Food and Drug Administration for irritability and aggression in patients with ASD (Table 3). Although their efficacy is supported by meta-analyses of randomized clinical trials, the medications have adverse effects that include hyperglycemia, dyslipidemia, and weight gain. The effect size of risperidone, compared with placebo, for the outcome of irritability and aggression was 1.10 (95% CI, 0.8-1.3) in a meta-analysis of 372 participants with ASD and the effect size of aripiprazole, compared with placebo, was 1.20 (95% CI, 0.8-1.5) in a meta-analysis of 808 participants with ASD. Psychostimulants (methylphenidate) and nonpsychostimulants (atomoxetine and guanfacine) were effective for managing ADHD symptoms in ASD. For example, in a meta-analysis of 4 clinical trials including 97 participants with ASD, compared with placebo, methylphenidate had an effect size of 0.6 (95% CI, 0.2-1.0) for hyperactivity symptoms,[71] and in a meta-analysis of 4 clinical trials including 204 people with ASD, compared with placebo, atomoxetine had an effect size of 0.5 (95% CI, 0.2-0.8) for hyperactivity symptoms.[71] In a randomized clinical trial of 62 people with ASD, compared with placebo, extended-release guanfacine had an effect size of 1.2 (95% CI, 0.7-1.8) for overall ADHD symptoms. However, people with both ADHD and ASD are more likely to experience behavioral activation, consisting of restlessness and disinhibition, with psychostimulants compared with individuals without ASD.[71] Melatonin may be useful for sleep problems in people with ASD.[89] In a randomized clinical trial of 125 children and adolescents in which 97% of participants had ASD, prolonged-release melatonin, compared with placebo, increased sleep duration by 32 minutes (P = .03; effect size, 0.4) and reduced sleep-onset latency by 25 minutes (P = .01; effect size, 0.5).[74] Fluoxetine, an SSRI, did not improve obsessive-compulsive behaviors compared with placebo in a 16-week clinical trial of 146 children and adolescents (effect size, −0.38 [95% CI, −0.76 to −0.004]).[90] Similarly, in a meta-analysis of 7 randomized clinical trials that included 519 people, SSRIs overall were not associated with reduced restricted, repetitive behaviors in ASD (effect size, 0.1 [95% CI, −0.2 to 0.4]).[73]

Because of the lack of effective pharmacotherapies for ASD, families are frequently interested in complementary and alternative approaches. Some supplements, such as N-acetylcysteine[75] and sulforaphane,[91] have been studied in randomized clinical trials and demonstrated efficacy for emotional and behavioral symptoms. However, current evidence does not support any supplement for ASD symptoms of speech delay, poor social interaction, or restricted or repetitive behaviors.[75,91] In a randomized clinical trial of 150 children and adolescents with ASD that compared cannabis extract–containing cannabidiol with tetrahydrocannabinol administered in a 20:1 ratio (whole-plant cannabis extract), purified cannabinoid-containing cannabidiol and tetrahydrocannabinol administered in a 1:1 ratio (pure cannabinoid), and placebo, changes in the primary outcome of the child's noncompliant behavior at home did not differ among the 3 groups. However, the whole-plant cannabis extract significantly improved disruptive behaviors, a second primary outcome, compared with placebo (49% responder rate vs 21% responder rate; P < .01), while pure cannabinoids did not differ from placebo in the same measure (38% responder rate vs 21% responder rate; P = .08).[92]

ASD in the Clinic/Office Setting

Adults with ASD are more likely to report lower satisfaction with patient-clinician communication[93] and have lower health care self-efficacy[94] in the general clinic setting than adults without ASD. People with ASD are more likely to visit the emergency department for medical care than people without ASD.[95] A cross-sectional analysis of the Nationwide Emergency Department Sample database on emergency department visits from the years 2006 to 2011, data that were created for the Healthcare Cost and Utilization Project, reported that adults with ASD were more likely to have a psychiatric visit (15% vs 4.2%; P < .01) as well as an injury visit (16.1% vs 13.6%; P < .01) compared with adults without ASD.

The Academic Autism Spectrum Partnership in Research and Education, a project funded by the National Institutes of Health, developed a health toolkit for adults with ASD and their primary care physicians[96] and provides physicians with information to improve care. Examples include giving patients extra time to answer questions, scheduling longer appointments, using natural or dim light in the examination room, and ensuring that the examination room is quiet and calming.

Transitions of care between pediatric and adult medicine are important, because those with ASD may have difficulty finding adult clinicians familiar with ASD when they become too old for pediatric practices.[97] A national survey of 56 014 people with special health care needs revealed that only 23% of youth (individuals aged 12-17 y) with ASD received health care transition services to improve their health care knowledge and encouraged independent management of their health care needs in transitioning from pediatric to adult services, compared with 50% for youth with other special health care needs.[98]

Prognosis

With respect to social, psychological, and health outcomes, adults with ASD less frequently live independently, are more frequently unemployed, and have higher needs and higher use of mental health services than people without ASD.[99] Better cognitive abilities during childhood are associated with higher levels of independence, education, and employment later in life, but are not associated with higher rates of friendship or well-being reported by caregivers.[100]

Premature mortality rates are approximately 2-fold higher for individuals with ASD than for the general population.[101] Mortality risk in people with ASD is increased by the coexistence of neurologic disorders, such as seizure (mortality rates of 1.1% vs 0.2%), and co-occurring mental/behavioral disorders, such as mood disorders (mortality rates of 0.4% vs 0.2%).[102] Suicide attempts and death by suicide are more common in individuals with ASD than in the general population. In a population-based study of 6 559 266 people in Denmark,[103] incident rates for suicide attempts were 266 per 100 000 person-years in people with ASD and 63 per 100 000 person-years in those without ASD (incident rate ratio after adjusting for sex, age, and period, 3.2 [95% CI, 2.9-3.5]). Similarly, incident rates for death by suicide were 24 per 100 000 person-years in people with ASD and 14 per 100 000 person-years in those

without ASD (incident rate ratio after adjusting for sex, age, and period, 3.8 [95% CI, 2.9-4.9]).

Limitations

This review has several limitations. First, the search was restricted to English-language publications. Second, the quality of included literature was not formally evaluated. Third, some relevant papers may have been missed.

Conclusions

Autism spectrum disorder affects approximately 2.3% of children aged 8 years and 2.2% of adults in the US. First-line therapy consists of behavioral interventions delivered by a multidisciplinary team, while co-occurring mental health conditions, such as anxiety or aggression, may be treated with specific behavioral therapy or medications.

ARTICLE INFORMATION

Accepted for Publication: December 6, 2022.

Conflict of Interest Disclosures: None reported.

Submissions: We encourage authors to submit papers for consideration as a Review. Please contact Mary McGrae McDermott, MD, at mdm608@northwestern.edu.

REFERENCES

1. *Diagnostic and Statistical Manual of Mental Disorders*. 5th ed. American Psychiatric Association; 2013.

2. Autism spectrum disorder (ASD). Centers for Disease Control and Prevention. Accessed November 28, 2022. https://www.cdc.gov/ncbddd/autism/index.html

3. Lai MC, Kassee C, Besney R, et al. Prevalence of co-occurring mental health diagnoses in the autism population: a systematic review and meta-analysis. *Lancet Psychiatry*. 2019;6(10):819-829. doi:10.1016/S2215-0366(19)30289-5

4. Rydzewska E, Dunn K, Cooper SA. Umbrella systematic review of systematic reviews and meta-analyses on comorbid physical conditions in people with autism spectrum disorder. *Br J Psychiatry*. 2021;218(1):10-19. doi:10.1192/bjp.2020.167

5. Maenner MJ, Shaw KA, Bakian AV, et al. Prevalence and characteristics of autism spectrum disorder among children aged 8 years: Autism and Developmental Disabilities Monitoring Network, 11 sites, United States, 2018. *MMWR Surveill Summ*. 2021;70(11):1-16. doi:10.15585/mmwr.ss7011a1

6. Kim YS, Leventhal BL, Koh YJ, et al. Prevalence of autism spectrum disorders in a total population sample. *Am J Psychiatry*. 2011;168(9):904-912.

7. Saito M, Hirota T, Sakamoto Y, et al. Prevalence and cumulative incidence of autism spectrum disorders and the patterns of co-occurring neurodevelopmental disorders in a total population sample of 5-year-old children. *Mol Autism*. 2020;11(1):35. doi:10.1186/s13229-020-00342-5

8. Hansen SN, Schendel DE, Parner ET. Explaining the increase in the prevalence of autism spectrum disorders: the proportion attributable to changes in reporting practices. *JAMA Pediatr*. 2015;169(1):56-62. doi:10.1001/jamapediatrics.2014.1893

9. Zeidan J, Fombonne E, Scorah J, et al. Global prevalence of autism: a systematic review update. *Autism Res*. 2022;15(5):778-790. doi:10.1002/aur.2696

10. Fombonne E. Camouflage and autism. *J Child Psychol Psychiatry*. 2020;61(7):735-738. doi:10.1111/jcpp.13296

11. Lai MC, Lombardo MV, Ruigrok AN, et al; MRC AIMS Consortium. Quantifying and exploring camouflaging in men and women with autism. *Autism*. 2017;21(6):690-702. doi:10.1177/1362361316671012

12. Warrier V, Greenberg DM, Weir E, et al. Elevated rates of autism, other neurodevelopmental and psychiatric diagnoses, and autistic traits in transgender and gender-diverse individuals. *Nat Commun*. 2020;11(1):3959. doi:10.1038/s41467-020-17794-1

13. Angell AM, Empey A, Zuckerman KE. A review of diagnosis and service disparities among children with autism from racial and ethnic minority groups in the United States. In: Hodapp RM, Fidler DJ, eds. *International Review of Research in Developmental Disabilities*. Vol 55. Academic Press; 2018:145-180. doi:10.1016/bs.irrdd.2018.08.003

14. Ozonoff S, Young GS, Carter A, et al. Recurrence risk for autism spectrum disorders: a Baby Siblings Research Consortium study. *Pediatrics*. 2011;128(3):e488-e495. doi:10.1542/peds.2010-2825

15. Palmer N, Beam A, Agniel D, et al. Association of sex with recurrence of autism spectrum disorder among siblings. *JAMA Pediatr*. 2017;171(11):1107-1112. doi:10.1001/jamapediatrics.2017.2832

16. Wu Y, Cao H, Baranova A, et al. Multi-trait analysis for genome-wide association study of five psychiatric disorders. *Transl Psychiatry*. 2020;10(1):1-11. doi:10.1038/s41398-020-00902-6

17. Sanders SJ, He X, Willsey AJ, et al; Autism Sequencing Consortium. Insights into autism spectrum disorder genomic architecture and biology from 71 risk loci. *Neuron*. 2015;87(6):1215-1233. doi:10.1016/j.neuron.2015.09.016

18. Klei L, Sanders SJ, Murtha MT, et al. Common genetic variants, acting additively, are a major source of risk for autism. *Mol Autism*. 2012;3(1):9. doi:10.1186/2040-2392-3-9

19. Willsey HR, Willsey AJ, Wang B, State MW. Genomics, convergent neuroscience and progress in understanding autism spectrum disorder. *Nat Rev Neurosci*. 2022;23(6):323-341. doi:10.1038/s41583-022-00576-7

20. Zarrei M, Burton CL, Engchuan W, et al. A large data resource of genomic copy number variation across neurodevelopmental disorders. *NPJ Genom Med*. 2019;4(1):26. doi:10.1038/s41525-019-0098-3

21. Cross-Disorder Group of the Psychiatric Genomics Consortium. Genomic relationships, novel loci, and pleiotropic mechanisms across eight psychiatric disorders. *Cell*. 2019;179(7):1469-1482.e11. doi:10.1016/j.cell.2019.11.020

22. Willsey HR, Exner CRT, Xu Y, et al. Parallel in vivo analysis of large-effect autism genes implicates cortical neurogenesis and estrogen in risk and resilience. *Neuron*. 2021;109(5):788-804.e8. doi:10.1016/j.neuron.2021.01.002

23. Kim JY, Son MJ, Son CY, et al. Environmental risk factors and biomarkers for autism spectrum disorder: an umbrella review of the evidence. *Lancet Psychiatry*. 2019;6(7):590-600. doi:10.1016/S2215-0366(19)30181-6

24. Wu S, Wu F, Ding Y, Hou J, Bi J, Zhang Z. Advanced parental age and autism risk in children: a systematic review and meta-analysis. *Acta Psychiatr Scand*. 2017;135(1):29-41. doi:10.1111/acps.12666

25. Zerbo O, Yoshida C, Gunderson EP, Dorward K, Croen LA. Interpregnancy interval and risk of autism spectrum disorders. *Pediatrics*. 2015;136(4):651-657. doi:10.1542/peds.2015-1099

26. Christensen J, Grønborg TK, Sørensen MJ, et al. Prenatal valproate exposure and risk of autism spectrum disorders and childhood autism. *JAMA*. 2013;309(16):1696-1703. doi:10.1001/jama.2013.2270

27. Kaplan YC, Keskin-Arslan E, Acar S, Sozmen K. Maternal SSRI discontinuation, use, psychiatric disorder and the risk of autism in children: a meta-analysis of cohort studies. *Br J Clin Pharmacol*. 2017;83(12):2798-2806. doi:10.1111/bcp.13382

28. Jain A, Marshall J, Buikema A, Bancroft T, Kelly JP, Newschaffer CJ. Autism occurrence by MMR vaccine status among US children with older siblings with and without autism. *JAMA*. 2015;313(15):1534-1540. doi:10.1001/jama.2015.3077

29. Hviid A, Hansen JV, Frisch M, Melbye M. Measles, mumps, rubella vaccination and autism: a nationwide cohort study. *Ann Intern Med*. 2019;170(8):513-520. doi:10.7326/M18-2101

30. Committee to Review Adverse Effects of Vaccines; Institute of Medicine. *Adverse Effects of Vaccines: Evidence and Causality*. National Academies Press; 2011.

31. Chawarska K, Shic F, Macari S, et al. 18-Month predictors of later outcomes in younger siblings of children with autism spectrum disorder: a baby siblings research consortium study. *J Am Acad Child Adolesc Psychiatry*. 2014;53(12):1317-1327.e1. doi:10.1016/j.jaac.2014.09.015

32. Autism spectrum disorder: signs and symptoms. Centers for Disease Control and Prevention. Updated March 28, 2022. Accessed November 28, 2022. https://www.cdc.gov/ncbddd/autism/signs.html

33. 16 Early signs of autism by 16 months. Baby Navigator. Accessed November 28, 2022. https://babynavigator.com/lookbooks/english/earlysigns/#16-early-signs-autism/1https://babynavigator.com/16-early-signs-of-autism-by-16-months-chinese/

34. Ludwig NN, Jashar DT, Sheperd K, et al. Considerations for the identification of autism spectrum disorder in children with vision or hearing impairment: a critical review of the literature and

recommendations for practice. *Clin Neuropsychol*. 2021;0(0):1-20. doi:10.1080/13854046.2021.2002933

35. Do B, Lynch P, Macris EM, et al. Systematic review and meta-analysis of the association of autism spectrum disorder in visually or hearing impaired children. *Ophthalmic Physiol Opt*. 2017;37(2):212-224. doi:10.1111/opo.12350

36. Tonnsen BL, Boan AD, Bradley CC, Charles J, Cohen A, Carpenter LA. Prevalence of autism spectrum disorders among children with intellectual disability. *Am J Intellect Dev Disabil*. 2016;121(6):487-500. doi:10.1352/1944-7558-121.6.487

37. Richards C, Jones C, Groves L, Moss J, Oliver C. Prevalence of autism spectrum disorder phenomenology in genetic disorders: a systematic review and meta-analysis. *Lancet Psychiatry*. 2015;2(10):909-916. doi:10.1016/S2215-0366(15)00376-4

38. Pilling S, Baron-Cohen S, Megnin-Viggars O, Lee R, Taylor C; Guideline Development Group. Recognition, referral, diagnosis, and management of adults with autism: summary of NICE guidance. *BMJ*. 2012;344:e4082. doi:10.1136/bmj.e4082

39. Mutluer T, Aslan Genç H, Özcan Morey A, et al. Population-based psychiatric comorbidity in children and adolescents with autism spectrum disorder: a meta-analysis. *Front Psychiatry*. Published online May 23, 2022. doi:10.3389/fpsyt.2022.856208.

40. McKenzie K, Milton M, Smith G, Ouellette-Kuntz H. Systematic review of the prevalence and incidence of intellectual disabilities: current trends and issues. *Curr Dev Disord Rep*. 2016;3(2):104-115. doi:10.1007/s40474-016-0085-7

41. O'Halloran L, Coey P, Wilson C. Suicidality in autistic youth: a systematic review and meta-analysis. *Clin Psychol Rev*. 2022;93:102144. doi:10.1016/j.cpr.2022.102144

42. Volkmar F, Siegel M, Woodbury-Smith M, King B, McCracken J, State M; American Academy of Child and Adolescent Psychiatry (AACAP) Committee on Quality Issues (CQI). Practice parameter for the assessment and treatment of children and adolescents with autism spectrum disorder. *J Am Acad Child Adolesc Psychiatry*. 2014;53(2):237-257. doi:10.1016/j.jaac.2013.10.013

43. Amiet C, Gourfinkel-An I, Bouzamondo A, et al. Epilepsy in autism is associated with intellectual disability and gender: evidence from a meta-analysis. *Biol Psychiatry*. 2008;64(7):577-582. doi:10.1016/j.biopsych.2008.04.030

44. Fiest KM, Sauro KM, Wiebe S, et al. Prevalence and incidence of epilepsy: a systematic review and meta-analysis of international studies. *Neurology*. 2017;88(3):296-303. doi:10.1212/WNL.0000000000003509

45. Mayes SD, Zickgraf H. Atypical eating behaviors in children and adolescents with autism, ADHD, other disorders, and typical development. *Res Autism Spectr Disord*. 2019;64:76-83. doi:10.1016/j.rasd.2019.04.002

46. Carlsson LH, Norrelgen F, Kjellmer L, Westerlund J, Gillberg C, Fernell E. Coexisting disorders and problems in preschool children with autism spectrum disorders. *ScientificWorldJournal*. 2013;2013:213979. doi:10.1155/2013/213979

47. Zwicker JG, Missiuna C, Harris SR, Boyd LA. Developmental coordination disorder: a review and update. *Eur J Paediatr Neurol*. 2012;16(6):573-581. doi:10.1016/j.ejpn.2012.05.005

48. Holingue C, Newill C, Lee LC, Pasricha PJ, Daniele Fallin M. Gastrointestinal symptoms in autism spectrum disorder: a review of the literature on ascertainment and prevalence. *Autism Res*. 2018;11(1):24-36. doi:10.1002/aur.1854

49. Happé F. Why are savant skills and special talents associated with autism? *World Psychiatry*. 2018;17(3):280-281. doi:10.1002/wps.20552

50. Howlin P, Goode S, Hutton J, Rutter M. Savant skills in autism: psychometric approaches and parental reports. *Philos Trans R Soc Lond B Biol Sci*. 2009;364(1522):1359-1367. doi:10.1098/rstb.2008.0328

51. Hughes JEA, Ward J, Gruffydd E, et al. Savant syndrome has a distinct psychological profile in autism. *Mol Autism*. 2018;9(1):53. doi:10.1186/s13229-018-0237-1

52. Hyman SL, Levy SE, Myers SM; COUNCIL ON CHILDREN WITH DISABILITIES, SECTION ON DEVELOPMENTAL AND BEHAVIORAL PEDIATRICS. Identification, evaluation, and management of children with autism spectrum disorder. *Pediatrics*. 2020;145(1):e20193447. doi:10.1542/peds.2019-3447

53. Siu AL, Bibbins-Domingo K, Grossman DC, et al; US Preventive Services Task Force (USPSTF). Screening for autism spectrum disorder in young children: US Preventive Services Task Force Recommendation Statement. *JAMA*. 2016;315(7):691-696. doi:10.1001/jama.2016.0018

54. Autism spectrum disorder in young children: screening. US Preventive Services Task Force. Updated June 4, 2021. Accessed November 28, 2022. https://www.uspreventiveservicestaskforce.org/uspstf/draft-update-summary/autism-spectrum-disorder-young-children-1

55. Robins DL, Casagrande K, Barton M, Chen CMA, Dumont-Mathieu T, Fein D. Validation of the modified checklist for autism in toddlers, revised with follow-up (M-CHAT-R/F). *Pediatrics*. 2014;133(1):37-45. doi:10.1542/peds.2013-1813

56. Lebersfeld JB, Swanson M, Clesi CD, O'Kelley SE. Systematic review and meta-analysis of the clinical utility of the ADOS-2 and the ADI-R in diagnosing autism spectrum disorders in children. *J Autism Dev Disord*. 2021;51(11):4101-4114. doi:10.1007/s10803-020-04839-z

57. Baranek GT, Parham LD, Bodfish JW. Sensory and motor features in autism: assessment and intervention. In. *Handbook of Autism and Pervasive Developmental Disorders*. 3rd ed. John Wiley & Sons; 2005:831-857. doi:10.1002/9780470939352.ch6.

58. Havdahl KA, Hus Bal V, Huerta M, et al. Multidimensional influences on autism symptom measures: implications for use in etiological research. *J Am Acad Child Adolesc Psychiatry*. 2016;55(12):1054-1063.e3. doi:10.1016/j.jaac.2016.09.490

59. Schaefer GB, Mendelsohn NJ; Professional Practice and Guidelines Committee. Clinical genetics evaluation in identifying the etiology of autism spectrum disorders: 2013 guideline revisions. *Genet Med*. 2013;15(5):399-407. doi:10.1038/gim.2013.32

60. Lai MC, Anagnostou E, Wiznitzer M, Allison C, Baron-Cohen S. Evidence-based support for autistic people across the lifespan: maximising potential, minimising barriers, and optimising the person-environment fit. *Lancet Neurol*. 2020;19(5):434-451. doi:10.1016/S1474-4422(20)30034-X

61. National Standards Project. National Autism Center. Accessed November 28, 2022. https://nationalautismcenter.org/

62. Herscu P, Handen BL, Arnold LE, et al; Autism Speaks Autism Clinical Trials Network. The SOFIA study: negative multi-center study of low dose fluoxetine on repetitive behaviors in children and adolescents with autistic disorder. *J Autism Dev Disord*. 2020;50(9):3233-3244. doi:10.1007/s10803-019-04120-y

63. Siafis S, Çıray O, Wu H, et al. Pharmacological and dietary-supplement treatments for autism spectrum disorder: a systematic review and network meta-analysis. *Mol Autism*. 2022;13(1):10. doi:10.1186/s13229-022-00488-4

64. Sandbank M, Bottema-Beutel K, Crowley S, et al. Project AIM: autism intervention meta-analysis for studies of young children. *Psychol Bull*. 2020;146(1):1-29. doi:10.1037/bul0000215

65. Rogers SJ, Yoder P, Estes A, et al. A multisite randomized controlled trial comparing the effects of intervention intensity and intervention style on outcomes for young children with autism. *J Am Acad Child Adolesc Psychiatry*. 2021;60(6):710-722. doi:10.1016/j.jaac.2020.06.013

66. Wang X, Zhao J, Huang S, et al. Cognitive behavioral therapy for autism spectrum disorders: a systematic review. *Pediatrics*. 2021;147(5):e2020049880. doi:10.1542/peds.2020-049880

67. Wood JJ, Kendall PC, Wood KS, et al. Cognitive behavioral treatments for anxiety in children with autism spectrum disorder: a randomized clinical trial. *JAMA Psychiatry*. 2020;77(5):474-483. doi:10.1001/jamapsychiatry.2019.4160

68. Russell A, Gaunt DM, Cooper K, et al. The feasibility of low-intensity psychological therapy for depression co-occurring with autism in adults: the Autism Depression Trial (ADEPT): a pilot randomised controlled trial. *Autism*. 2020;24(6):1360-1372. doi:10.1177/1362361319889272

69. Zheng S, Kim H, Salzman E, Ankenman K, Bent S. Improving social knowledge and skills among adolescents with autism: systematic review and meta-analysis of UCLA PEERS for adolescents. *J Autism Dev Disord*. 2021;51(12):4488-4503. doi:10.1007/s10803-021-04885-1

70. Salazar de Pablo G, Pastor Jordá C, Vaquerizo-Serrano J, et al. Systematic review and meta-analysis: efficacy of pharmacological interventions for irritability and emotional dysregulation in autism spectrum disorder and predictors of response. *J Am Acad Child Adolesc Psychiatry*. Published online April 22, 2022. doi:10.1016/j.jaac.2022.03.033

71. Rodrigues R, Lai MC, Beswick A, et al. Practitioner review: pharmacological treatment of attention-deficit/hyperactivity disorder symptoms in children and youth with autism spectrum disorder: a systematic review and meta-analysis. *J Child Psychol Psychiatry*. 2021;62(6):680-700. doi:10.1111/jcpp.13305

72. Scahill L, McCracken JT, King BH, et al; Research Units on Pediatric Psychopharmacology Autism Network. Extended-release guanfacine for hyperactivity in children with autism spectrum

disorder. *Am J Psychiatry*. 2015;172(12):1197-1206. doi:10.1176/appi.ajp.2015.15010055

73. Zhou MS, Nasir M, Farhat LC, Kook M, Artukoglu BB, Bloch MH. Meta-analysis: pharmacologic treatment of restricted and repetitive behaviors in autism spectrum disorders. *J Am Acad Child Adolesc Psychiatry*. 2021;60(1):35-45. doi:10.1016/j.jaac.2020.03.007

74. Gringras P, Nir T, Breddy J, Frydman-Marom A, Findling RL. Efficacy and safety of pediatric prolonged-release melatonin for insomnia in children with autism spectrum disorder. *J Am Acad Child Adolesc Psychiatry*. 2017;56(11):948-957.e4. doi:10.1016/j.jaac.2017.09.414

75. Lee TM, Lee KM, Lee CY, Lee HC, Tam KW, Loh EW. Effectiveness of *N*-acetylcysteine in autism spectrum disorders: a meta-analysis of randomized controlled trials. *Aust N Z J Psychiatry*. 2021;55(2):196-206. doi:10.1177/0004867420952540

76. Sikich L, Kolevzon A, King BH, et al. Intranasal oxytocin in children and adolescents with autism spectrum disorder. *N Engl J Med*. 2021;385(16):1462-1473. doi:10.1056/NEJMoa2103583

77. Schreibman L, Dawson G, Stahmer AC, et al. Naturalistic developmental behavioral interventions: empirically validated treatments for autism spectrum disorder. *J Autism Dev Disord*. 2015;45(8):2411-2428. doi:10.1007/s10803-015-2407-8

78. National Research Council. *Educating Children with Autism*. National Academies Press; 2001.

79. Green J, Leadbitter K, Ellis C, et al. Combined social communication therapy at home and in education for young autistic children in England (PACT-G): a parallel, single-blind, randomised controlled trial. *Lancet Psychiatry*. 2022;9(4):307-320. doi:10.1016/S2215-0366(22)00029-3

80. Nevill RE, Lecavalier L, Stratis EA. Meta-analysis of parent-mediated interventions for young children with autism spectrum disorder. *Autism*. 2018;22(2):84-98. doi:10.1177/1362361316677838

81. Watkins L, Ledbetter-Cho K, O'Reilly M, Barnard-Brak L, Garcia-Grau P. Interventions for students with autism in inclusive settings: a best-evidence synthesis and meta-analysis. *Psychol Bull*. 2019;145(5):490-507. doi:10.1037/bul0000190

82. Virues-Ortega J, Julio FM, Pastor-Barriuso R. The TEACCH program for children and adults with autism: a meta-analysis of intervention studies. *Clin Psychol Rev*. 2013;33(8):940-953. doi:10.1016/j.cpr.2013.07.005

83. Ambrose K, Simpson K, Adams D. The relationship between social and academic outcomes and anxiety for children and adolescents on the autism spectrum: A systematic review. *Clin Psychol Rev*. 2021;90:102086. doi:10.1016/j.cpr.2021.102086

84. White SW, Simmons GL, Gotham KO, et al. Psychosocial treatments targeting anxiety and depression in adolescents and adults on the autism spectrum: review of the latest research and recommended future directions. *Curr Psychiatry Rep*. 2018;20(10):82. doi:10.1007/s11920-018-0949-0

85. Picci G, Scherf KS. A two-hit model of autism: adolescence as the second hit. *Clin Psychol Sci*. 2015;3(3):349-371. doi:10.1177/2167702614540646

86. Wolstencroft J, Robinson L, Srinivasan R, Kerry E, Mandy W, Skuse D. A systematic review of group social skills interventions, and meta-analysis of outcomes, for children with high functioning ASD. *J Autism Dev Disord*. 2018;48(7):2293-2307. doi:10.1007/s10803-018-3485-1

87. White SW, Smith IC, Miyazaki Y, Conner CM, Elias R, Capriola-Hall NN. Improving transition to adulthood for students with autism: a randomized controlled trial of STEPS. *J Clin Child Adolesc Psychol*. 2021;50(2):187-201. doi:10.1080/15374416.2019.1669157

88. Petty S, Tunstall L, Richardson H, Eccles N. Workplace adjustments for autistic employees: what is 'reasonable'? *J Autism Dev Disord*. Published online January 12, 2022. doi:10.1007/s10803-021-05413-x

89. Rossignol DA, Frye RE. Melatonin in autism spectrum disorders: a systematic review and meta-analysis. *Dev Med Child Neurol*. 2011;53(9):783-792. doi:10.1111/j.1469-8749.2011.03980.x

90. Reddihough DS, Marraffa C, Mouti A, et al. Effect of fluoxetine on obsessive-compulsive behaviors in children and adolescents with autism spectrum disorders: a randomized clinical trial. *JAMA*. 2019;322(16):1561-1569. doi:10.1001/jama.2019.14685

91. Zimmerman AW, Singh K, Connors SL, et al. Randomized controlled trial of sulforaphane and metabolite discovery in children with autism spectrum disorder. *Mol Autism*. 2021;12(1):38. doi:10.1186/s13229-021-00447-5

92. Aran A, Harel M, Cassuto H, et al. Cannabinoid treatment for autism: a proof-of-concept randomized trial. *Mol Autism*. 2021;12(1):6. doi:10.1186/s13229-021-00420-2

93. Raymaker DM, McDonald KE, Ashkenazy E, et al. Barriers to healthcare: instrument development and comparison between autistic adults and adults with and without other disabilities. *Autism*. 2017;21(8):972-984. doi:10.1177/1362361316661261

94. Brice S, Rodgers J, Ingham B, et al. The importance and availability of adjustments to improve access for autistic adults who need mental and physical healthcare: findings from UK surveys. *BMJ Open*. 2021;11(3):e043336. doi:10.1136/bmjopen-2020-043336

95. Vohra R, Madhavan S, Sambamoorthi U. Emergency department use among adults with autism spectrum disorders (ASD). *J Autism Dev Disord*. 2016;46(4):1441-1454. doi:10.1007/s10803-015-2692-2

96. AASPIRE healthcare took kit: primary care resources for adults on the autism spectrum and their primary care providers. AASPIRE. Accessed November 28, 2022. https://autismandhealth.org/

97. Shattuck PT, Lau L, Anderson KA, Kuo AA. A national research agenda for the transition of youth with autism. *Pediatrics*. 2018;141(suppl 4):S355-S361. doi:10.1542/peds.2016-4300M

98. Cheak-Zamora NC, Yang X, Farmer JE, Clark M. Disparities in transition planning for youth with autism spectrum disorder. *Pediatrics*. 2013;131(3):447-454. doi:10.1542/peds.2012-1572

99. Mason D, Capp SJ, Stewart GR, et al. A meta-analysis of outcome studies of autistic adults: quantifying effect size, quality, and meta-regression. *J Autism Dev Disord*. 2021;51(9):3165-3179. doi:10.1007/s10803-020-04763-2

100. Pickles A, McCauley JB, Pepa LA, Huerta M, Lord C. The adult outcome of children referred for autism: typology and prediction from childhood. *J Child Psychol Psychiatry*. 2020;61(7):760-767. doi:10.1111/jcpp.13180

101. Catalá-López F, Hutton B, Page MJ, et al. Mortality in persons with autism spectrum disorder or attention-deficit/hyperactivity disorder: a systematic review and meta-analysis. *JAMA Pediatr*. 2022;176(4):e216401. doi:10.1001/jamapediatrics.2021.6401

102. Schendel DE, Overgaard M, Christensen J, et al. Association of psychiatric and neurologic comorbidity with mortality among persons with autism spectrum disorder in a Danish population. *JAMA Pediatr*. 2016;170(3):243-250. doi:10.1001/jamapediatrics.2015.3935

103. Kölves K, Fitzgerald C, Nordentoft M, Wood SJ, Erlangsen A. Assessment of suicidal behaviors among individuals with autism spectrum disorder in Denmark. *JAMA Netw Open*. 2021;4(1):e2033565. doi:10.1001/jamanetworkopen.2020.33565

Received: 2 August 2023 | Revised: 27 January 2024 | Accepted: 30 January 2024

DOI: 10.1002/mco2.497

MedComm Open Access WILEY

REVIEW

Autism spectrum disorder: pathogenesis, biomarker, and intervention therapy

Hongbin Zhuang[1,#] | **Zhiyuan Liang**[1,#] | **Guanwei Ma**[1] | **Ayesha Qureshi**[1] | **Xiaoqian Ran**[1] | **Chengyun Feng**[2] | **Xukun Liu**[1] | **Xi Yan**[1] | **Liming Shen**[1,3,*]

[1]College of Life Science and Oceanography, Shenzhen University, Shenzhen, P. R. China

[2]Maternal and Child Health Hospital of Baoan, Shenzhen, P. R. China

[3]Shenzhen-Hong Kong Institute of Brain Science-Shenzhen Fundamental Research Institutions, Shenzhen, P. R. China

[*]**Correspondence**
Liming Shen, College of Life Sciences and Oceanography, Shenzhen University, Shenzhen 518060, P. R. China.
Email: slm@szu.edu.cn

[Correction added on 2 April 2024, after first online publication: correction in changing the heading numbers]

Funding information
National Natural Science Foundation of China, Grant/Award Number: 31870825; Shenzhen-Hong Kong Institute of Brain Science-Shenzhen Fundamental Research Institutions, Grant/Award Number: 2023SHIBS0003; Shenzhen Bureau of Science, Technology, and Information, Grant/Award Number: JCYJ20170412110026229

Abstract

Autism spectrum disorder (ASD) has become a common neurodevelopmental disorder. The heterogeneity of ASD poses great challenges for its research and clinical translation. On the basis of reviewing the heterogeneity of ASD, this review systematically summarized the current status and progress of pathogenesis, diagnostic markers, and interventions for ASD. We provided an overview of the ASD molecular mechanisms identified by multi-omics studies and convergent mechanism in different genetic backgrounds. The comorbidities, mechanisms associated with important physiological and metabolic abnormalities (i.e., inflammation, immunity, oxidative stress, and mitochondrial dysfunction), and gut microbial disorder in ASD were reviewed. The non-targeted omics and targeting studies of diagnostic markers for ASD were also reviewed. Moreover, we summarized the progress and methods of behavioral and educational interventions, intervention methods related to technological devices, and research on medical interventions and potential drug targets. This review highlighted the application of high-throughput omics methods in ASD research and emphasized the importance of seeking homogeneity from heterogeneity and exploring the convergence of disease mechanisms, biomarkers, and intervention approaches, and proposes that taking into account individuality and commonality may be the key to achieve accurate diagnosis and treatment of ASD.

KEYWORDS
autism spectrum disorder, biomarker, intervention therapy, molecular mechanisms, multi-omics

[#]Hongbin Zhuang and Zhiyuan Liang have contributed equally to this study.

1 | INTRODUCTION

Autism spectrum disorder (ASD) is a group of developmental neurological disorders characterized by early onset of abnormal social communication and restricted repetitive behaviors and interests. Since ASD was first discovered and defined, researchers have not stopped studying and exploring it (Figure S1).[1–4] Currently, the percentage of children with ASD has steadily increased since the 1970s, when it was less than 0.4%. It is currently estimated to be between 1% and 2%.[5–7] The rate of ASD in 8-year-old children in the United States has increased from one in 44 in 2018 to one in 36 in 2020.[8,9] In China, the incidence of ASD in children aged 6–12 years is ~0.7%.[10,11] As a result, ASD have attracted widespread societal attention.

The etiology of ASD is extremely complex. Twin studies suggest that genes play a key role in the pathogenesis of ASD, and its heritability estimates range from 64% to 91%.[12] In families with children with ASD, the average rate of ASD recurrence is estimated to be 15%–25% for male newborns and 5%–15% for female newborns.[13,14] Besides, environmental factors are also implicated in the development of ASD, including prenatal/perinatal, microbial–gut–brain axis, and others. Prenatal/perinatal causes included maternal age >35 years, maternal characteristics of metabolic syndrome, use of antidepressant valproic acid (VPA) medications, and the effects of infection and inflammation.[15,16] Environmental factors can directly influence specific susceptibility genes, prompting epigenetic modifications such as DNA methylation and histone changes (phosphorylation and acetylation), which increase the risk of developing ASD.[17] ASD arises from a complex interplay of genetic and environmental factors, leading to changes in brain structure and function that manifest as behavioral abnormalities (Figure 1).

Moreover, the heterogeneity of ASD impedes both pinpointing underlying mechanisms and tailoring effective therapies. Interestingly, the previous studies have shown that the function of ASD-associated genes converges with the affected cell type[18–23] and that the affected brain has a characteristic molecular pathology.[22] ASD-specific molecular changes are mainly concentrated in central nervous system (CNS).[18–23] Besides, individuals with ASD have different comorbidities, but all share the same social communication deficits and repetitive stereotyped behavioral phenotypes, implying a common underlying biological mechanism among them.[24] The heterogeneity of ASD does not preclude the possibility of finding common features or mechanisms that could lead to breakthroughs in the pathogenesis, diagnosis, and treatment of ASD. Efforts have been made to identify biomarkers, pathological mechanisms, and drug targets, and to explore the possibility of defining ASD subgroups by biological features.

In this review, we summarized the heterogeneity of ASD and explore its underlying disease mechanisms based on genes and multi-omics studies. We focused on searching convergent disease pathways under genetic backgrounds and comorbidities. In addition, the mechanisms associated with common physiological and metabolic abnormalities and the gut microbiota were reviewed. An overview of research advances in ASD biomarkers was provided, and its role in early diagnosis was emphasized. Advances in behavioral interventions and pharmacological studies of ASD were also reviewed.

2 | HETEROGENEITY OF ASD

Heterogeneity in etiology, phenotype, and outcome are hallmarks of ASD.[25] These factors contribute to a clinical heterogeneity, which manifest as diverse deficits or impairments in behavioral features and communicative functioning. The remarkable heterogeneity of ASD complicates and diversifies the clinical diagnosis and the individualization of treatment for ASD, which involves a combination of multiple genes, environmental factors, and mental health disorders. Heterogeneity of genes, comorbidity in ASD, and gender bias contribute to the heterogeneity of ASD.[25]

2.1 | The challenge from heterogeneity of genes

With the application of genome-wide linkage and association analysis, copy number variant analysis, candidate gene resequencing and association analysis, and exome sequencing, many genes associated with ASD have been identified. Over 1200 genes have been recorded in the SFARI autism gene database (https://www.sfari.org/). More than 100 risk genes have been identified, including de novo mutations, genomic copy number variants, and single base mutations. Notably, children with ASD are genetic heterogeneous, with genetic variants detected in about 10%–20% of cases, but no single gene or mutation can cause more than 1% of cases,[26] and genetic testing is still not available to accurately predict or diagnose ASD.

2.2 | Comorbidity in ASD

In addition to core symptoms, children with ASD often have learning difficulties, intellectual disabilities (IDs), and other behavioral problems that may manifest as aggression, self-injurious behavior, impulsivity, irritability, hyperactivity, anxiety, and mood symptoms.[27] The severity of clinical symptoms and behavioral difficulties varies

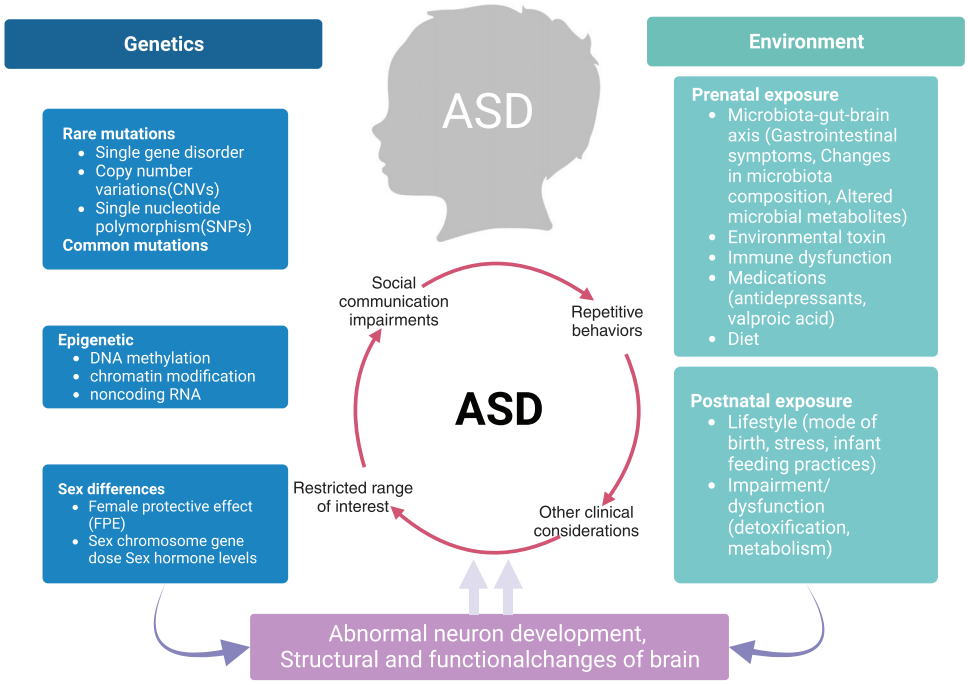


FIGURE 1 Potential influences of autism spectrum disorder (ASD). ASD is a heterogenous group of neurodevelopmental disorders characterized by social communication impairments, repetitive behaviors, restricted range of interest, and other clinical considerations. ASD is a multifactorial disease that involves the interactions of genetic and environmental factors. The genetic factors include genetics (single gene disorder, copy number variations and single-nucleotide polymorphism), epigenetic (DNA methylation, chromatin modification and noncoding RNA), and sex differences factors (female protective effect and sex chromosome gene dose sex hormone levels). In contrast, the environmental factors comprise prenatal exposure (microbiota–gut–brain axis, environmental toxin, immune dysfunction, medications, and diet) and postnatal exposure (lifestyle and impairment/dysfunction). These factors lead to abnormal neuron development, changes in the structure and function of the brain, resulting in ASD.

from person to person with autism and can have a severe or mild impact on daily life. Individuals with ASD are also more likely to have comorbid developmental and psychiatric problems such as attention deficit hyperactivity disorder (ADHD), anxiety and depression, ID, and specific disorders such as epilepsy, motor coordination, feeding difficulties, sleep disturbances, and gastrointestinal problems.[28] About 29% of individuals with ASD are likely to have savant skills.[29] The situation is complicated by changes in behavior and symptoms throughout development and maturity, as well as comorbidities that occur simultaneously.

2.3 | Gender bias in ASD

Male preponderance is a highly replicated finding in ASD despite striking heterogeneity in symptoms and severity. The ratio of male to female prevalence was 4:1.[30] In different studies, it has been reported that ASD is more prevalent in males possibly due to sex-specific single-nucleotide polymorphisms, single-nucleotide variants, micro-deletions, copy number variants, and proteins.[31–36] The findings of these studies have, however, not been consistently replicated in studies of the highly heterogeneous ASD.[37] ASD preponderance and severity differences between males and females are explained by the female protective effect (FPE) theory.[26] As part of the FPE, the greater variability model is included. Which asserts that males are more genetically variable, resulting in a higher incidence and decreased severity of ASD.[38,39] Additionally, the FPE incorporates a liability threshold model, which is based on the hypothesis that females who fulfill diagnostic thresholds for autism are more likely to carry mutations than males, and relatives of females with ASD tend to be more affected than relatives of males with autism.[40] Other studies examining groups of people with ASDs and siblings of those with the disorder neither find an increase in the genetic burden of females with the disorder nor an increased incidence in female relatives of those with the disorder.[37,41,42] It is possible that these differences can be attributed to the heterogeneity in the samples and the different methodologies employed. The future will require replication with larger groups.

3 | POTENTIAL PATHOGENESIS OF ASD

Here, we reviewed the underlying mechanisms with the association of ASD risk genes, omics studies, ASD occurrence in different genetic backgrounds, and its common mechanisms between ASD and its comorbidities. We also summarized the mechanisms associated with important physiological and metabolic abnormalities, as well as gut microbiota.

3.1 | Pathway networks associated with ASD risk genes based on SFARI database

Single gene mutations merely account for 1%–2% of autism cases and they act through distinct molecular pathways.[43,44] We gathered the ASD risk genes from SFARI database and categorized them into three groups based on risk level. The Gene Ontology (GO) analysis was conducted on three groups, respectively. In the first set, most of risk genes were enriched in histone modification, cognition, as well as regulation of transporter activity pathway. Regulation of neurological system process, synapse organization, and social behavior pathways were placed in a prominent position within pathway network (Figure S2A). These results implicated that impairment of cognition is the most obvious character. Individuals with autism spectrum conditions or rare mutation related to ASD have profound impairments in the interpersonal social domain.[45–48] In the second set, a majority of the risk genes exhibited enrichment in modulation of synaptic transmission, synapse organization, and learning or memory (Figure S2B). Additionally, some pathways involved human traits and actions were found, including learning or memory, social behavior, mating, circadian rhythm, sleep, and locomotory behavior. The change of these human action may be potential indication for ASD.[49–53] In the third set, many risk genes were enriched in cellular response to peptide, regulation of cell growth, and modulation of synaptic transmission (Figure S2C).

3.2 | Multiple omics revealed pathological mechanism of ASD

Omics techniques allowed an in-depth study of ASD from a wide range of samples. The advantage of omics approaches is that they provide a complete overview of biological "features" (genes/transcripts/proteins/metabolites). It provided the most appropriate stratification of diseases or identification of new biomarkers. Meanwhile, multi-omics can integrate information across different populations, validate them against each other, identify key genes, proteins and metabolic pathways, explore pathological mechanisms, and provide a scientific basis for the disease diagnosis and treatment. Here, we reviewed the omics studies related to ASD and the signaling pathways, in particular the convergent signaling pathways (Table S1) which associated with synaptic dysfunction, glutamatergic and GABAergic synapse imbalance, and postsynaptic density (PSD), as well as important physiological and metabolic abnormalities.

3.2.1 | The signaling pathways of synaptic dysfunction

The main signaling pathways involved in synaptic dysfunction include phosphatidylinositol 3-kinase/Protein kinase B/Mammmalian target of rapamycin (PI3K/Akt/mTOR) signal and abnormal autophagy, extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) signal, Janus kinase and microtubule interacting protein 1 (JAKMIP1) pathway, and calcium signaling. Among them, dysregulation of the PI3K/Akt/mTOR pathway was considered as a point of convergence ASD.[54–56] mTORC1 severed as a key role to tightly coordinates synaptic signaling pathways downstream of glutamate and neurotrophic receptors.[57] An unbiased proteomic showed that a brief repression of mTORC1 activity causes a significant remodeling of proteins resided in the PSD.[58] A rat fetal brain transcriptome demonstrated prominent maternal immune activation (MIA)-induced transcriptional dysregulation of mTOR and EIF4E-dependent signaling.[59] The significant proteins from S-nitrosylation proteomics could be enriched in mTORC1 upstream pathway in InsG3680(+/+) ASD mouse models.[60] DEPs from frontal cortex (FC) and hippocampus of Tsc1+/− mouse model were involved in myelination, dendrite, and oxidative stress, an up-regulation of ribosomal proteins and the mTOR kinase.[61] In addition, a leukocyte transcriptomics identified a perturbed gene network involved with PI3K/AKT and its downstream pathways such as mTOR, autophagy, viral translation, and FC receptor signaling were enriched from 1−4-year-old male toddlers with ASD or typical development.[62] Likewise, autophagy dysfunction meditated by PI3K/AKT/mTOR pathway is a causative factor for ASD.[55,63,64]

Accumulating evidence suggested ERK/MAPK signaling as a downstream mediator of divergent genetic mutations linked to certain forms of autism.[65–68] It also could be a converge on mTOR signaling pathway.[69] A global down-regulation of the MAPK/ERK pathway and decrease in phosphorylation level of ERK1/2 were

found in Fmr1-KO cell lines.[70,71] NMDA NR1-knockdown mouse show the abnormalities of ERK signaling pathway in FC and hippocampus.[72] MAPKAPK3 and MRPL33 in human blood were associated with a higher risk of ASD, and MAPK/ERK signaling pathways and mitochondrial dysfunction play key roles in the pathogenesis of ASD.[73]

The alteration of JAKMIP1 could be found in individuals with distinct syndromic forms of ASD, fragile X syndrome, and 15q duplication syndrome.[74] A previous study found that CYFIP1 play a role in regulating two dysregulated genes, JAKMIP1 and GPR155 compared the mRNA expression profile in lymphoblastoid cells from autism.[75] An enriched network from interactome showed that JAKMIP1 interacted with proteins related to signaling and interaction, nervous system development and function, and protein synthesis. Notably, its loss affected neuronal translation and glutamatergic N-methyl-D-aspartate receptor (NMDAR) signaling.[74]

Calcium signaling has a prominent effect on pathogenesis of ASD.[76] An action of calcium ion plays an essential role for neurodevelopment.[77] ERK signaling has also been found to be greatly linked to calcium channels to cause abnormal synaptic functions, chromatin remodeling, and ion channel activity.[78,79] Ca^{2+}/calmodulin-dependent protein kinase II is considered as key node in synaptic plasticity of ASD.[80] Its interactome identified proteins related to NMDARs, synaptic scaffolds, myosins, tubulin and microtubules, actin cytoskeleton, ribosome and translation, mitochondria, and others.[81] Synaptic fraction contained more CaMKII-associated proteins including scaffolding, microtubule organization, actin organization, ribosomal function, vesicle trafficking, and others.[81] Activated CaMKII phosphorylates multiple substrates in the PSD, including scaffold protein PSD-95, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) receptor targeting subunit stargazing, and proteins involved in cytoskeleton rearrangement.[82]

3.2.2 | Imbalance between glutamatergic and GABAergic synapse

Accumulating evidence supported a hypothesis that the imbalance between excitation and inhibition (E/I) caused by changes in the availability of glutamate and/or GABA signal transmission contribute to pathological synaptic transmission and neural circuits in ASD.[83–87] A broad transcriptomics from postmortem samples with ASD demonstrated that both rare and common ASD-associated genetic variation converge within a down-regulated synaptic signaling.[88] Previous study found a decrease of AMPA-type glutamate receptors, glutamate transporters, and density of GABAA receptors in the cerebellum and anterior cingulate cortex of ASD.[89] An orthogonal selected reaction monitoring assays validated the proteomics results in NMDA NR1-knockdown mouse to show the abnormalities of synaptic long-term potentiation and myelination in FC and hippocampus.[72] Another proteomics study showed up-regulation of glutamatergic ion channels and down-regulation of neurofilament proteins in ASD brain.[90] Similarly, a cortical transcriptome of ASD exhibited analogous cortical–striatal hyperconnectivity at the protein level with mTOR or TSC2.[91] A single-cell transcriptomics from *Chd8* heterozygote mice strengthen the E/I balance hypothesis of ASD in general.[92]

Interestingly, previous metabolomics studies found that ASD often suffer from dysregulated amino acid metabolism and glutamate urinary level was lower compared with their unaffected siblings.[93,94] The reduced pyridoxal phosphate in urine from ASD children implicated the dysregulation of biotransformation of glutamate into GABA.[95] Similarly, a strongly reduced glycine level would primarily affect NMDAR excitatory tone, overall impairing downstream glutamatergic, and GABAergic transmissions.[96]

3.2.3 | Essential role of postsynaptic density in neural communication

The PSD of synapses is a wide range of scaffolding proteins, receptors, and signaling molecules that acts as a switchboard of neurotransmitter molecular and have strong association to ASD.[97,98] Glutamate receptor levels could be regulated by endocytosis of PSD scaffolding proteins.[99] In general, E/I balance required the integrity of PSD to transmit signal between neuros.[100–102] Several genes encoding PSD have been identified disruptive mutations in psychiatric disorder patients, including ASD.[98,103]

Synaptic protein/pathways resource (SyPPRes) was identified as the prioritization of ASD risk factors across 41 in vivo interactome, which show a larger number of shared protein associations to Psd95/Dlgap1/Shank3 indicating a role of core–PSD scaffolds interactions.[104] The alteration of macromolecular complex proteins such as SHANK3 can cause ASD.[105] To quantify the proteins in PSD fractions, the most altered levels of proteins exhibiting ionotropic glutamate receptor activity, cell–cell signaling, and cytoskeleton organization as the results of SHANK3 deficiency.[106] A zebrafish embryo model of ASD induced by VPA showed the significant decrease of *Shank3* in transcriptome.[107] Striatal regions of *Shank2*-mutant mice showed distinct patterns from transcriptomic including synapse, ribosome, mitochondria, spliceosome, and extracellular matrix.[108] The transcriptomic from hippocampal

showed strongly enriched GO terms associated with PSD, synapse, and postsynaptic membrane.[108] Other omics studies related to ASD risk genes have achieved similar results, such as SAP97 gene,[109] p140Cap gene,[110–112] Pten gene,[113] and nSR100 gene.[114]

3.2.4 | Others

Previous omics studies have also revealed that physiological and metabolic abnormalities such as mitochondrial dysfunction, oxidation, and inflammation are associated with ASD. The mitochondrial deficiency is expected to explain the underlying damage mechanism in ASD. ASD were described as mitochondrial diseases and its potential mechanism was identified through phosphoproteomics.[115] The alternated pathways in brain of autistic subjects were associated with energy metabolism, synaptic vesicle regulation as well as myelination.[116] The change of mitochondrial function, energy metabolism, EIF2 signaling, immune functions, ubiquitination, and DNA repair were found in global proteomics of peripheral blood-derived lymphoblasts with homozygous HERC2 variants.[117] A transcriptome suggested that mitochondrial function, ribosome, and spliceosome components were down-regulated in postmortem brain of ASD.[118]

A metabolic profiling of lymphoblastoid cells revealed a decreased tryptophan metabolism in ASD and showed a reduced generation of nicotinamide adenine dinucleotide (NADH), a critical energy carrier in mitochondria.[119] The metabolic clusters containing lactate or pyruvate, succinate, α-ketoglutarate, glycine, ornithine, and 4-hydroxyproline highlighted potential dysregulation in amino acid and energy metabolism in ASD plasma.[120] Importantly, a metabolomics in cerebrospinal fluid analysis from ASD showed that L-cysteine, adenine, and dodecanoic acid were important metabolites for ASD.[121] Additionally, amino acid and energy metabolism pathways were most disrupted in all neurodevelopment disorders.[121] A previous study performed proteomics and metabolomics on amniotic fluids from pregnant woman with male fetuses and premutation in FMR1 gene. The result showed the mitochondrial dysfunction induced by the deficits in prenatal serine biosynthesis underlie.[122] A wide range of aberrant mitochondria-related pathways, including respiratory electron transport chain, cellular response to stress, regulation of neuron apoptotic process, and reactive oxygen species (ROS) metabolic process were triggered by SHANK3 mutation in mouse cortex.[123] Untargeted metabolomics revealed that key metabolic mitochondrial/extramitochondrial pathways was up-regulated in mecp2-deficient mouse cortex.[124] VPA-induced alterations in metabolites of serum, urine, and brain cortex were associated with mitochondrial dysfunction metabolism and CNS disorders.[125]

A mechanistic modeling based on transcriptome suggested a direct link between inflammation and ASD in neurons.[118] Notedly, the MIA is a one of the common environmental risk factors of ASD pathology during pregnancy.[126–128] The adaptive immune pathway was enriched in maternal blood from mothers of children later diagnosed with ASD by transcriptome.[129] Maternal inflammation with elevated kynurenine metabolites is related to the risk of abnormal brain development in ASD.[130] Similarly, the increased paternal age at conception has been associated with ASD.[131,132]

In the metabolic profile, prostaglandin D2, which is a type of inflammatory mediators was increased in plasma of young boys with ASD and implicated with neuroinflammation.[133] In the liver of BTBR mouse model of autism, 12 differential metabolites suggested that bile acid-mediated activation of LXRα might contribute to metabolic dysfunction of lipid and leukotriene D4 produced by the activation of 5-LOX led to hepatic inflammation.[134] In ASD children brain, abnormal levels of N-acetyl-compounds, glutamate glutamine, creatine phosphocreatine (Cr), or choline-compounds (Cho) implicated that neuron or glial density, mitochondrial energetic metabolism, and/or inflammation contribute to ASD neuropathology.[135] The consistent appearance of inflammation regulation in proteomics from Mecp2-mutant mouse, cells generated from induced pluripotent stem cells (iPSC) in Rett syndrome (RTT), and RTT peripheral samples implied that it contributed to the destruction of the nervous system.[136]

In summary, the above-mentioned signal pathways play a significant role in the typical neurodevelopment process, and their dysfunction can lead to downstream alterations, such as an imbalance in excitatory and inhibitory synapses. This can result in the transmission of erroneous signals within neural circuits, may be caused by inflammation and reoxidation. The maintenance of stable neural communication is contingent upon the integration of synapse construction, such as PSD, and the provision of sustainable energy from mitochondria. As a result, a series of aberrant signaling molecules, excitatory and inhibitory imbalances, PSD, mitochondrial dysfunction, and inflammation ultimately lead to neural immaturity and damage in ASD pathology (Figure 2).

3.3 | Studies on the pathogenesis of ASD in different genetic backgrounds

The search for "commonalities" among children with ASD has become a focus of current research and a breakthrough

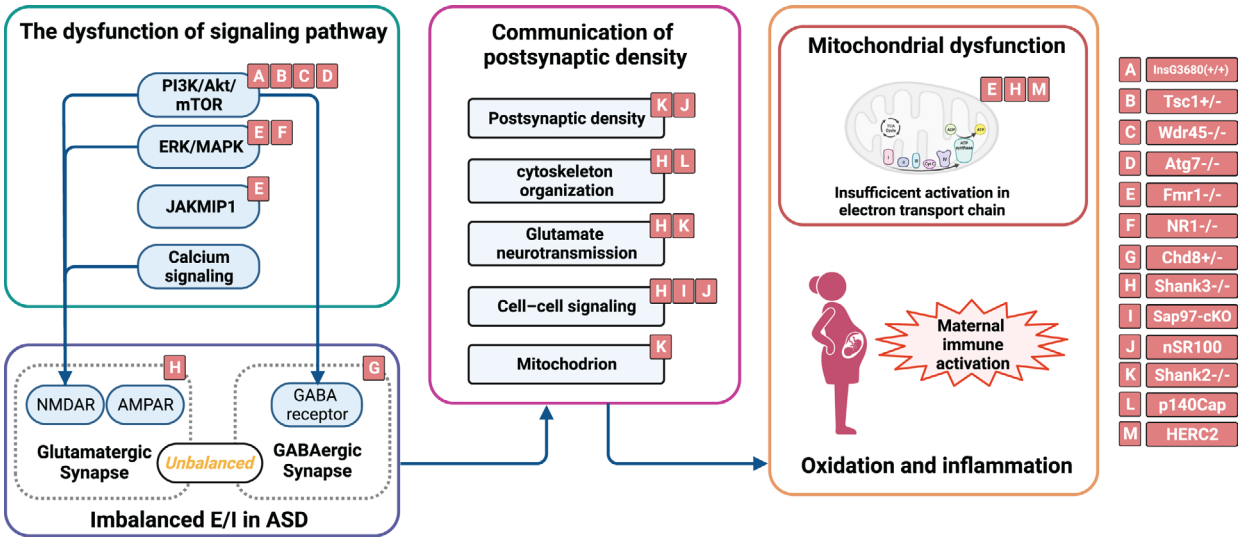


FIGURE 2 The graphical abstract of potential pathology of autism spectrum disorder (ASD) investigated by multi-omics methods. The dysregulation of signaling pathways in neuron lead to abnormal balance between excitatory and inhibition. Many genes mutation influenced the postsynaptic density including the cytoskeleton organization, glutamate neurotransmission, cell–cell signaling, and mitochondrial function. The accumulation of negative effect further impacts the downstream of synapse. The maternal immune activation and mitochondrial dysregulation are associated with oxidation and inflammation. The red labels indicates that the genes are associated with the process in the studies. E/I, excitation and inhibition.

point.[137–140] ASD-related syndromes with a clear genetic cause for the autism phenotype offer the best opportunity to elucidate the underlying mechanisms of ASD and to identify possible therapeutic targets[141] and diagnostic markers.[142] In recent years, there has been notable advancement in the identification of genes closely linked to ASD. These genes exhibited distinct molecular functions but may share biological pathways. In the context of known genes, the research on genes and pathological mechanisms, diagnostic markers, and even imaging is conducive to finding the commonality between different genes (Table 1).

A recent study of iPSC-derived "brain-like organs" from children carrying three different ASD risk genes showed that although each gene acts through a unique underlying molecular mechanism, they have similar overall defects that affect similar aspects of neurogenesis and the same type of neurons.[24] Using iPSC, Pintacuda et al. constructed protein–protein interaction networks among 13 ASD-related genes in human excitatory neurons, revealing the neuron-specific biology associated with ASD.[150] Three animal experiments with known genetic backgrounds suggest that synapses play a key role in the pathogenesis of ASD.[144,146] Among them, Jordan and coworkers compared the synaptic proteomes of five mouse models of autism revealing convergent molecular similarities, including defects in oxidative phosphorylation and Rho GTPase signaling.[146] They also compared synaptic proteomes of seven mouse models of autism revealing molecular subtypes and defects in Rho GTPase signaling.[146] Another study investigated seven animal models of ASD and showed that there is great heterogeneity between models. However, high-dimensional measurements of synaptic protein networks may allow a promising avenue for subtype differentiation of ASD with common molecular pathology. Notably, this approach demonstrated convergence between the glutamate synapse protein interaction networks of the VPA and TSC2 mouse models, both converging on a putative "mTOR" cluster.[144]

Similarly, a previous study identified distinct and overlapping phenotypes at the level of behavior, brain structure and circuitry by analyzing the function of 10 autism genes in zebrafish. The study observed that the forebrain contributed most to brain size differences between ASD genes, that brain activity phenotypes were concentrated in regions involved in sensory-motor control, that dopaminergic and microglia abnormalities were the confluence of two genes (SCN2A and DYRK1A), and implied that neuroimmune dysfunction was associated with autism biology.[151] In addition, Willsey et al. employed parallel in vivo analyses and systems biology approaches to examine 10 genes linked to ASD by utilizing tropical African clawed toads. The results suggested that cortical neurogenesis served as a convergence vulnerability site in ASDs. Moreover, estrogen is a restorative factor for several different autism genes and they revealed a conserved role for estrogen in inhibiting sonic Hedgehog signaling.[152]

TABLE 1 Studies on different genotypes of autism spectrum disorder (ASD).

No.	Author	Sample	Genotype	Method	Major finding
1	Ellegood et al. (2015)[143]	Mouse brain	15q11-13, 16p11, AndR, BALB/c, BTBR, CNTNAP2, En2, FMR1, GTF2i, ITGβ3, Mecp2, NLGN3, NRXN1α, SLC6A4, SHANK3, XO	MRI	26 different mouse models were examined, the parieto-temporal lobe, cerebellar cortex, frontal lobe, hypothalamus, and the striatum are the abnormal regions, unknown connections between Nrxn1α, En2, Fmr1, Nlgn3, BTBR, and Slc6A4 were identified.
2	Brown et al. (2018)[144]	Mouse FC, HC	CNTNAP2, FMR1, Shank3B, Shank3Δex4-9, TSC2, Ube3a2xTG,	QMI	A unique set of disrupted interactions was displayed by each model, but synaptic activity-related interactions were disrupted. Potential relationships among models and deficits in AKT signaling in Ube3a2xTG mice were confirmed.
3	Jin et al. (2020)[145]	Mouse CPN, CIN, AC, OC, MG	Adnp, Ank2, Arid1b, Ash1l, Asxl3, Chd2, Chd8, Cntnb1, Cul3, Ddx3x, Dscam, Dyrk1a, Fbxo11, Gatad2b, Kdm5b, Larp4b, Mbd5, Med13l, Mll1, Myst4, Pogz, Pten, Qrich1, Satb2, Scn2a1, Setd2, Setd5, Spen, Stard9, Syngap1, Tcf20, Tcf712, Tnrc6b, Upf3b, Wac	In vivo Perturb-Seq	In vivo Perturb-Seq can serve as a tool to reveal cell-intrinsic functions at single-cell resolution in complex tissues, which can be applied across diverse and tissues in the intact organism.
4	Carbonell et al. (2021)[146]	Mouse HC	Anks1b, BTBR, Cntnap2, Cacna1c, Fmr1, Pten, Shank3	TMT, SDS-PAGE, LC–MS	Hippocampal synaptic proteomes from seven mouse models were identified, common altered cellular and molecular pathways at the synapse were also identified.
5	Zerbi et al. (2021)[147]	Mouse brain	16p11.2, BTBRT, CDKL5, CHD8, CNTNAP2, En2, FRM1.1, FRM1.2, Het, IL6, Mecp2, SGSH, SHANK3b, Syn2, TREM2	MRI	ASD-associated etiologies cause a broad spectrum of connectional abnormalities, etiological variability is a key determinant of connectivity heterogeneity in ASD, identification of etiologically relevant connectivity subtypes could improve diagnostic label accuracy in the non-syndromic ASD population.
6	Willsey et al. (2021)[148]	Xenpus tropicalis brain	ARID1B, ADNP, ANK2, CHD8, CHD2, DYRK1A, NRXN1, POGZ, SCN2A, SYNGAP1	LWI	Mutations lead to an increase in the ratio of neural progenitor cells to maturing neurons, systematic small molecule screening identifies that estrogen rescues the convergent phenotype and mitigate a broad range of ASD genetic risks.
7	Shen et al. (2022)[149]	Human blood	ASH1L, DDX3X, GIGYF2, NAA15, SCN2A	iTRAQ, LC–MS/MS, ELISA	The DEPs and differential metabolites of plasma could distinguish the cases form controls. Proteomic results highlighted complement, inflammation, immunity, mitochondrial dysfunction, proteasome, ubiquitin-mediated proteolysis, and ER stress in the pathogenesis of ASD.

(Continues)

TABLE 1 (Continued)

No.	Author	Sample	Genotype	Method	Major finding
8	Paulsen et al. (2022)[24]	Human CC	ARID1B, CHD8, SUV420H1	IC, WB	Cell-type-specific neurodevelopmental abnormalities that are shared across ASD risk genes and are modulated by human genomic context were uncovered, convergence in the neurobiological basis of how different risk genes contribute to ASD pathology were found.
9	Pintacuda et al. (2023)[150]	Human brain excitatory iNs	ARID1B, ANK2, ADNP, CTNNB1, CHD8, DYRK1A, GIGYF1, MED13L, PTEN, SCN2A, SYNGAP1, SHANK3, TLK2	WB, LC–MS/MS	The ASD-linked brain-specific isoform of ANK2 is important for its interactions with synaptic proteins and to characterize a PTEN–ANKAP8L interaction that influences neuronal growth, the IGF2BP1–3 complex emerged as a convergent point in the network that may regulate a transcriptional circuit of ASD-associated genes.
10	Carbonell et al. (2023)[146]	Mouse HC	Anks1b, BTBR, Cntnap2, Fmr1, Pten	TMT, LC–MS	Changes in oxidative phosphorylation and Rho family small GTPase signaling were revealed, the ANKS1B model displays altered Rac1 activity counter to that observed in other models was confirmed.
11	Mendes et al. (2023)[151]	Zebrafish brain	CHD8, CNTNAP2, CUL3, DYRK1A, GRIN2B, KATNAL2, KDM5B, SCN2A, TBR1, POGZ	IC, CI, RNA-Seq	A global increase in microglia resulting from ASD gene loss of function in select mutants, implicates neuroimmune dysfunction as a key pathway relevant to ASD biology.

Abbreviations: AC, astrocytes; CC, cerebral cortex; CI, confocal imaging; CIN, cortical inhibitory neurons; CPN, cortical projection neurons; ELISA, Enzyme-linked immuno sorbent assay; ER, Endoplasmic reticulum; FC, frontal cortex; HC, hippocampus; IC, immunohistochemistry; iNs, induced neurons; LC–MS/MS, liquid chromatography–tandem mass spectrometry; LWI, large-scale whole-brain imaging; MG, microglia; MRI, magnetic resonance imaging; OC, oligodendrocytes; QMI, quantitative multiplex co-immunoprecipitation; SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis;TMT, tandem mass tag system; WB, western blot.

In vivo Perturb-Seq technology based on CRISPR-Cas9 and single-cell RNA sequencing technology developed a high-throughput genetic screening method to study the function of numerous genes in complex tissues at single-cell resolution. Recently, Zhang and coworkers applied this method to analyze the effects of 35 ASD/ND risk genes on brain development in mice. The authors identified cell type specific and evolutionarily conserved gene modules from neuronal and glial cell categories.[145]

These studies exemplify the examination of genetic heterogeneity in ASD by conducting studies of common features of ASD and controls based on known genetic backgrounds. The findings suggested that ASD-associated susceptibility genes ultimately converge on common signaling pathways and that these convergence sites are key to understand ASD pathology. Therefore, categorizing genes based on shared biology despite their heterogeneity might represent a path toward precision medicine in ASD, bridging the gap between gene discovery and actionable biological mechanisms.[151]

Moreover, similar results have been obtained in imaging studies under different genetic backgrounds. Functional magnetic resonance imaging analysis of 16 mouse mutants with ASD-related mutations identified brain connectivity subtypes among the mutants despite the presence of distinct phenotypes.[147] Likewise, although mouse mutants with 26 ASD genes exhibited heterogeneous neuroanatomical phenotypes, clustering of these mutants by shared features allowed identification of gene subgroups.[143]

Overall, these studies suggest that conducting research on the convergent mechanisms among ASD-related genes and elucidating the shared pathways could provide information to unravel the mechanisms of ASD and explore potential therapeutic targets and diagnostic biomarkers.

3.4 | Common mechanisms associated with ASD and its comorbidities

The comorbidities in most children with ASD is a notable attribute, contributing to its diverse and intricate nature.[153] Thus, investigating common mechanisms between ASD and comorbidities, as well as the specific genes and mechanisms that lead to their respective occurrence, is a topic of interest in the field of ASD research, and its study contributes to the diagnosis and treatment of ASD. Previous studies have shown some common mechanisms between these comorbidities and ASD.[153] For example, recent studies have highlighted points of convergence between ASD and neurodevelopmental disorders (NDD) genes.[154] Chromosomal microarray and sequencing studies have identified significant genetic overlap between ASD and other NDD and neurological disorders, including ID, epilepsy, and schizophrenia.[155,156] Two meta-analyses of genome-wide associations have also shown that ASD shares a common genetic background in neuropsychiatric disorders.[157,158] Genes involved in synaptic structure and function are implicated in a variety of disorders, including schizophrenia, ASDs, and other NDDs.[159–161] The gene discovery can help to distinguish this complexity by analyzing the genetic structure and risk gene associations of different subtypes or comorbidities. In addition, several environmental factors have been found to be associated with ASD and its comorbidities, such as MIA in the prenatal environment, stress, drug exposure, and malnutrition,[126,127,162–164] as well as gastrointestinal dysfunction and disruption of intestinal flora.[165–167] These studies suggest that although the heterogeneity of ASD is complicated by the occurrence of comorbidities, common mechanisms may still be found between ASD and its comorbidities.

3.5 | Mechanisms associated with important physiological and metabolic abnormalities

As mentioned above, immune dysregulation, inflammation, oxidative stress, and mitochondrial dysfunction are closely associated with ASD and are important physiological and metabolic abnormalities in ASD.[128,138,168–170] They may be the intersection of genetic and environmental factors and contribute to ASD.

Immunity and neuroinflammation play a key role in the development of ASD.[171–173] Immune dysfunction in ASD involves a network of interactions between several cell types from the innate and adaptive immune response. Multiple immune factors mediate the effects of CNS function. Some cytokines inhibit neurogenesis and promote neuronal death, whereas others promote the growth and proliferation of neurons and oligodendrocytes. Complement proteins and microglia can be involved in synaptic scaling and pruning, while brain-reactive autoantibodies can alter neuronal development or function.[172] Active microglia and astrocytes have been observed in the brains of ASD. Activation of microglia in different brain regions was observed, including an increase in cell number or cell density, morphological changes, and phenotypic alterations.[174] Activation of microglia releases inflammatory cytokines and chemokines such as interleukin (IL)-6, IL-12, IL-β, and tumor necrosis factor-alpha (TNF-α). Excessive induction of nitric oxide synthase (NOS) and ROS affects synaptic plasticity and produces behavioral abnormalities associated with ASD.[175]

Oxidative stress is associated with mitochondrial dysfunction. Decreased endogenous antioxidant capacity, particularly reduced total glutathione (tGSH) levels and altered glutathione peroxidase (GPx), superoxide dismutase, and catalase activities, have been reported in ASD, which is consistent with elevated oxidative stress indicators in children with ASD.[176] The prevalence of mitochondrial disease in ASD is 4%−5%, which is significantly higher than in the general population (about 0.01%).[177,178] Mitochondrial abnormalities such as increased hydrogen peroxide, decreased NADH, and mitochondrial DNA overreplication have been observed in lymphocytes isolated from subjects with ASD.[179] Mitochondria produce adenosine triphosphate (ATP). Reduced ATP production and elevated levels of lactate and pyruvate in individuals with ASD may indicate mitochondrial dysfunction in autism.

These physiologic and pathologic processes interact with each other, and multiple mechanisms are interrelated.[128,170,180,181] Oxidative stress can lead to mitochondrial dysfunction, and abnormal mitochondrial function leads to increased ROS metabolism and oxidative stress, creating a vicious cycle. The association between gut flora and MIA is also reflected in the pathogenesis of ASD.[128,180] MIA induces an immune response in pregnant women, leading to further inflammation and oxidative stress, as well as mitochondrial dysfunction in the placenta and fetal brain. These negative factors lead to neurodevelopmental deficits in the developing fetal brain, which subsequently lead to symptoms of behavioral disorders in the offspring.[128] In summary, accumulating research into these common pathophysiologic mechanisms will enhance our comprehension of ASD diagnosis and treatment, while provide insight into general or subgroup-specific processes that may contribute to the development of ASD and other psychiatric disorders.[168]

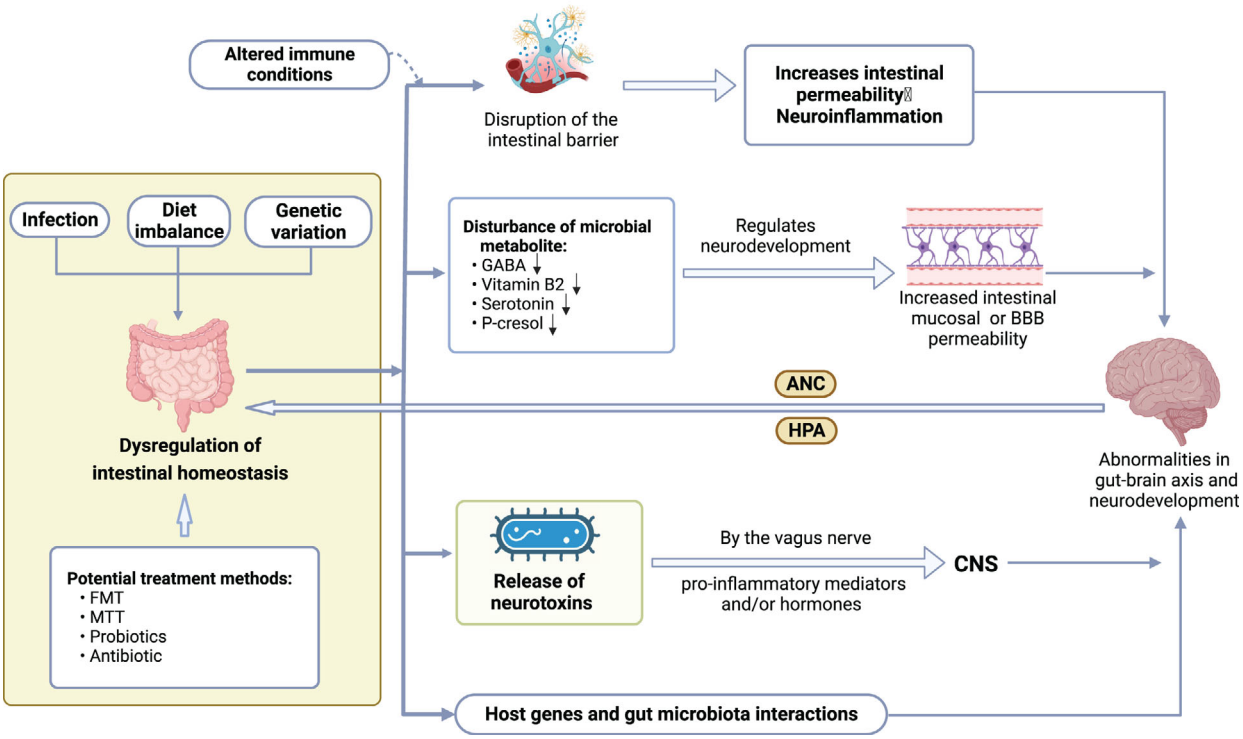


FIGURE 3 Potential mechanisms of gut microbiota imbalance and autism spectrum disorder (ASD) occurrence. The gut flora and brain can interact through immune, metabolic, and gut nervous system pathways and ultimately leading to abnormalities in gut brain axis and neural development. Gut flora alternation causes metabolism changes and its dysbiosis linked to greater intestinal mucosa and blood–brain barrier (BBB) permeability. Feedback regulation exists in gene expression, dietary preference, and gut flora. ANC, autonomic nervous system; CNS, central nervous system; FMT, fecal microbiota transplantation; HPA, hypothalamic–pituitary–adrenal; MTT, microbiota transfer therapy.

3.6 | Pathological mechanisms of ASD associated with gut microbiota

A series of studies have reported significant differences in the composition of gut microbiota between ASD cases and healthy controls (Figure 3). Changes in gut microbiota cause changes in metabolism. Several animal experiments have demonstrated an association between ASD and gut microbiota. Transplantation of gut flora from the individuals with ASD into germ-free mice leads to autism-like symptoms in the mice, which may be related to the regulation of tryptophan and 5-hydroxytryptaminergic synaptic metabolism[182] or it may lead to alterations in neuroactive metabolites.[183] It has also been found that changes in the gut microbiota of children with ASD affect glutathione (GSH) synthesis[184] and degradation of organic toxins, lacking biosynthetic pathways for several neurotransmitters[185] or vitamins.[186] In addition, some bacterial metabolites may contribute to the development of autism-like behaviors, such as elevated acetaminophen sulfate levels.[187,188] The presence of gut dysbiosis has also been linked to heightened permeability of the intestinal mucosa or the blood–brain barrier. For example, abnormal metabolism of some short-chain fatty acids (SCFAs) affected tight junction proteins associated with blood–brain barrier permeability.[189] The neurotoxins are released by a variety of harmful bacteria that are delivered via the enteric vagus nerve to the CNS.[182] The permitting pro-inflammatory mediators and/or hormones enter the circulation and to be transported from bloodstream to the brain, where they may ultimately affected the CNS neurodevelopment and/or function.[182]

Studies on Cntnap2 double knockout[190] and CHD8 single knockout autism model mice[191] have shown that the combined effects of host genes and gut flora in interacting with each other lead to behavioral abnormalities in autism. Genetic factors and dietary habits can alter the composition of the gut microbiota, while imbalances in the gut microbiota can also trigger aberrant gene expression and influence dietary preferences.[192] Moreover, the nervous system can act on the gastrointestinal tract and its microbiota through specific pathways (e.g., the autonomic nervous system axis and the hypothalamic–pituitary–adrenal axis) to regulate intestinal motility and

secretion, and to influence gut microbial composition and function.

Furthermore, studies of gut microbiota have also revealed that pathological inflammation of ASD occurs not only in the CNS and periphery, but also in the gut. The damaged, inflamed, and permeable epithelia are the predominant routes utilized by commensal bacteria to migrate to the bloodstream.[193] The microbial metabolites are likely the most significant contributors to systemic inflammation and subsequent neuroinflammation.[193,194] The occurrence of abnormal oxidation or unsuitable activation of immune led to subsequent inflammation and neuroinflammation in CNS, periphery, and gut of ASD.

Taken together, dysbiosis of the gut microbiota may be an important contributor to ASD, leading to disruptions in gut–brain axis connectivity and neurodevelopment caused by bacterial metabolites, the enteric nervous system, and the systemic immune system. An in-depth exploration of the possible molecular mechanisms by which gut microbes influence behavioral changes in ASD offers great potential for intervention, diagnosis, and therapeutic evaluation in ASD. Notably, to date, the relationship between the gut microbiota and autism symptom severity is difficult to determine, and no specific bacterial group could be identified as being solely responsible.[195]

4 | STUDY ON DIAGNOSTIC BIOMARKERS OF ASD

A widely accepted consensus in clinical practice is that timely identification and diagnosis play a crucial role in facilitating early intervention and prognostic outcomes. To achieve this goal, the American Academy of Pediatrics recommends that all children should be screened for autism for the first time at 9 months of age and at routine developmental monitoring centers at 18, 24, and 30 months of age.[196] In China, there are similar consensus or norms.[197] Therefore, it is necessary to identify early behavioral features of ASD that can be used for early diagnosis. Moreover, there is a need to investigate biomarkers for objective diagnosis. Measurable laboratory biomarkers may be an opportunity to identify risks that not only provide an earlier and more reliable diagnosis, but further differentiate the autism spectrum based on common pathophysiological features, allowing for individualized treatment and response monitoring, and increasing the chances of success of future drug development programs.[198] To date, some consensus has raised on the early behavioral features of ASD.[28,196] Although progress has been made in the study of diagnostic markers, most biomarkers have not yet been validated and further research is required.

A recent study conducted a systematic review of diagnostic molecular markers for ASD.[199] The majority of these markers are measured peripherally via blood, and although there is considerable variation between and within individual biomarkers, two major groups are apparent, one consisting of cytokines and growth factors (e.g., IL-6, brain-derived neurotrophic factor) and the other consisting of amino acids, neurotransmitters (e.g., cysteine, serotonin, GABA), and hormones (e.g., vitamin D). In between these two groups are molecules related to reduction/oxidation (redox), including GSH, which is the most frequently detected molecule. Most papers also report an association between molecular markers and ASD diagnostic status.

In this section, we provide an overview in terms of non-targeted omics and targeted research of ASD diagnostic markers, as well as research on diagnostic markers associated with important physiological and metabolic abnormalities of ASD, and gut microbiota.

4.1 | The identification of potential biomarkers by non-targeted omics

Genetic testing, proteomics, and metabolomics were employed in previous study to screen a number of genes, proteins, peptides, and metabolites that have the potential to be diagnostic markers for ASD.[18] Protein and metabolite-based tests provided the highest diagnostic accuracy for ASD, which combined with multiple features may further improve diagnostic accuracy.[200]

There have been several reports and reviews on the proteomics of ASD protein diagnostic markers, mainly including blood, urine, and saliva studies. Overall, candidate proteins obtained from proteomic studies have little or no reproducibility in independent cohorts.[201] However, bioinformatics analysis showed that the majority of proteins in different studies were associated with complement and coagulation cascades, focal adhesion, platelet activation, vitamin digestion and absorption, immune response, inflammatory response, cholesterol metabolism, lipid metabolism, oxidative stress, and energy metabolism. These mechanisms are evidently prevalent in individuals with ASD, thus indicating a convergence of protein-associated mechanisms that hold promise as potential diagnostic markers.[201–205]

Metabolism-based analyses have the advantage of being sensitive to the interactions between genomic, gut microbiome, dietary, and environmental factors. The metabolite differences between disease and normal states has received increasing attention in recent years. Studies of blood and urine metabolomics in children with

autism versus controls have shown that although fewer metabolisms show consistent changes across studies, the mechanisms by which they are associated are convergent and correlate with common pathogenesis and pathophysiological changes in ASD. Changes in blood metabolites are mainly associated with mitochondrial dysfunction, oxidative stress, fatty acid metabolism, energy metabolism, cholesterol metabolism, neurotransmitters, and mammalian–microbial co-metabolism pathway.[18,206] Most of the changes in urinary metabolites are related to amino acid metabolism, energy metabolism, oxidative stress, intestinal flora, and neurotransmission. The metabolism of some amino acids (e.g., tryptophan and branched-chain amino acids) and neurotransmitters (e.g., glutamate, ROS, and lipids) may play an important role in the pathogenesis of ASD.[18,206]

A recent study analyzed blood and urine metabolites from the same group of children with autism and found decreased urinary taurine and catechol levels and increased plasma taurine and catechol levels.[207] Another urine metabolomics study in twins found that phenylpyruvate and taurine were elevated in the autistic group, while carnitine was decreased, and arginine and proline metabolic pathways were enriched. In twins, there was a significant positive correlation between indole-3-acetate and autistic traits.[208] In addition, in some recent omics studies,[209–212] machine learning methods have been used to screen diagnostic markers from omics data.

The combined multi-omics approach has been reported in several studies of diagnostic markers for ASD.[142,213,214] For example, using metabolomic and transcriptomic approaches, Dai et al. revealed that blood uric acid levels were significantly lower in children with ASD and the expression levels of some genes related to purine metabolism differed between children with ASD and controls.[213] Integrated proteome and metabolome analysis, another study found that six signaling pathways were significantly enriched in ASD, three of which were correlated with impaired neuroinflammation (GSH metabolism, metabolism of xenobiotics by cytochrome P450, and transendothelial migration of leukocyte).[214] Although further validation is needed, in combination with proteomic and metabolomic data, a previous study suggests that glycerophospholipid metabolism and N-glycan biosynthesis may play a key role in the pathogenesis of ASD.[142]

Moreover, to explore the effect of ASD gene heterogeneity on the study and application of diagnostic markers, Shen et al. preliminarily detected five children with ASD carrying risk genes for ASD from 126 cases through gene-targeted testing, proteomic, and metabolomics in plasma and peripheral blood mononuclear cells (PBMCs) compared to healthy controls.[142] The results showed that although the children with ASD differed in their expression patterns of total proteins and metabolites, the differential proteins and metabolites identified were still able to distinguish cases from controls well, and the mechanisms of association were consistent with those reported in previous studies.[18] Based on this, they added the group of children clinically diagnosed with ASD but not detected as carrying risk genes to further the study and obtained similar conclusions.[215] These findings support that, despite the presence of genetic heterogeneity, it is possible to identify markers for diagnosis among children with different genetic backgrounds.

4.2 | Targeted research and application of diagnostic markers

The targeted validation and detection of diagnostic markers, especially using some high-throughput methods (e.g., targeted proteomics, metabolomics), is convenient and important. This is primarily due to the utilization of multiple markers in the combined diagnosis of multifactorial diseases, which typically results in enhanced diagnostic accuracy and specificity compared to single diagnostic marker. Here, we focus on targeted proteomics and metabolomics studies. However, in reality, any study that addresses the common pathophysiological mechanisms associated with ASD is also a targeted study, such as studies that have selected a panel of cytokines for peripheral blood testing based on literature reports.[216,217] Studies targeting a particular class of biomarkers related to oxidative stress, mitochondria, gut microbiota, etc., are also in line with this idea. They are reviewed in Sections 4.3 and 4.4. Indeed, genetic testing with a panel consisting of known ASD-related genes should also be included.[161,218,219]

4.2.1 | Targeted proteomics research

Applying targeted proteomics multiple reaction monitoring technology, we have previously performed targeting studies on the proteins of ASD plasma complement and coagulation cascades, and combined with machine learning methods, we obtained a set of 12 differential protein combinations with diagnostic potential.[212] The complement system composed of more than 40 proteins served as an important component of the human immune system. The expression of complement or complement and coagulation cascade-related proteins has been frequently reported alteration in the peripheral blood of ASD since the first proteomic studies on peripheral

blood in ASD,[18,142,211,220–222] while changes in the brain have also been reported.[221,222] The association of complement with neuropsychiatric disorders has recently attracted attention.[221,222] The correlation between alterations of complement proteins in brain and periphery of children with ASD remains unclear, and the underlying mechanisms are not comprehensively understood, thus necessitating further research.

4.2.2 | Targeted metabolomics studies

Metabolomics is capable of identifying biochemical imbalances that are frequently present in children with ASD, primarily involving amino acids, reactive oxidative stress, neurotransmitters, and the microbial–gut–brain axis,[206,223] and their changes further support the association of these mechanisms with ASD. Studies on the targeted metabolomics of ASD are progressing rapidly, including those on the targeted metabolomics of body fluids such as blood and urine. We have summarized them in Table 2.

At present, targeted detection of metabolites altered in blood include amino acids (tyrosine, tryptophan, arginine, proline, methionine, cysteine, and taurine), lipids (phospholipids, sphingolipids, and fatty acids), and metabolites in the urea cycle and xenobiotics metabolism.[142,235] The metabolites associated with branched-chain amino acid (BCAA) metabolism,[236] fatty acid metabolism (free carnitine, short- and long-chain acylcarnitine),[227] tricarboxylic acid (TCA) cycle, fatty acids, oxidative phosphorylation, mitochondrial dysfunction, gut microbiome metabolism,[142,237] and neurotransmitter metabolism[238] in the plasma of ASD are also involved.

Similarly, in targeted metabolomics studies of urine, previous studies have targeted the abnormalities of reactive oxidative stress, gut bacteria metabolism,[239] amino acid (tyrosine, tryptophan, arginine, proline, methionine, cysteine, and taurine), lipid (phospholipid, sphingolipid, and fatty acid), urea cycle, xenobiotics metabolism,[239,240] TCA cycle, and glutamate metabolism[240] in urine of ASD. Additional studies have also observed abnormalities of ornithine (urea) cycle, methionine, lysine, reactive oxidative stress, and tryptophan–serotonin metabolism in urine of children with ASD.[239] Of interest, a prior study applied a targeted metabolomics approach to examine markers of oxidative stress and gut microbiota dysbiosis reported in previous studies and determined that levels of methylguanidine and n-acetylarginine, which are associated with oxidative stress, and the gut bacterial metabolites indolol sulfate and indole-3-acetic acid were elevated in the urine of children with ASD.[241]

4.3 | Study of biomarkers associated with important physiological and metabolic abnormalities in ASD

4.3.1 | Biomarkers associated with immunity/inflammation

The mounting evidence of altered central and peripheral immune system function supports to the notion that a subgroup of ASD may exhibited some form of immune system dysregulation.[242] The levels of different cytokines in the peripheral blood of ASD have been extensively investigated, and several meta-analyses have reviewed the relationship.[243–246] A systematic review and meta-analysis showed that the pro-inflammatory cytokines interferon (IFN)-γ, IL-1β, and IL-6 were elevated in blood of children with ASD, while the anti-inflammatory cytokine transforming growth factor-β1 was decreased. Levels of several chemokines associated with recruitment of inflammatory cells, including eotaxin, IL-8, and monocyte chemotactic protein-1 (MCP-1), were elevated. Another meta-analysis showed that individuals with autism had lower levels of the anti-inflammatory cytokines IL-10 and IL-1Ra, and higher concentrations of the pro-inflammatory cytokines IFN-γ, IL-1β, IL-6, and TNF-α than controls.[245] Also, meta-regression analyses point to the interaction of latitude, age, and gender with peripheral alterations of associated pro-inflammatory cytokines.[244] A recent meta-analysis found that the levels of peripheral IL-6, IL-1b, IL-12p70, MIF, eotaxin-1, MCP-1, IL-8, IL-7, IL-2, IL-12, TNF-α, IL-17, and IL-4 were significantly changed in ASD compared with controls. These findings reinforce the clinical evidence that ASD is associated with an abnormal inflammatory response. These cytokines may be a series of potential biomarkers in the peripheral blood of ASD.[246] Besides, previous studies have reported that levels of some pro- and anti-inflammatory cytokines and chemokines are associated with severity of abnormal behavior and impaired developmental and adaptive functioning.[247–249] For example, IL-6 has been extensively studied and its levels are elevated in ASD and correlate with severity.[199] Indeed, cytokine changes have also been reported in postmortem brain tissue[250,251] and PBMCs.[248] The changes in mRNA expression of some cytokines were found in whole blood from subjects with ASD.[252]

4.3.2 | Oxidative stress-related biomarkers

In terms of markers associated with oxidative stress, a recent meta-analysis showed that blood levels of oxidized glutathione (GSSG), malondialdehyde, homocysteine,

TABLE 2 Research on potential biomarkers of autism spectrum disorder (ASD) based on targeted metabolomics.

No.	Author	Sample	Method	Related metabolites	Metabolic process involved
1	West et al. (2014)[224]	Blood	GC–MS, LC–HRMS	Decreased[a]: homocitrulline, citric acid, lactic acid, heptadecanoic acid, myristic acid Increased[a]: aspartic acid, serine, glutamic acid, glutaric acid, soleucine acid, 2-hydroxyvaleric, 3-aminoisobutyric acid, 5-hydroxynorvaline	Mitochondrial dysfunction, abnormal gut microbiome metabolism
2	Anwar et al. (2018)[225]	Blood	LC–MS/MS	Decreased[b]: FL, G-H1, NFK Increased[b]: CMA, AASA, GSA, arginine, glutamic	Abnormal protein glycosylation, protein oxidative metabolism
3	Delaye et al. (2018)[226]	Blood	Ion exchange chromatography	Decreased[b]: glutamate, serine, ornithine, proline	Glutamate neurotransmission, gastrointestinal abnormalities
4	Lv et al. (2018)[227]	Blood	MS/MS	Decreased[a]: free carnitine, glutaricyl carnitine, octyl carnitine, 24 carbonyl carnitine, carnosyl carnitine	Mitochondrial dysfunction, abnormal fatty acid metabolism
5	Smith et al. (2019)[228]	Blood	LC–MS/MS, MRM	Decreased[a]: leucine, isoleucine, valine Increased[a]: glutamine, glycine, ornithine	Protein synthesis, neurotransmission, AA/BCAA metabolism
6	Brister et al. (2022)[229]	Blood	LC–MS/MS	Decreased[b]: Nε-fructosyl-lysine Increased[b]: Nω-carboxymethylarginine, Nε-(1-carboxyethyl) lysine, glutamic semialdehyde, 3-nitrotyrosineα-aminoadipic semialdehyde	Energy metabolism, amino acid neurotransmitter metabolism, branched-chain amino acid metabolism, nicotinamide metabolism, aminoacyl tRNA biosynthesis
7	Shen et al. (2022)[149]	Blood	LC–MS/MS	Decreased[b]: L-glutamate, pyridoxamine, O-phospho-4-hydroxy-L-threonine, L-aspartate, 4-pyridoxate, phosphatidylethanolamine, 2-oxoglutaramate Increased[b]: L-glutamine, creatineacetylglycine, serylserine, 1-acyl-sn-glycero3phosphocholine, ornithine, phosphatidylserine	Mitochondrial dysfunction, oxidative stress, energy metabolism, amino acid, vitamin, lipid metabolism
8	Kaluzna-Czaplinska et al. (2010)[230]	Urine	GC–MS	Increased[a]: urine homovanillic acid, vanilla mandelic acid	Neurotransmitter metabolism, visual perception/memory, repetitive behavior, emotional disorders
9	Mavel et al. (2013)[231]	Urine	^{1}H-^{13}C NMR	Decreased[b]: creatine, 3-methylhistidine Increased[b]: glycine, taurine, succinate, β-alanine	Taurine and succinic acid
10	Emond et al. (2013)[232]	Urine	GC–MS	Decreased[b]: 1H-indole-3-acetate, phosphate, palmitate, stearate, 3-methyladipate, hippurate, vanillylhydracrylate, 4-hydroxyphenyl-2-hydroxyacetate, 3-hydroxyphenylacetate Increased[b]: succinate, glycolate	Intestinal bacteria microbial pathways
11	Nadal-Desbarats et al. (2014)[233]	Urine	^{1}H-NMR, ^{1}H-^{13}C HSQC-NMR	Decreased[b]: glutamate, creatine, 3-methylhistidine Increased[b]: succinate	Energy metabolism disorder, mitochondrial dysfunction, amino acid metabolism of gut microbiota

(Continues)

TABLE 2 (Continued)

No.	Author	Sample	Method	Related metabolites	Metabolic process involved
12	Liu et al. (2019)[234]	Urine	LC–MS/MS	Decreased[a]: Lys, Thr, Car, Pro, EtN, Hcy, Aad, Cit, Ans, 5Ava, Asp Increased[a]: MetS, Harg, 3MHis, Cr, Arg, 5HT, Hyp	Oxidative stress, abnormal ornithine cycle, abnormal lysine metabolism, abnormal 5HT metabolism, E/I balance

Abbreviations: 3MHis, 3-methyl-histidine; 5Ava, 5-aminovaleric acid; 5HT, 5-hydroxytryptamine; Aad, α-aminoadipic acid; AA/BCAA, amino acids/branched-chain amino acid; AASA, α-aminoadipic semialdehyde; Ans, anserine; Arg, arginine; Asp, aspartic acid; Car, carnosine; Cit, citrulline; CMA, Nω-carboxymethylarginine; Cr, creatinine; E/I, excitation and inhibition; EtN, ethanolamine; FL, Nε-fructosyl-lysine; GC–MS, gas chromatography–mass spectrometry; G-H1, hydroimidazol one; GSA, glutamic semialdehyde; Harg, homoarginine; Hcy, homocysteine; HSQC-NMR, heteronuclear singular quantum correlation-nuclear magnetic resonance; Hyp, 4-hydroxyproline; LC–HRMS, liquid chromatography–tandem high-resolution mass spectrometry; LC–MS/MS, liquid chromatograph–tandem mass spectrometry; Lys, lysine; MetS, methionine sulfoxide; MRM, multiple reaction monitoring; NFK, N-formylkynurenine; Pro, proline; TD, typically developing; Thr, threonine.

[a]ASD compared to TD.

[b]ASD compared to Ctrl.

S-adenosylhomocysteine, nitric oxide, and copper were higher in children with ASD than in healthy controls, whereas GSH, tGSH, GSH/GSSG, tGSH/GSSG, methionine, cysteine, vitamin B9, vitamin D, vitamin B12, vitamin E, S-adenosylmethionine/S-adenosylhomocysteine, and calcium concentrations were decreased.[253] Given the consistent and large effective size, GSH metabolism biomarkers have the potential to inform early diagnosis of ASD.[253]

Biomarkers of oxidative stress associated with ASD have recently been reviewed.[254] GSH is an important antioxidant in the human body, it is converted to GSSG by GPx and reduced back to GSH by GSH reductase. Elevated levels of oxidative stress in ASD cause increased GSH depletion, which disrupts the dynamic balance between GSH and GSSG. The increased GSH/GSSG ratio is consistent with various pertinent studies, indicating that its efficacy as a reliable indicator of oxidative stress.[254] In addition, blood levels of vitamin B9 and B12 were significantly lower in children with autism than in controls,[253,255,256] and this deficiency resulted in decreased homocysteine remethylation and increased homocysteine levels. Vitamin B12 deficiency may lead to hypomethylation and affect brain development.[257] Vitamin deficiencies in children with ASD may be due to poor nutrition, poor digestion, and absorption, or dysbiosis of the intestinal flora.[95,254] These results clarified blood oxidative stress profile in children with ASD, strengthening clinical evidence of increased oxidative stress implicating in pathogenesis of ASD.

4.3.3 | Mitochondria-related diagnostic markers

A meta-analysis showed that the regulation of mitochondrial biomarkers (including lactate, pyruvate, carnitine, and ubiquinone) was decreased in ASD, and that some of these markers correlated with ASD severity.[177]

4.4 | Biomarkers associated with gut microbiota

Changes in the gut microbiota and metabolites may lead to changes in metabolites in blood and urine, providing an opportunity to develop diagnostic tests for early detection of ASD. For example, studies have shown that combining Veillonella and Enterobacteriaceae and 17 bacterial metabolic functions to create diagnostic models can effectively differentiate between ASD and healthy children.[258] Several studies have shown that high levels of p-cresol are detected in stool, blood, and urine of children with ASD.[18,224,259–262] Of interest, p-cresol is only produced in the gastrointestinal tract and correlates with autistic behavior and ASD severity.[263] In addition, other gut microbial metabolites including SCFAs, free amino acids, indoles, and lipopolysaccharides, have been detected in the blood and urine from children with ASD.[263,264] The analysis of gut microbes and the detection of microbial-derived metabolites in stool, as well as the detection of gut microbial-derived metabolites in blood and urine, may provide an alternative method for the early diagnosis of ASD and is worthy of initiating research (Table S2).

Overall, current research on diagnostic biomarkers for ASD suggests that despite the presence of heterogeneity in ASD, it is still possible to find diagnostic biomarkers. The mechanisms involved in the candidate diagnostic biomarkers identified in the existing studies are convergent. In the high-throughput screening stage, there is still a lack of unified research methods, especially unified experimental conditions, and some studies need to overcome the shortage of small sample sizes. The targeted detection methods is beneficial for the practical application and translation of potential diagnostic biomarkers. It may be a panel composed of biomarkers involved in different mechanisms, or biomarkers related to a certain type of important physiological and metabolic changes.

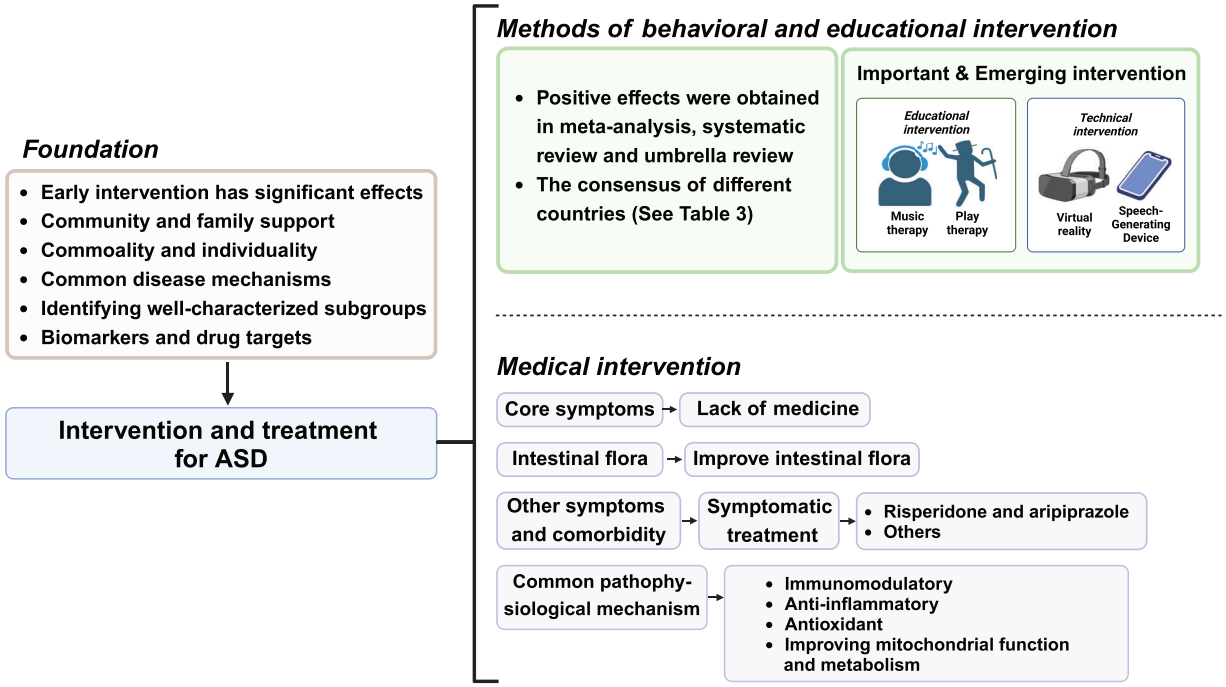


FIGURE 4 The summary of intervention therapy in autism spectrum disorder (ASD). Interventions for ASD mainly include behavioral and educational interventions, and we provide an overview of recent meta-analyses, reviews, and consensus on them, as well as other important and emerging interventions. In terms of pharmacologic interventions, there are still no medications that target the core symptoms, and drug treatment is mainly for other abnormal symptoms or neuropsychiatric comorbidities of ASD. Treatments for common pathophysiology and gut flora are under investigation. Overall, early intervention has a significant effect, with community and family support being important. Given the characteristics of ASD, intervention and treatment need to take into account both commonalities and individuality. Finding common disease mechanisms and identifying well-characterized subgroups will provide the basis for disease diagnosis and treatment, and disease markers and drug targets can influence and inform each other.

5 | INTERVENTION AND TREATMENT OF ASD

Early detection and early intervention are effective for ASD. To date, more than 100 interventions for ASD have been developed, but there is a lack of interventions that target their core symptoms (Figure 4). The goal of ASD treatment is to improve the individual's functioning and well-being. Intervention therapy is more effective in improving ASD-related symptoms (e.g., effective use of language) than ASD characteristics. Early interventions based on mature behavior analysis can help ASD acquire specific skills to address problem behaviors. Here, we reviewed recent meta-analyses, reviews, and consensus on intervention approaches, focusing on approaches that are evidence based and have positive outcomes in some respects (Table 3). In addition, there are many ASD interventions that overlap with each other in terms of operationalization, and there is a tendency for interventions to learn from and integrate with each other, and for each class of approaches to be divided into different "subcategories," as well as some important or emerging approaches (Figure 4). We also made a review in this section.

5.1 | Advances in behavioral and educational interventions

A recent review summarized evidence-supported intervention approaches, including behavioral approaches (e.g., early intensive behavioral intervention [EIBI], discrete trial training), developmental approaches (e.g., developmental, individual differences, relationship-based/Floortime model, preschool autism communication trial [PACT]), naturalistic developmental behavioral intervention (NDBI) (e.g., Early Start Denver Model [ESDM], pivotal response treatment [PRT], JASPER, Project ImPACT), treatment and education of autistic and related communication children (TEACCH), psychotherapy (cognitive behavioral therapy [CBT]), and group social skills interventions.[28] In this review, the authors highlight NDBI, parent-mediated interventions, CBT, and the fact that school-aged children with ASD can often receive behavioral, speech, integration, and physical therapy in the school setting.[28]

A recent umbrella review identified several psychosocial interventions that are expected to improve symptoms associated with ASD at different stages of life, such as

TABLE 3 Recommended behavioral and educational interventions for autism spectrum disorder.

No.	Author	Title	Recommended interventions
1	Xu et al. (2017)[265]	Expert consensus on early identification, screening and early intervention of children with autism spectrum disorders	① ABA ② TEACCH ③ ESDM ④ PRT ⑤ PACT ⑥ RIT ⑦ JA
2	Howes et al. (2018)[266]	Autism spectrum disorder: consensus guidelines on assessment, treatment, and research from the British Association for Psychopharmacology	① Psychological approaches: social learning program, behavioral and life-skills interventions, cognitive-behavioral interventions, facilitated communication ② Pharmacological treatment: serotonergic agents, glutamatergic agents, GABAergic agents, dopamine receptor blockers ③ Non-pharmacological approaches: social-communication interventions, behavioral interventions, alternative interventions
3	Sandbank et al. (2020)[267]	Project aim: autism intervention meta-analysis for studies of young children	① Behavioral approaches: EIBI, DTI, PECS, PBS, ABA ② Developmental approaches: DF, HM ③ NDBI: ESDM, EMT, PRT, JA, SP, EG, RG ④ TEACCH ⑤ Sensory-based interventions ⑥ Animal-assisted interventions: EAAT ⑦ Technology-based interventions: CAI, TTDVD
4	Hyman et al. (2020)[268]	Identification, evaluation, and management of children with autism spectrum disorder	① ABA ② Developmental relationship-focus intervention ③ NDBI ④ Parent-mediated treatment or training ⑤ Educational interventions: LEAP, TEACCH ⑥ Other therapeutic interventions: SLI, MT, HM
5	Gosling et al. (2022)[269]	Efficacy of psychosocial interventions for autism spectrum disorder: an umbrella review	① PMI ② TECH ③ SSG ④ DEV ⑤ CBT ⑥ NDBI ⑦ TEACCH
6	Hirota et al. (2023)[28]	Autism spectrum disorder: a review	① Behavioral approaches: EIBI, DTI ② Developmental approaches: FM, PACT ③ NDBI: ESDM, PRT, JASPER, PI ④ TEACCH ⑤ CBT ⑥ GSSIs ⑦ Pharmacological interventions: aripiprazole, risperidone, methylphe-nidate, atomoxetine, extended-release guanfacine, melatonin, oxytocin

Abbreviations: ABA, applied behavior analysis; CAI, computer-assisted instruction; CBT, cognitive behavioral therapy; DEV, developmental interventions; DF, DIR/Floortime; DTI, discrete tracking instruction; EAAT, equine-assisted activities and therapy; EG, engagement; EIBI, early intensive behavioral intervention; EMT, enhanced milieu teaching; ESDM, Early Start Denver Model; FM, Floortime model; GSSI, group social skills intervention; HM, Hanen models; JA, joint attention; JASPER, Joint Attention, Symbolic Play, Engagement and Regulation;LEAP, learning experiences and alternative programs for preschoolers and their parents; MT, motor therapies; NDBI, naturalistic developmental behavioral intervention; PACT, preschool autism communication trial; PBS, positive behavioral supports; PECS, picture exchange communication system; PI, parent involvement; PMI, parent-mediated interventions; PRT, pivotal response treatment; RG, regulation; RIT, reciprocal imitation training; SLI, speech and language interventions; SP, symbolic play; SSG, social skill groups; TEACCH, treatment and education of autistic and related communication children; TECH, technology-mediated interventions; TTDVD, the transportersTM DVD series.

early reinforcement behavioral interventions, developmental interventions, natural developmental behavioral interventions, and parent-mediated interventions that improve social communication deficits, overall cognitive abilities, and adaptive behaviors in children with ASD in preschool-age children. The effectiveness of social skills groups in improving social communication deficits and overall ASD symptoms in school-aged children and adolescents is supported by suggestive evidence.[269] Another umbrella review identified positive therapeutic effects of behavioral interventions, developmental interventions, NDBI, technology-based interventions, and CBT for several child and family outcomes.[270]

Moreover, a recent systematic review and meta-analysis summarized the effects of seven early intervention types (behavioral, developmental, NDBI, TEACCH, sensory based, animal assisted, and technology based, aged between 0 and 8 years).[267] Of these, significant positive effects were found for behavioral, developmental, and NDBI intervention types. When effect size estimates were limited to studies with a randomized controlled trial (RCT) design, there was evidence of positive summary effects for developmental and NDBI intervention types only. When effect estimates were limited to RCT designs and outcomes without detectable risk of bias, no intervention type showed a significant effect on any outcome.[267] Together, despite the availability of multiple intervention models for children with ASD, many have still failed to demonstrate effectiveness in clinical trials. More well-designed RCTs are still needed to gain a clearer understanding of the efficacy of these interventions[269,271] (Table 4).

When developing a consensus, Chinese experts selected and recommended methods that are supported by randomized controlled studies, have a high level of evidence-based medical evidence, and have a recommendation rating of "strongly recommended" for children with ASD under the age of 3 years and are eligible for implementation in China. The early intervention methods that are supported by randomized controlled studies have evidence-based ratings and "strongly recommended" ratings for children with ASD under 3 years of age and are eligible for implementation in China, including ESDM, PRT, PACT, reciprocal imitation training, and joint attention (JA) training.[265]

Furthermore, it is also worth mentioning a recent report by the Lancet Commission, which states that individualized, stepped care strategies can meet an individual's needs throughout the life course, leading to effective assessment and care. The importance of community and family supports in lifelong intervention and treatment for individuals with autism. It further describes the broad spectrum of autism and introduces the concept of "profound autism"; that is, "profound autism" should be paid attention to.[285]

5.2 | Methods of behavioral intervention

5.2.1 | Applied behavior analysis

Over the past decades, applied behavior analysis (ABA) has been at the forefront of these interventions and has been recommended as a scientifically validated intervention in different countries.[286] Due to its high level of acceptance, ABA interventions have also become the benchmark for existing and subsequently developed interventions. In most studies, this approach has shown positive improvements in cognition, language development, social skills and communication, and adaptive behavior in children with ASD, along with reductions in problem behaviors.[287]

EIBI was the first intensive ABA therapy proposed for ASD, focusing on eliminating atypical behaviors and building learning capacity. Since then, treatments for ASD have weakened structural features while focusing on more complex cognitive and social skills.[288,289] The EIBI model relies heavily on discrete tracking instruction (DTI), which focuses on reducing extraneous details and teaching skills and learning content in a repetitive and streamlined manner. Ongoing data collection and analysis are key components of DTI,[290,291] and these data are an important reference for determining how quickly children progress and whether program modifications are needed. In general, DTI is more appropriate for developing JA, play, or imitation skills in children around 2 years of age,[292] and may also be of shorter duration as conditions improve to address more complex social behaviors.[293]

One of the earliest alternative forms of ABA for ASD was the Natural Language Paradigm (NLP), the earliest natural language training strategy,[294] whose main advantage was the integration of therapy into natural, ongoing social, and play activities. PRT[295] and ESDM are the naturalistic language strategies with the most empirical evidence to support their effectiveness. As an extension of NLP, the training goals of PRT focus on motivation to interact with others, self-management, self-regulation, and response to multiple cues.[296] Its validity has been supported by several studies.

5.2.2 | Physical exercise

Studies have found that children with ASD spend significantly less time per day participating in moderate to vigorous physical activity compared to normally growing children.[297] Physical activity of appropriate intensity is a remedy to reduce physical–motor deficits, stereotypic and aggressive behaviors, and improve cognitive functioning in individuals with ASD.[298–300] In recent years, there has been an explosion of systematic evaluations and

TABLE 4 Related clinical trials related to interventions for autism spectrum disorders (ASD).

No.	Author	Study type	Clinical trial number	Sample size	Conclusion
1	Gabriels et al. (2015)[272]	Retrospective case	NCT 02301195	116	The study further establishes the evidence base supporting EAAT as a viable therapeutic option for children and adolescents with ASD. Further research is needed to examine the joint attention and movement experiences are key THR mechanisms to observe behavioral and social communication improvements in the ASD population.
2	Bearss et al. (2015)[273]	Retrospective case	NCT 01233414	30	Significant improvement (>12 units) in two patients and minor improvement (8–12 units) in eight patients.
3	Bieleninik et al. (2017)[274]	Retrospective case	ISRCTN 78923965	167	CBT was efficacious for children with ASD and interfering anxiety, an adapted CBT approach showed additional advantages. CBT can be considered as a professional reference for psychological treatment of autistic children.
4	Sharda et al. (2018)[275]	Retrospective case	ISRCTN 26821793	51	The study provides the first evidence that 8—12 weeks of individual music intervention can indeed improve social communication and function brain connectivity.
5	Grimaldi et al. (2018)[276]	Retrospective case	NCT 02720900	61	After 1 week of medication, all patients had significant improvements in abnormal behavior and irritability scores, with the risperidone group showing significant improvement at each assessment period.
6	DeVane et al. (2019)[277]	Retrospective case	NCT 01333072	364	ASD children who underwent improvisational music therapy and enhanced standard care showed improvement in scale assessment results, but compared with the two methods there was no significant difference in symptom severity based on the ADOS social affect domain over 5 months, indicating that the effect of using improvisational music therapy to reduce symptoms in ASD children was not significant.
7	Voss et al. (2019)[278]	Retrospective case	NCT 03569176	71	In terms of socialization, children who received the wearable intervention improved significantly than those who received only standard-of-care behavioral treatments, indicating potential for digital home therapy.
8	Malow et al. (2020)[279]	Retrospective case	NCT 01906866	80	Nightly pediatric prolonged-release melatonin at optimal dose of 2, 5, or 10 mg is safe and effective for long-term treatment in children and adolescents with ASD and insomnia, which has no detrimental effects on children's growth and pubertal development.
8	Wood et al. (2020)[280]	Retrospective case	NCT 02028247	150	A whole-plant extract BOL-DP-O-01-W which contains CBD and THC in a 20:1 ratio improved disruptive behaviors on one primary outcome measures and on a secondary outcome, an index of ASD core symptoms, with acceptable adverse events.
9	Sikich et al. (2021)[281]	Retrospective case	NCT 01944046	277	In this trial involving children and adolescents with ASD, 24 weeks of daily intranasal oxytocin treatment, as compared with placebo, did not improve social interaction or other measures of social function related to ASD.

(Continues)

TABLE 4 (Continued)

No.	Author	Study type	Clinical trial number	Sample size	Conclusion
10	Aran et al. (2021)[282]	Retrospective case	NCT 02956226	30	Children on exclusion diets were less likely to report gastrointestinal abnormalities and had lower abundance of the Bifidobacterium and Veillonellaceae families but higher presence of Faecalibacterium and Bacteroidetes. A combined dietary approach resulted in significant changes in gut microbiota composition and metabolism.
11	Scahill et al. (2022)[283]	Retrospective case	NCT 02483910	83	On CELF, DI + TAU did not meet the prespecified difference from TAU. When adjusted for IQ, DI + TAU was superior to TAU on CELF at end point. DI + TAU was superior to TAU on CGI-I.
12	Chu et al. (2023)[284]	Retrospective case	ChiCTR 2100053165	78	Potentially positive effects of nonwearable digital therapy plus LSP on core symptoms associated with ASD were found in the study, which leading to a modest improvement in the function of sensory, motor and response inhibition, while reducing impulsivity and hyperactivity in preschoolers with both ASD and ADHD, and VR-CBT was found to be an effective and feasible adjunctive digital tool.

Abbreviations: ADHD, attention deficit hyperactivity disorder; ADOS, autism diagnostic observation schedule; CBD, cannabidiol; CBT, cognitive behavioral therapy; CELF, clinical evaluation of language fundamentals; CGI-I, clinical global impressions-improvement scale; DI, direct instruction language for learning; EAAT, equine-assisted activities and therapies; LSP, learning style profile; TAU, treatment as usual; THC, tetrahydrocannabinol; THR, therapeutic horseback riding; VR-CBT, virtual reality-incorporated cognitive behavioral therapy.

meta-analyses of exercise interventions on stereotypic behaviors, executive functions, and cognitive abilities in children and adolescents with ASD.[298,299,301–303] Stereotypical behavior patterns of individuals with ASD are alleviated through exercise intervention.[304,305] It is also beneficial to enhance overall cognitive flexibility and inhibitory control,[301] and reduce the deficits of social interaction.[299] Different types of exercise all play a positive role in alleviating stereotypical behaviors in people with ASD.[306–318] Although the molecular mechanisms involved in the beneficial effects of exercise on ASD remission are still unknown, the thesis that cytokines released after exercise play an important role in regulating neuronal metabolism, neuroinflammation, and neuroplasticity has been confirmed,[316,319,320] which may be related to the improvement of symptoms in children with ASD and associated comorbidities.[316]

5.3 | Methods of educational intervention

5.3.1 | Music therapy intervention

There is a long history of using music or music therapy services for non-musical goals (including social skills) for people with ASD.[321] Currently, most music therapy applications for ASD are focused on children and adolescents; they are thought to have positive effects on social skills, including engagement behaviors,[322] increased emotional involvement,[323] improved social interactions,[324,325] increased social greeting routines,[326] JA behaviors,[327,328] peer interactions,[329] communication skills,[274,330,331] and cognitive social skills.[332]

There are also differences in the effectiveness of different types of music therapy for people with ASD. Improvisational music therapy (IMT) is one of the most studied music therapies for children with ASD.[327,333–336] Family-centered music therapy as an important variant of IMT improves social interactions in families, communities, and parent–child relationships.[337] However, studies have also reported contradictory results or no improvement in some areas.[338,339] Nevertheless, the feasibility of music therapy interventions for children with ASD has received preliminary support, at least in terms of improving social interaction, verbal communication, initiating behavior, and social–emotional reciprocity.

5.3.2 | Play therapy intervention

Providing children with ASD the opportunity to engage in play activities can strengthen their connections with others and improve social interaction deficits. Patient-centered play therapy is considered an effective evidence-based intervention to improve core issues related to ASD, such

as social skills, communication, emotion regulation, and JA,[340–343] while a reduction in repetitive behaviors is a strong reason for the validation of the effectiveness of play therapy.[344]

5.3.3 | Family involved intervention

Research has shown that involving parents in interventions reinforces the effectiveness of the intervention and the prevalence of skills outside of the school setting.[345] Family–school partnerships (FSPs) are a child-centered approach, where families and school collaborate and coordinate to produce positive student outcomes in the social, emotional, behavioral, and academic domains.[346,347] Active parental involvement in education and intervention can have a significant impact on children's learning and development, children's cognitive and language skills,[348] school participation, academic achievement,[349] and children's problem-solving skills can be improved and enhanced.[350] In addition, parental involvement can lead to positive outcomes in prosocial behavior,[351] peer interaction, and self-regulatory skills.[352] In addition to the FSPs mentioned above, parent involvement (PI) is also applicable to family-level interventions and education for children with ASD. Unlike FSP, PI focuses more on the structure and process of activities.[353,354] Numerous studies have shown that in children with ASD, improvements in social communication and reductions in restrictive and repetitive behaviors occur after interventions using the PI model.[355–357]

5.4 | The interventions derived from technical devices for ASD

With the rapid development of modern technology, a number of assistive devices for the rehabilitation of people with autism have been developed and put into use. These devices have shown some effectiveness in ASD interventions and deserve further study and evaluation.

5.4.1 | Speech-generating device intervention application

Speech-generating device (SGD) is a portable electronic device that displays various graphic symbols or written language and generates digital or synthetic speech.[356,357] For children with ASD, whose communication skills are severely lacking, the motor skills tolerance of SGD, the popularity of the output language, and the large storage space make it more socially acceptable.[358,359] At the same time, the SGD's ability to request, tag, comment, and answer questions extends its scope of application.[360] Previous studies have shown that SGD can improve participants' communication skills,[358,361,362] while the acquisition of communication skills is a top priority in early intervention programs for ASD.

5.4.2 | Virtual reality technology application

Virtual reality (VR) is a realistic and immersive three-dimensional virtual environment created by interactive software and hardware and is a product of multidisciplinary integration. With the increasing sophistication of VR technology, researchers have successfully applied it to the treatment of people with autism.[363–368] On this basis, immersive virtual reality has been developed, which is able to reproduce real objects and scenes to a higher degree.[369–372] However, VR still has some shortcomings, such as the current VR technologies used in ASD treatment are homogeneous and usually can target only one characteristic, and VR simulation scenes are still different from reality. It is expected that VR technology will continue to overcome its limitations and meet the individual needs of people with autism.

5.4.3 | Social bots' application

In contrast to VR, another more tangible technological development, humanoid robot, is also being used for the treatment of ASD. There is growing evidence that robotic assistance has a positive effect on the improvement of the condition of individuals with ASD.[373–377] Unlike humans, robots operating in predictable and legitimate systems provide a highly structured learning environment for people with ASD, enabling structured and standardized interventions that will help them focus on relevant stimuli, and certain social behaviors may be simulated in the standardized social contexts created by such structured interactions.[378,379] In the field of autism, there are precedents for the use of robots to assist in the diagnostic process, improve eye contact and spontaneous interaction, turn-taking activities, mimicry, emotion recognition, JA, triadic interactions, etc.[373,376,380] The results of an induction training for people with ASD involving android robots are also encouraging and make other approaches to intervention using robots worth trying.[381]

6 | MEDICAL INTERVENTION AND POTENTIAL DRUG TARGET

Currently, there is still a lack of drugs to treat the core symptoms of ASD, and research on them is difficult.[382,383]

Here, we provide an overview of existing pharmacologic therapies for ASD as well as those that target its common pathophysiology and gut microbiota. With the rapid growth of genomics and systems neuroscience, a variety of new molecular targets are surfacing.[384]

6.1 | Drug treatment for ASD

There are currently no medications available worldwide that specifically target the core symptoms of ASD. More commonly, existing antipsychotics are used to alleviate anxiety,[385] depression,[386,387] or obsessive–compulsive disorder[388] in order to ameliorate certain symptoms of ASD, such as ADHD.[389,390] The US Food and Drug Administration (FDA) has approved two medications, risperidone and aripiprazole, for the pharmacologic treatment of ASD-related irritability and aggression.[391] However, while there are medications that can alleviate several specific conditions of ASD, the side effects should not be underestimated. For example, aripiprazole can cause side effects, such as drowsiness/sedation, increased sleep duration, and weight gain.[392] In addition, selective 5-hydroxytryptamine reuptake inhibitors have been approved by the FDA for a wide range of other disorders, and as a result they are frequently and increasingly used in the treatment of ASD.[393] A recent review based on RCTs suggests that the following medications improve at least one core symptom area compared to placebo: aripiprazole, atoxetine, bumetanide, and risperidone for children/adolescents, and fluoxetine, fluvoxamine, oxytocin, and risperidone for adults.[394,395]

Consequently, finding common mechanisms to screen for access to targeted drugs remains important and possible.[396,397] For example, and clinical trials are attempting to use the GABAergic system as a therapeutic strategy for ASD,[396] and a recent study showed that a clinically relevant selective ERK pathway inhibitor reverses the core deficits in a mouse model of autism.[397]

6.2 | Interventions and treatments related to common pathological mechanisms

Given that inflammation and immunity, oxidative stress, and mitochondrial dysfunction are common pathophysiological mechanisms of ASD, there are a number of proposals and studies targeting them, including antioxidant, anti-inflammatory, immunomodulatory, and improving mitochondrial function and metabolism.[398–400]

Several clinical studies on antioxidant therapy for ASD have been reported, including radicicicol,[401] resveratrol,[402] coenzyme Q10,[403] N-acetylcysteine (NAC),[404] omega-3 fatty acids,[405] arachidonic acid, and docosahexaenoic acid (DHA),[406] all of which showed beneficial effects except for resveratrol, whose role is uncertain. Of these, NAC appears to be the most effective antioxidant therapy.[400] In addition, some studies have demonstrated that supplementation with micronutrients related to redox metabolism (e.g., methyl B12) can be helpful for children with autism.[407] Other studies have evaluated antioxidant-rich foods, including broccoli,[408] camel's milk,[409] and dark chocolate.[410] Notably, there are antioxidants, such as radicicchioidin, resveratrol, naringenin, curcumin, and guanidinium that are not only antioxidants, but also activators of Nrf2, a transcription factor involved in immune dysregulation, inflammation, oxidative stress, and mitochondrial dysfunction.[411]

However, many of the oxidative stress treatment groups in the study showed strong individual differences, reflecting the heterogeneity of ASD.[412] Therefore, assessing and identifying physiological changes associated with ASD and taking targeted and personalized interventions are more likely to produce positive treatment outcomes.[398,412] As mentioned in a recent review,[398] folic acid supplementation has a positive effect in individuals with ASD identified by autoantibodies to the folate receptor,[413] whereas methylcobalamin has significant clinical utility when impaired methylation capacity.[414,415] Mitochondrial regulatory cofactors should be considered when mitochondrial dysfunction is evident. Multivitamin/multimineral formulas, as well as biotin, appear to be appropriate when metabolic abnormalities have been identified, as well as the use of low-dose suramin antipurinergic therapy.[416]

In addition, many antioxidant molecules available in nature show anti-inflammatory activity.[417] Some natural antioxidants have been carried out in human studies, such as GSH, vitamin C, NAC, flavonoids, luteolin, quercetin, rutin,[418,419] palmitoylethanolamide and luteolin,[420] DHA, eicosapentaenoic acid (EPA),[421] and Ginkgo biloba extract 761.[422] The most common of the inflammatory signaling pathways is nuclear factor-κB (NF-κB), MAPK, and JAK–STAT pathways.[423] Several preclinical studies have been initiated targeting these pathways, including resveratrol,[424] palmitoylethanolamide, and luteolin[420] against the NF-κB pathway, and luteolin, diosmine,[425] and quercetine[426] against the janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway, as well as IL-17A antibody against ERK/MAPK pathway.[427,428]

Marchezan et al. classified immune and inflammatory interventions for ASD into two broad categories: (1) using radicicicol, celecoxib, lenalidomide, hexacosanolide, spironolactone, flavonoid lignocerotonin, corticosteroids, oral immunoglobulins, intravenous immunoglobulins, and cellular therapy. (2) Other ASD therapies that have been used or are being studied that are initially

characterized as neither anti-inflammatory nor immunomodulatory at first, but exhibit immunomodulatory capabilities throughout the course of treatment: risperidone, vitamin D, omega-3, ginkgo biloba, l-creatinine, n-acetylcysteine, and microbiome recovery.[429] Another narrative review of randomized controlled placebo trials summarizes how immunomodulatory/anti-inflammatory therapeutic agents such as prednisolone, pregnenolone, celecoxib, minocycline, n-acetylcysteine, radicicic acid, and/or omega-3 fatty acids may be useful in the core management of (e.g., stereotypic behaviors) and related (e.g., irritability, hyperactivity, lethargy) symptoms in individuals with autism.[430] Likewise, a review based on RCTs concluded that myostatin, haloperidol, folinic acid, guanfacine, omega-3 fatty acids, probiotics, radicicic acid, sodium alginate, and sodium valproate showed some signs of improvement, but were imprecise and unreliable.[431]

Overall, among the several intervention approaches described above, attention needs to be paid to individualization, targeting interventions to subgroups of ASDs with associations with these pathophysiological mechanisms, and improving the efficacy of interventions.[412,432] The literature on intervention efficacy is limited, and large-scale RCTs are still needed to provide strong evidence as well as the use of biomarkers.[430]

6.3 | Interventions for ASD targeting the microbiota-gut-brain axis

As research into the mechanisms associated with gut microbial imbalance and the development of ASD has intensified, probiotics, prebiotics, fecal microbiota transplantation (FMT), microbiota transfer therapy, antibiotics, and diet dietary adjustment methods received considerable attention.[433] The beneficial effects of probiotics in improving mood and regulating host behavior have been explored, with specific probiotic therapy reducing the severity of ASD symptoms and developing strategies to manage typical social impairment, communication disorders, perceptual impairment, and behavioral limitations.[434–439] FMT has been shown to be an established and effective treatment for recurrent *Clostridium difficile* infection.[440] It has also been proposed as a safe and effective strategy to modulate the symbiosis of the gastrointestinal microbiota and improve behavioral symptoms in children with ASD.[440,441] Recently, a study showed that FMT improved VPA-induced ASD mice by modulating 5-hydroxytryptaminergic and glutamatergic synaptic signaling pathways.[442] Modified FMT therapy for children with ASD resulted in significant improvements in gastrointestinal symptoms and ASD symptoms, and follow-up of these individuals after 2 years showed that most of the improvements in gastrointestinal symptoms were maintained, including significant increases in bacterial diversity and relative abundance of beneficial bacteria such as bifidobacterial.[440,443] However, due to the complexity of the intestinal microbiota, FMT therapies are still highly heterogeneous with respect to donor selection, material preparation, ideal dosing regimen, and cost-effectiveness, and are still a long way from clinical application. In addition, although these therapeutic modalities have been shown to be safe and effective for short-term supplementation, the safety over long periods of time remains uncertain needs to be validated by additional studies.[195] Overall, autism interventions targeting the gut–brain axis have the potential to be an effective treatment for ASD and are expected to have a positive effect on the improvement of ASD symptoms.[444]

7 | CONCLUSION AND PERSPECTIVES

ASDs have become a common neurological developmental disorder in children. Early detection and early intervention are highly effective. Heterogeneity is a distinctive feature of children with ASD. In addition to the core symptoms, children with ASD are accompanied by different behavioral abnormalities and comorbidities with varying degrees of severity, which exacerbates its complexity and poses challenges for its research and clinical translation.

In this paper, based on the review of the pathological mechanisms of ASD, the progress of its diagnostic markers and intervention methods are reviewed. ASD is caused by genetic and environmental factors and their interactions, its signaling pathways, and mechanisms are convergent. Oxidative stress, inflammation and immunity, mitochondrial dysfunction, and intestinal flora dysregulation are common pathophysiological mechanisms, and they are interrelated. There are also common mechanisms between ASD and comorbidities. These provide the basis for the diagnosis and treatment, at least on a stratified or subclass-based basis. Stratified biomarkers are objective measures used to define subgroups of individuals with common biological characteristics. The treatment and management of children with ASD often involves the management of associated medical problems and psychopathological comorbidities. Therefore, it is important to consider both commonalities and individuality in diagnosis, treatment, and intervention of ASD. Priority is given to personalized diagnosis and treatment for different individuals to improve the precision and efficacy of ASD diagnosis, treatment, and rehabilitation. With the application of high-throughput omics, such as genomics, proteomics, metabolomics, transcriptomics, as well as in-depth mechanism studies, it is expected to find common mechanisms

among individuals with ASD subjects and find specific early diagnostic biomarkers and drug therapeutic targets, which is a key research direction in the future. In terms of ASD intervention and treatment, large-scale RCT-based clinical studies need to be strengthened. In this context, maximizing the sample pools, designing studies with more diverse populations, increasing the number of subjects in RCTs, and defining more accurate patient codifies using gold standard diagnostic instruments are common themes for future developments in the field.

In summary, ASD is a highly heterogeneous, and it is particularly important to improve the understanding of the biological basis of the inherent heterogeneity of ASD, to search for potential common or convergent mechanisms, and to explore the "homogeneity" within the "heterogeneity." On this basis, the development of diagnosis, intervention and treatment is an effective way to achieve accurate diagnosis and treatment of ASD.

AUTHOR CONTRIBUTIONS

All authors were involved in the conceptualization and design of this paper. Preparation of relevant materials, data collection, and analysis were also performed by all authors. The first draft of the manuscript was written by H.Z., Z.L., G.M., and A.Q. L.S., X.R., X.L., X.Y., and C.F. undertook the revision of the manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGMENTS

We are grateful to the BioRender website (https://www.biorender.com/) and PowerPoint for providing the image materials. We also thank the Instrument Analysis Center of Shenzhen University. This work was jointly supported by the National Natural Science Foundation of China (No. 31870825), the Shenzhen-Hong Kong Institute of Brain Science-Shenzhen Fundamental Research Institutions (No. 2023SHIBS0003), and the Shenzhen Bureau of Science, Technology, and Information (No. JCYJ20170412110026229).

CONFLICT OF INTEREST STATEMENT

All authors read and approved the final manuscript and declare they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data availability is not applicable to this review as no new data were created or analyzed in this study.

ETHICS STATEMENT

Not applicable.

ORCID

Hongbin Zhuang https://orcid.org/0000-0003-2661-3182
Liming Shen https://orcid.org/0000-0003-0074-8556

REFERENCES

1. Kanner L. Autistic disturbances of affective contact. *Acta Paedopsychiatr*. 1968;35(4):100-136.
2. Asperger H. Die "Autistischen psychopathen" im kindesalter. *Arch Psychiat Nervenkrankheiten*. 1944;117(1):76-136.
3. Wing L, Gould J. Systematic recording of behaviors and skills of retarded and psychotic children. *J Autism Child Schizophr*. 1978;8(1):79-97.
4. APA. Diagnostic and Statistical Manual of Mental Disorders. *American Psychiatric Association*; 2013.
5. Fombonne E. Editorial: the rising prevalence of autism. *J Child Psychol Psychiatry*. 2018;59(7):717-720.
6. Lyall K, Croen L, Daniels J, et al. The changing epidemiology of autism spectrum disorders. *Annu Rev Public Health*. 2017;38:81-102.
7. Zeidan J, Fombonne E, Scorah J, et al. Global prevalence of autism: a systematic review update. *Autism Res*. 2022;15(5):778-790.
8. Maenner MJ, Shaw KA, Bakian AV, et al. Prevalence and characteristics of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 Sites, United States, 2018. *MMWR Surveill Summ*. 2021;70(11):1-16.
9. Maenner MJ, Warren Z, Williams AR, et al. Prevalence and characteristics of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 Sites, United States, 2020. *MMWR Surveill Summ*. 2023;72(2):1-14.
10. Zhou H, Xu X, Yan W, et al. Prevalence of autism spectrum disorder in China: a nationwide multi-center population-based study among children aged 6 to 12 years. *Neurosci Bull*. 2020;36(9):961-971.
11. Zhang ZC, Han J. The first national prevalence of autism spectrum disorder in China. *Neurosci Bull*. 2020;36(9):959-960.
12. Tick B, Bolton P, Happé F, Rutter M, Rijsdijk F. Heritability of autism spectrum disorders: a meta-analysis of twin studies. *J Child Psychol Psychiatry*. 2016;57(5):585-595.
13. Constantino JN, Zhang Y, Frazier T, Abbacchi AM, Law P. Sibling recurrence and the genetic epidemiology of autism. *Am J Psychiatry*. 2010;167(11):1349-1356.
14. Palmer N, Beam A, Agniel D, et al. Association of sex with recurrence of autism spectrum disorder among siblings. *JAMA Pediatr*. 2017;171(11):1107-1112.
15. Miles JH, Takahashi TN, Bagby S, et al. Essential versus complex autism: definition of fundamental prognostic subtypes. *Am J Med Genet A*. 2005;135(2):171-180.
16. Cohen D, Pichard N, Tordjman S, et al. Specific genetic disorders and autism: clinical contribution towards their identification. *J Autism Dev Disord*. 2005;35(1):103-116.
17. Fakhoury M. Autistic spectrum disorders: a review of clinical features, theories and diagnosis. *Int J Dev Neurosci*. 2015;43:70-77.
18. Shen L, Liu X, Zhang H, Lin J, Feng C, Iqbal J. Biomarkers in autism spectrum disorders: current progress. *Clin Chim Acta*. 2020;502:41-54.
19. Won H, Mah W, Kim E. Autism spectrum disorder causes, mechanisms, and treatments: focus on neuronal synapses. *Front Mol Neurosci*. 2013;6:19.

20. Voineagu I, Wang X, Johnston P, et al. Transcriptomic analysis of autistic brain reveals convergent molecular pathology. *Nature*. 2011;474(7351):380-384.
21. Parikshak NN, Swarup V, Belgard TG, et al. Genome-wide changes in lncRNA, splicing, and regional gene expression patterns in autism. *Nature*. 2016;540(7633):423-427.
22. Velmeshev D, Magistri M, Mazza EMC, et al. Cell-type-specific analysis of molecular pathology in autism identifies common genes and pathways affected across neocortical regions. *Mol Neurobiol*. 2020;57(5):2279-2289.
23. Velmeshev D, Schirmer L, Jung D, et al. Single-cell genomics identifies cell type-specific molecular changes in autism. *Science*. 2019;364(6441):685-689.
24. Paulsen B, Velasco S, Kedaigle AJ, et al. Autism genes converge on asynchronous development of shared neuron classes. *Nature*. 2022;602(7896):268-273.
25. Masi A, DeMayo MM, Glozier N, Guastella AJ. An overview of autism spectrum disorder, heterogeneity and treatment options. *Neurosci Bull*. 2017;33(2):183-193.
26. Jeste SS, Geschwind DH. Disentangling the heterogeneity of autism spectrum disorder through genetic findings. *Nat Rev Neurol*. 2014;10(2):74-81.
27. Rosen TE, Mazefsky CA, Vasa RA, Lerner MD. Co-occurring psychiatric conditions in autism spectrum disorder. *Int Rev Psychiatry*. 2018;30(1):40-61.
28. Hirota T, King BH. Autism spectrum disorder: a review. *JAMA*. 2023;329(2):157-168.
29. Howlin P, Goode S, Hutton J, Rutter M. Savant skills in autism: psychometric approaches and parental reports. *Philos Trans R Soc Lond B Biol Sci*. 2009;364(1522):1359-1367.
30. Geschwind DH. Advances in autism. *Annu Rev Med*. 2009;60:367-380.
31. Werling DM, Geschwind DH. Sex differences in autism spectrum disorders. *Curr Opin Neurol*. 2013;26(2):146-153.
32. Carayol J, Schellenberg GD, Dombroski B, Genin E, Rousseau F, Dawson G. Autism risk assessment in siblings of affected children using sex-specific genetic scores. *Mol Autism*. 2011;2(1):17.
33. Steeb H, Ramsey JM, Guest PC, et al. Serum proteomic analysis identifies sex-specific differences in lipid metabolism and inflammation profiles in adults diagnosed with Asperger syndrome. *Mol Autism*. 2014;5(1):4.
34. Sato D, Lionel AC, Leblond CS, et al. SHANK1 deletions in males with autism spectrum disorder. *Am J Hum Genet*. 2012;90(5):879-887.
35. Tropeano M, Ahn JW, Dobson RJ, et al. Male-biased autosomal effect of 16p13.11 copy number variation in neurodevelopmental disorders. *PLoS One*. 2013;8(4):e61365.
36. Tropeano M, Howley D, Gazzellone MJ, et al. Microduplications at the pseudoautosomal SHOX locus in autism spectrum disorders and related neurodevelopmental conditions. *J Med Genet*. 2016;53(8):536-547.
37. Mitra I, Tsang K, Ladd-Acosta C, et al. Pleiotropic mechanisms indicated for sex differences in autism. *PLoS Genet*. 2016;12(11):e1006425.
38. Wing L. Sex ratios in early childhood autism and related conditions. *Psychiatry Res*. 1981;5(2):129-137.
39. Kreiser NL, White SW. ASD in females: are we overstating the gender difference in diagnosis? *Clin Child Fam Psychol Rev*. 2014;17(1):67-84.
40. Tsai L, Stewart MA, August G. Implication of sex differences in the familial transmission of infantile autism. *J Autism Dev Disord*. 1981;11(2):165-173.
41. Goin-Kochel RP, Abbacchi A, Constantino JN. Lack of evidence for increased genetic loading for autism among families of affected females: a replication from family history data in two large samples. *Autism*. 2007;11(3):279-286.
42. Messinger DS, Young GS, Webb SJ, et al. Early sex differences are not autism-specific: a Baby Siblings Research Consortium (BSRC) study. *Mol Autism*. 2015;6:32.
43. Gazzellone MJ, Zhou X, Lionel AC, et al. Copy number variation in Han Chinese individuals with autism spectrum disorder. *J Neurodev Disord*. 2014;6(1):34.
44. Rosti RO, Sadek AA, Vaux KK, Gleeson JG. The genetic landscape of autism spectrum disorders. *Dev Med Child Neurol*. 2014;56(1):12-18.
45. Lombardo MV, Barnes JL, Wheelwright SJ, Baron-Cohen S. Self-referential cognition and empathy in autism. *PLoS One*. 2007;2(9):e883.
46. Damaj L, Lupien-Meilleur A, Lortie A, et al. CACNA1A haploinsufficiency causes cognitive impairment, autism and epileptic encephalopathy with mild cerebellar symptoms. *Eur J Hum Genet*. 2015;23(11):1505-1512.
47. Fernandes JM, Cajão R, Lopes R, Jerónimo R, Barahona-Corrêa JB. Social cognition in schizophrenia and autism spectrum disorders: a systematic review and meta-analysis of direct comparisons. *Front Psychiatry*. 2018;9:504.
48. Chen G, Yu B, Tan S, et al. GIGYF1 disruption associates with autism and impaired IGF-1R signaling. *J Clin Invest*. 2022;132(19):e159806.
49. Goh S, Peterson BS. Imaging evidence for disturbances in multiple learning and memory systems in persons with autism spectrum disorders. *Dev Med Child Neurol*. 2012;54(3):208-213.
50. Connolly S, Anney R, Gallagher L, Heron EA. Evidence of assortative mating in autism spectrum disorder. *Biol Psychiatry*. 2019;86(4):286-293.
51. Ballester P, Richdale AL, Baker EK, Peiró AM. Sleep in autism: a biomolecular approach to aetiology and treatment. *Sleep Med Rev*. 2020;54:101357.
52. Abdul F, Sreenivas N, Kommu JVS, et al. Disruption of circadian rhythm and risk of autism spectrum disorder: role of immune-inflammatory, oxidative stress, metabolic and neurotransmitter pathways. *Rev Neurosci*. 2022;33(1):93-109.
53. Yenen AS, Çak HT. Melatonin and circadian rhythm in autism spectrum disorders. *Turk Psikiyatri Derg*. 2020;31(3):201-211.
54. Ganesan H, Balasubramanian V, Iyer M, et al. mTOR signalling pathway—a root cause for idiopathic autism? *BMB Rep*. 2019;52(7):424-433.
55. Zhang J, Zhang JX, Zhang QL. PI3K/AKT/mTOR-mediated autophagy in the development of autism spectrum disorder. *Brain Res Bull*. 2016;125:152-158.
56. Yeung KS, Tso WWY, Ip JJK, et al. Identification of mutations in the PI3K-AKT-mTOR signalling pathway in patients with macrocephaly and developmental delay and/or autism. *Mol Autism*. 2017;8:66.

57. Costa-Mattioli M, Monteggia LM. mTOR complexes in neurodevelopmental and neuropsychiatric disorders. *Nat Neurosci*. 2013;16(11):1537-1543.
58. Niere F, Namjoshi S, Song E, et al. Analysis of proteins that rapidly change upon mechanistic/mammalian target of rapamycin complex 1 (mTORC1) repression identifies Parkinson protein 7 (PARK7) as a novel protein aberrantly expressed in tuberous sclerosis complex (TSC). *Mol Cell Proteomics*. 2016;15(2):426-444.
59. Lombardo MV, Moon HM, Su J, Palmer TD, Courchesne E, Pramparo T. Maternal immune activation dysregulation of the fetal brain transcriptome and relevance to the pathophysiology of autism spectrum disorder. *Mol Psychiatry*. 2018;23(4):1001-1013.
60. Mencer S, Kartawy M, Lendenfeld F, et al. Proteomics of autism and Alzheimer's mouse models reveal common alterations in mTOR signaling pathway. *Transl Psychiatry*. 2021;11(1):480.
61. Wesseling H, Elgersma Y, Bahn S. A brain proteomic investigation of rapamycin effects in the Tsc1(+/–) mouse model. *Mol Autism*. 2017;8:41.
62. Gazestani VH, Pramparo T, Nalabolu S, et al. A perturbed gene network containing PI3K-AKT, RAS-ERK and WNT-β-catenin pathways in leukocytes is linked to ASD genetics and symptom severity. *Nat Neurosci*. 2019;22(10):1624-1634.
63. Wan H, Wang Q, Chen X, et al. WDR45 contributes to neurodegeneration through regulation of ER homeostasis and neuronal death. *Autophagy*. 2020;16(3):531-547.
64. Tang G, Gudsnuk K, Kuo SH, et al. Loss of mTOR-dependent macroautophagy causes autistic-like synaptic pruning deficits. *Neuron*. 2014;83(5):1131-1143.
65. Pucilowska J, Vithayathil J, Pagani M, et al. Pharmacological inhibition of ERK signaling rescues pathophysiology and behavioral phenotype associated with 16p11.2 chromosomal deletion in mice. *J Neurosci*. 2018;38(30):6640-6652.
66. Eichler EE, Zimmerman AW. A hot spot of genetic instability in autism. *N Engl J Med*. 2008;358(7):737-739.
67. Murari K, Abushaibah A, Rho JM, Turner RW, Cheng N. A clinically relevant selective ERK-pathway inhibitor reverses core deficits in a mouse model of autism. *EBioMedicine*. 2023;91:104565.
68. Li Q, Shi Y, Li X, et al. Proteomic-based approach reveals the involvement of apolipoprotein A-I in related phenotypes of autism spectrum disorder in the BTBR mouse model. *Int J Mol Sci*. 2022;23(23):15290.
69. Gilman SR, Iossifov I, Levy D, Ronemus M, Wigler M, Vitkup D. Rare de novo variants associated with autism implicate a large functional network of genes involved in formation and function of synapses. *Neuron*. 2011;70(5):898-907.
70. Matic K, Eninger T, Bardoni B, Davidovic L, Macek B. Quantitative phosphoproteomics of murine Fmr1-KO cell lines provides new insights into FMRP-dependent signal transduction mechanisms. *J Proteome Res*. 2014;13(10):4388-4397.
71. D'Incal C, Broos J, Torfs T, Kooy RF, Vanden Berghe W. Towards kinase inhibitor therapies for fragile X syndrome: tweaking twists in the autism spectrum kinase signaling network. *Cells*. 2022;11(8):1325.
72. Wesseling H, Guest PC, Lee CM, Wong EH, Rahmoune H, Bahn S. Integrative proteomic analysis of the NMDA NR1 knockdown mouse model reveals effects on central and peripheral pathways associated with schizophrenia and autism spectrum disorders. *Mol Autism*. 2014;5:38.
73. Yang J, He X, Qian L, et al. Association between plasma proteome and childhood neurodevelopmental disorders: a two-sample Mendelian randomization analysis. *EBioMedicine*. 2022;78:103948.
74. Berg JM, Lee C, Chen L, et al. JAKMIP1, a novel regulator of neuronal translation, modulates synaptic function and autistic-like behaviors in mouse. *Neuron*. 2015;88(6):1173-1191.
75. Nishimura Y, Martin CL, Vazquez-Lopez A, et al. Genome-wide expression profiling of lymphoblastoid cell lines distinguishes different forms of autism and reveals shared pathways. *Hum Mol Genet*. 2007;16(14):1682-1698.
76. Pourtavakoli A, Ghafouri-Fard S. Calcium signaling in neurodevelopment and pathophysiology of autism spectrum disorders. *Mol Biol Rep*. 2022;49(11):10811-10823.
77. Hutchins BI, Li L, Kalil K. Wnt-induced calcium signaling mediates axon growth and guidance in the developing corpus callosum. *Sci Signal*. 2012;5(206):pt1.
78. Reilly J, Gallagher L, Leader G, Shen S. Coupling of autism genes to tissue-wide expression and dysfunction of synapse, calcium signalling and transcriptional regulation. *PLoS One*. 2020;15(12):e0242773.
79. Wen Y, Alshikho MJ, Herbert MR. Pathway network analyses for autism reveal multisystem involvement, major overlaps with other diseases and convergence upon MAPK and calcium signaling. *PLoS One*. 2016;11(4):e0153329.
80. Daroles L, Gribaudo S, Doulazmi M, et al. Fragile X mental retardation protein and dendritic local translation of the alpha subunit of the calcium/calmodulin-dependent kinase II messenger RNA are required for the structural plasticity underlying olfactory learning. *Biol Psychiatry*. 2016;80(2):149-159.
81. Baucum AJ 2nd, Shonesy BC, Rose KL, Colbran RJ. Quantitative proteomics analysis of CaMKII phosphorylation and the CaMKII interactome in the mouse forebrain. *ACS Chem Neurosci*. 2015;6(4):615-631.
82. Bezprozvanny I, Hiesinger PR. The synaptic maintenance problem: membrane recycling, Ca^{2+} homeostasis and late onset degeneration. *Mol Neurodegener*. 2013;8:23.
83. Guimarães-Souza EM, Joselevitch C, Britto LRG, Chiavegatto S. Retinal alterations in a pre-clinical model of an autism spectrum disorder. *Mol Autism*. 2019;10:19.
84. Rubinstein M, Westenbroek RE, Yu FH, Jones CJ, Scheuer T, Catterall WA. Genetic background modulates impaired excitability of inhibitory neurons in a mouse model of Dravet syndrome. *Neurobiol Dis*. 2015;73:106-117.
85. Rubenstein JL, Merzenich MM. Model of autism: increased ratio of excitation/inhibition in key neural systems. *Genes Brain Behav*. 2003;2(5):255-267.
86. Nelson SB, Valakh V. Excitatory/inhibitory balance and circuit homeostasis in autism spectrum disorders. *Neuron*. 2015;87(4):684-698.
87. Han S, Tai C, Westenbroek RE, et al. Autistic-like behaviour in Scn1a+/– mice and rescue by enhanced GABA-mediated neurotransmission. *Nature*. 2012;489(7416):385-390.
88. Gandal MJ, Haney JR, Wamsley B, et al. Broad transcriptomic dysregulation occurs across the cerebral cortex in ASD. *Nature*. 2022;611(7936):532-539.

89. Purcell AE, Jeon OH, Zimmerman AW, Blue ME, Pevsner J. Postmortem brain abnormalities of the glutamate neurotransmitter system in autism. *Neurology*. 2001;57(9):1618-1628.
90. Abraham JR, Szoko N, Barnard J, et al. Proteomic investigations of autism brain identify known and novel pathogenetic processes. *Sci Rep*. 2019;9(1):13118.
91. Pagani M, Barsotti N, Bertero A, et al. mTOR-related synaptic pathology causes autism spectrum disorder-associated functional hyperconnectivity. *Nat Commun*. 2021;12(1):6084.
92. Hoffmann A, Spengler D. Single-cell transcriptomics supports a role of CHD8 in autism. *Int J Mol Sci*. 2021;22(6):3261.
93. Lussu M, Noto A, Masili A, et al. The urinary (1) H-NMR metabolomics profile of an Italian autistic children population and their unaffected siblings. *Autism Res*. 2017;10(6):1058-1066.
94. Bitar T, Mavel S, Emond P, et al. Identification of metabolic pathway disturbances using multimodal metabolomics in autistic disorders in a Middle Eastern population. *J Pharm Biomed Anal*. 2018;152:57-65.
95. Gevi F, Belardo A, Zolla L. A metabolomics approach to investigate urine levels of neurotransmitters and related metabolites in autistic children. *Biochim Biophys Acta Mol Basis Dis*. 2020;1866(10):165859.
96. Gagliano A, Murgia F, Capodiferro AM, et al. (1)H-NMR-based metabolomics in autism spectrum disorder and pediatric acute-onset neuropsychiatric syndrome. *J Clin Med*. 2022;11(21):6493.
97. Droogers WJ, MacGillavry HD. Plasticity of postsynaptic nanostructure. *Mol Cell Neurosci*. 2023;124:103819.
98. Jung S, Park M. Shank postsynaptic scaffolding proteins in autism spectrum disorder: mouse models and their dysfunctions in behaviors, synapses, and molecules. *Pharmacol Res*. 2022;182:106340.
99. Wise A, Schatoff E, Flores J, et al. Drosophila-Cdh1 (Rap/Fzr) a regulatory subunit of APC/C is required for synaptic morphology, synaptic transmission and locomotion. *Int J Dev Neurosci*. 2013;31(7):624-633.
100. Schmid A, Qin G, Wichmann C, et al. Non-NMDA-type glutamate receptors are essential for maturation but not for initial assembly of synapses at Drosophila neuromuscular junctions. *J Neurosci*. 2006;26(44):11267-11277.
101. Oliva C, Escobedo P, Astorga C, Molina C, Sierralta J. Role of the MAGUK protein family in synapse formation and function. *Dev Neurobiol*. 2012;72(1):57-72.
102. Davenport EC, Szulc BR, Drew J, et al. Autism and schizophrenia-associated CYFIP1 regulates the balance of synaptic excitation and inhibition. *Cell Rep*. 2019;26(8):2037-2051.
103. Iossifov I, O'Roak BJ, Sanders SJ, et al. The contribution of de novo coding mutations to autism spectrum disorder. *Nature*. 2014;515(7526):216-221.
104. Li J, Zhang W, Yang H, et al. Spatiotemporal profile of postsynaptic interactomes integrates components of complex brain disorders. *Nat Neurosci*. 2017;20(8):1150-1161.
105. Jin C, Lee Y, Kang H, et al. Increased ribosomal protein levels and protein synthesis in the striatal synaptosome of Shank3-overexpressing transgenic mice. *Mol Brain*. 2021;14(1):39.
106. Reim D, Distler U, Halbedl S, et al. Proteomic analysis of postsynaptic density fractions from Shank3 mutant mice reveals brain region specific changes relevant to autism spectrum disorder. *Front Mol Neurosci*. 2017;10:26.
107. Lee S, Chun HS, Lee J, et al. Plausibility of the zebrafish embryos/larvae as an alternative animal model for autism: a comparison study of transcriptome changes. *PLoS One*. 2018;13(9):e0203543.
108. Yoo YE, Yoo T, Kang H, Kim E. Brain region and gene dosage-differential transcriptomic changes in Shank2-mutant mice. *Front Mol Neurosci*. 2022;15:977305.
109. Gupta P, Uner OE, Nayak S, Grant GR, Kalb RG. SAP97 regulates behavior and expression of schizophrenia risk enriched gene sets in mouse hippocampus. *PLoS One*. 2018;13(7):e0200477.
110. Jaworski J, Kapitein LC, Gouveia SM, et al. Dynamic microtubules regulate dendritic spine morphology and synaptic plasticity. *Neuron*. 2009;61(1):85-100.
111. Alfieri A, Sorokina O, Adrait A, et al. Synaptic interactome mining reveals p140Cap as a new hub for PSD proteins involved in psychiatric and neurological disorders. *Front Mol Neurosci*. 2017;10:212.
112. Repetto D, Camera P, Melani R, et al. p140Cap regulates memory and synaptic plasticity through Src-mediated and citron-N-mediated actin reorganization. *J Neurosci*. 2014;34(4):1542-1553.
113. Thacker S, Eng C. Transcriptome-(phospho)proteome characterization of brain of a germline model of cytoplasmic-predominant Pten expression with autism-like phenotypes. *NPJ Genom Med*. 2021;6(1):42.
114. Quesnel-Vallières M, Dargaei Z, Irimia M, et al. Misregulation of an activity-dependent splicing network as a common mechanism underlying autism spectrum disorders. *Mol Cell*. 2016;64(6):1023-1034.
115. Walter C, Marada A, Suhm T, et al. Global kinome profiling reveals DYRK1A as critical activator of the human mitochondrial import machinery. *Nat Commun*. 2021;12(1):4284.
116. Broek JA, Guest PC, Rahmoune H, Bahn S. Proteomic analysis of post mortem brain tissue from autism patients: evidence for opposite changes in prefrontal cortex and cerebellum in synaptic connectivity-related proteins. *Mol Autism*. 2014;5:41.
117. Abraham JR, Barnard J, Wang H, et al. Proteomic investigations of human HERC2 mutants: insights into the pathobiology of a neurodevelopmental disorder. *Biochem Biophys Res Commun*. 2019;512(2):421-427.
118. Zhang P, Omanska A, Ander BP, Gandal MJ, Stamova B, Schumann CM. Neuron-specific transcriptomic signatures indicate neuroinflammation and altered neuronal activity in ASD temporal cortex. *Proc Natl Acad Sci U S A*. 2023;120(10):e2206758120.
119. Boccuto L, Chen CF, Pittman AR, et al. Decreased tryptophan metabolism in patients with autism spectrum disorders. *Mol Autism*. 2013;4(1):16.
120. Smith AM, Natowicz MR, Braas D, et al. A metabolomics approach to screening for autism risk in the children's autism metabolome project. *Autism Res*. 2020;13(8):1270-1285.
121. Brister D, Werner BA, Gideon G, et al. Central nervous system metabolism in autism, epilepsy and developmental delays: a cerebrospinal fluid analysis. *Metabolites*. 2022;12(5):371.
122. Nolin SL, Napoli E, Flores A, Hagerman RJ, Giulivi C. Deficits in prenatal serine biosynthesis underlie the mitochondrial

dysfunction associated with the autism-linked FMR1 gene. *Int J Mol Sci*. 2021;22(11):5886.

123. Kartawy M, Khaliulin I, Amal H. Systems biology reveals S-nitrosylation-dependent regulation of mitochondrial functions in mice with Shank3 mutation associated with autism spectrum disorder. *Brain Sci*. 2021;11(6):677.
124. Golubiani G, Lagani V, Solomonia R, Müller M. Metabolomic fingerprint of Mecp2-deficient mouse cortex: evidence for a pronounced multi-facetted metabolic component in Rett syndrome. *Cells*. 2021;10(9):2494.
125. Kim HY, Lee YJ, Kim SJ, et al. Metabolomics profiling of valproic acid-induced symptoms resembling autism spectrum disorders using 1H NMR spectral analysis in rat model. *J Toxicol Environ Health A*. 2022;85(1):1-13.
126. Meyer U. Neurodevelopmental resilience and susceptibility to maternal immune activation. *Trends Neurosci*. 2019;42(11):793-806.
127. Estes ML, McAllister AK. Maternal immune activation: implications for neuropsychiatric disorders. *Science*. 2016;353(6301):772-777.
128. Usui N, Kobayashi H, Shimada S. Neuroinflammation and oxidative stress in the pathogenesis of autism spectrum disorder. *Int J Mol Sci*. 2023;24(6):5487.
129. Zhu Y, Mordaunt CE, Durbin-Johnson BP, et al. Expression changes in epigenetic gene pathways associated with one-carbon nutritional metabolites in maternal blood from pregnancies resulting in autism and non-typical neurodevelopment. *Autism Res*. 2021;14(1):11-28.
130. Murakami Y, Imamura Y, Kasahara Y, et al. Maternal inflammation with elevated kynurenine metabolites is related to the risk of abnormal brain development and behavioral changes in autism spectrum disorder. *Cells*. 2023;12(7):1087.
131. Nevalainen T, Kananen L, Marttila S, et al. Increased paternal age at conception is associated with transcriptomic changes involved in mitochondrial function in elderly individuals. *PLoS One*. 2016;11(11):e0167028.
132. Buizer-Voskamp JE, Laan W, Staal WG, et al. Paternal age and psychiatric disorders: findings from a Dutch population registry. *Schizophr Res*. 2011;129(2-3):128-132.
133. Wang L, Zheng R, Xu Y, et al. Altered metabolic characteristics in plasma of young boys with autism spectrum disorder. *J Autism Dev Disord*. 2022;52(11):4897-4907.
134. Cao C, Wang D, Zou M, Sun C, Wu L. Untargeted metabolomics reveals hepatic metabolic disorder in the BTBR mouse model of autism and the significant role of liver in autism. *Cell Biochem Funct*. 2023;41(5):553-563.
135. O'Neill J, Bansal R, Goh S, Rodie M, Sawardekar S, Peterson BS. Parsing the heterogeneity of brain metabolic disturbances in autism spectrum disorder. *Biol Psychiatry*. 2020;87(2):174-184.
136. Marballi K, MacDonald JL. Proteomic and transcriptional changes associated with MeCP2 dysfunction reveal nodes for therapeutic intervention in Rett syndrome. *Neurochem Int*. 2021;148:105076.
137. Ayhan F, Konopka G. Regulatory genes and pathways disrupted in autism spectrum disorders. *Prog Neuropsychopharmacol Biol Psychiatry*. 2019;89:57-64.
138. Wang L, Wang B, Wu C, Wang J, Sun M. Autism spectrum disorder: neurodevelopmental risk factors, biological mechanism, and precision therapy. *Int J Mol Sci*. 2023;24(3):1819.
139. Guo H, Wang T, Wu H, et al. Inherited and multiple de novo mutations in autism/developmental delay risk genes suggest a multifactorial model. *Mol Autism*. 2018;9:64.
140. Xie Y, Xu Z, Xia M, et al. Alterations in connectome dynamics in autism spectrum disorder: a harmonized mega- and meta-analysis study using the autism brain imaging data exchange dataset. *Biol Psychiatry*. 2022;91(11):945-955.
141. Sztainberg Y, Zoghbi HY. Lessons learned from studying syndromic autism spectrum disorders. *Nat Neurosci*. 2016;19(11):1408-1417.
142. Shen L, Zhang H, Lin J, et al. A combined proteomics and metabolomics profiling to investigate the genetic heterogeneity of autistic children. *Mol Neurobiol*. 2022;59(6):3529-3545.
143. Ellegood J, Anagnostou E, Babineau BA, et al. Clustering autism: using neuroanatomical differences in 26 mouse models to gain insight into the heterogeneity. *Mol Psychiatry*. 2015;20(1):118-125.
144. Brown EA, Lautz JD, Davis TR, et al. Clustering the autisms using glutamate synapse protein interaction networks from cortical and hippocampal tissue of seven mouse models. *Mol Autism*. 2018;9:48.
145. Jin X, Simmons SK, Guo A, et al. In vivo Perturb-Seq reveals neuronal and glial abnormalities associated with autism risk genes. *Science*. 2020;370(6520):eaaz6063.
146. Carbonell AU, Freire-Cobo C, Deyneko IV, et al. Comparing synaptic proteomes across five mouse models for autism reveals converging molecular similarities including deficits in oxidative phosphorylation and Rho GTPase signaling. *Front Aging Neurosci*. 2023;15:1152562.
147. Zerbi V, Pagani M, Markicevic M, et al. Brain mapping across 16 autism mouse models reveals a spectrum of functional connectivity subtypes. *Mol Psychiatry*. 2021;26(12):7610-7620.
148. Willsey HR, Exner CRT, Xu Y, et al. Parallel in vivo analysis of large-effect autism genes implicates cortical neurogenesis and estrogen in risk and resilience. *Neuron*. 2021;109(5):e8.
149. Shen L, Zhang H, Lin J, et al. A combined proteomics and metabolomics profiling to investigate the genetic heterogeneity of autistic children. *Mol Neurobiol*. 2022;59(6):3529-3545.
150. Pintacuda G, Hsu Y-HH, Tsafou K, et al. Protein interaction studies in human induced neurons indicate convergent biology underlying autism spectrum disorders. *Cell Genomics*. 2023;3(3):100250.
151. Weinschutz Mendes H, Neelakantan U, Liu Y, et al. High-throughput functional analysis of autism genes in zebrafish identifies convergence in dopaminergic and neuroimmune pathways. *Cell Rep*. 2023;42(3):112243.
152. Willsey HR, Exner CRT, Xu Y, et al. Parallel in vivo analysis of large-effect autism genes implicates cortical neurogenesis and estrogen in risk and resilience. *Neuron*. 2021;109(8):1409.
153. Peng J, Zhou Y, Wang K. Multiplex gene and phenotype network to characterize shared genetic pathways of epilepsy and autism. *Sci Rep*. 2021;11(1):952.
154. Moyses-Oliveira M, Yadav R, Erdin S, Talkowski ME. New gene discoveries highlight functional convergence in autism and related neurodevelopmental disorders. *Curr Opin Genet Dev*. 2020;65:195-206.
155. Woodbury-Smith M, Bilder DA, Morgan J, et al. Combined genome-wide linkage and targeted association analysis of

head circumference in autism spectrum disorder families. *J Neurodev Disord*. 2017;9:5.
156. De Rubeis S, Buxbaum JD. Genetics and genomics of autism spectrum disorder: embracing complexity. *Hum Mol Genet*. 2015;24(R1):R24-R31.
157. Cross-Disorder Group of the Psychiatric Genomics Consortium. Genomic relationships, novel loci, and pleiotropic mechanisms across eight psychiatric disorders. *Cell*. 2019;179(7):1469-1482.e11.
158. Grove J, Ripke S, Als TD, et al. Identification of common genetic risk variants for autism spectrum disorder. *Nat Genet*. 2019;51(3):431-444.
159. Yu D, Sul JH, Tsetsos F, et al. Interrogating the genetic determinants of Tourette's syndrome and other tic disorders through genome-wide association studies. *Am J Psychiatry*. 2019;176(3):217-227.
160. Willsey HR, Willsey AJ, Wang B, State MW. Genomics, convergent neuroscience and progress in understanding autism spectrum disorder. *Nat Rev Neurosci*. 2022;23(6):323-341.
161. Choi L, An JY. Genetic architecture of autism spectrum disorder: lessons from large-scale genomic studies. *Neurosci Biobehav Rev*. 2021;128:244-257.
162. Bokobza C, Van Steenwinckel J, Mani S, Mezger V, Fleiss B, Gressens P. Neuroinflammation in preterm babies and autism spectrum disorders. *Pediatr Res*. 2019;85(2):155-165.
163. Rudolph MD, Graham AM, Feczko E, et al. Maternal IL-6 during pregnancy can be estimated from newborn brain connectivity and predicts future working memory in offspring. *Nat Neurosci*. 2018;21(5):765-772.
164. Han VX, Patel S, Jones HF, et al. Maternal acute and chronic inflammation in pregnancy is associated with common neurodevelopmental disorders: a systematic review. *Transl Psychiatry*. 2021;11(1):71.
165. Bundgaard-Nielsen C, Lauritsen MB, Knudsen JK, et al. Children and adolescents with attention deficit hyperactivity disorder and autism spectrum disorder share distinct microbiota compositions. *Gut Microbes*. 2023;15(1):2211923.
166. Mayer EA, Padua D, Tillisch K. Altered brain–gut axis in autism: comorbidity or causative mechanisms? *Bioessays*. 2014;36(10):933-939.
167. Zhang M, Chu Y, Meng Q, et al. A quasi-paired cohort strategy reveals the impaired detoxifying function of microbes in the gut of autistic children. *Sci Adv*. 2020;6(43):eaba3760.
168. Rossignol DA, Frye RE. A review of research trends in physiological abnormalities in autism spectrum disorders: immune dysregulation, inflammation, oxidative stress, mitochondrial dysfunction and environmental toxicant exposures. *Mol Psychiatry*. 2012;17(4):389-401.
169. Gevezova M, Sbirkov Y, Sarafian V, Plaimas K, Suratanee A, Maes M. Autistic spectrum disorder (ASD)—gene, molecular and pathway signatures linking systemic inflammation, mitochondrial dysfunction, transsynaptic signalling, and neurodevelopment. *Brain Behav Immun Health*. 2023;30:100646.
170. Pacheva I, Ivanov I. Targeted biomedical treatment for autism spectrum disorders. *Curr Pharm Des*. 2019;25(41):4430-4453.
171. Hughes HK, Moreno RJ, Ashwood P. Innate immune dysfunction and neuroinflammation in autism spectrum disorder (ASD). *Brain Behav Immun*. 2023;108:245-254.
172. Meltzer A, Van de Water J. The role of the immune system in autism spectrum disorder. *Neuropsychopharmacology*. 2017;42(1):284-298.
173. Onore C, Careaga M, Ashwood P. The role of immune dysfunction in the pathophysiology of autism. *Brain Behav Immun*. 2012;26(3):383-392.
174. Liao X, Yang J, Wang H, Li Y. Microglia mediated neuroinflammation in autism spectrum disorder. *J Psychiatr Res*. 2020;130:167-176.
175. Majhi S, Kumar S, Singh LA. Review on autism spectrum disorder: pathogenesis, biomarkers, pharmacological and non-pharmacological interventions. *CNS Neurol Disord Drug Targets*. 2023;22(5):659-677.
176. Bjørklund G, Meguid NA, El-Bana MA, et al. Oxidative stress in autism spectrum disorder. *Mol Neurobiol*. 2020;57(5):2314-2332.
177. Rossignol DA, Frye RE. Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis. *Mol Psychiatry*. 2012;17(3):290-314.
178. Singh K, Singh IN, Diggins E, et al. Developmental regression and mitochondrial function in children with autism. *Ann Clin Transl Neurol*. 2020;7(5):683-694.
179. Giulivi C, Zhang Y-F, Omanska-Klusek A, et al. Mitochondrial dysfunction in autism. *JAMA*. 2010;304(21):2389-2396.
180. Srikantha P, Mohajeri MH. The possible role of the microbiota–gut–brain-axis in autism spectrum disorder. *Int J Mol Sci*. 2019;20(9):2115.
181. Gevezova M, Sarafian V, Anderson G, Maes M. Inflammation and mitochondrial dysfunction in autism spectrum disorder. *CNS Neurol Disord Drug Targets*. 2020;19(5):320-333.
182. Xiao L, Yan J, Yang T, et al. Fecal microbiome transplantation from children with autism spectrum disorder modulates tryptophan and serotonergic synapse metabolism and induces altered behaviors in germ-free mice. *Msystems*. 2021;6(2):e01343-20.
183. Sharon G, Cruz NJ, Kang D-W, et al. Human gut microbiota from autism spectrum disorder promote behavioral symptoms in mice. *Cell*. 2019;177(6):1600-1618.
184. Zhang M, Chu Y, Meng Q, et al. A quasi-paired cohort strategy reveals the impaired detoxifying function of microbes in the gut of autistic children. *Sci Adv*. 2020;6(43):eaba3760.
185. Vuong HE, Hsiao EY. Emerging roles for the gut microbiome in autism spectrum disorder. *Biol Psychiatry*. 2017;81(5):411-423.
186. Zhu J, Hua X, Yang T, et al. Alterations in gut vitamin and amino acid metabolism are associated with symptoms and neurodevelopment in children with autism spectrum disorder. *J Autism Dev Disord*. 2022;52(7):3116-3128.
187. Sarkar A, Lehto SM, Harty S, Dinan TG, Cryan JF, Burnet PWJ. Psychobiotics and the manipulation of bacteria–gut–brain signals. *Trends Neurosci*. 2016;39(11):763-781.
188. Hsiao EY, McBride SW, Hsien S, et al. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell*. 2013;155(7):1451-1463.
189. Bjørklund G, Pivina L, Dadar M, et al. Gastrointestinal alterations in autism spectrum disorder: what do we know? *Neurosci Biobehav Rev*. 2020;118:111-120.
190. Buffington SA, Dooling SW, Sgritta M, et al. Dissecting the contribution of host genetics and the microbiome in complex behaviors. *Cell*. 2021;184(7):1740-1756.e16.

191. Yu Y, Zhang B, Ji P, et al. Changes to gut amino acid transporters and microbiome associated with increased E/I ratio in Chd8(+/−) mouse model of ASD-like behavior. *Nat Commun*. 2022;13(1):1151.
192. Liu X. The interaction of gut microbiota, genetic variation, and diet in autism spectrum disorder. *mLife*. 2022;1(3):241-244.
193. Panelli S, Capelli E, Lupo GFD, et al. Comparative study of salivary, duodenal, and fecal microbiota composition across adult celiac disease. *J Clin Med*. 2020;9(4):1109.
194. Bostick JW, Schonhoff AM, Mazmanian SK. Gut microbiome-mediated regulation of neuroinflammation. *Curr Opin Immunol*. 2022;76:102177.
195. Liu F, Li J, Wu F, Zheng H, Peng Q, Zhou H. Altered composition and function of intestinal microbiota in autism spectrum disorders: a systematic review. *Transl Psychiatry*. 2019;9(1):43.
196. Hyman SL, Levy SE, Myers SM. Identification, evaluation, and management of children with autism spectrum disorder. *Pediatrics*. 2020;145(1):e20193447.
197. General Office of the National Health Commission. *Notice of the General Office of the National Health Commission on exploring the Implementation of Special Services for the Prevention and Treatment of Depression and Senile Dementia*.
198. Cortese S, McGinn K, Højlund M, et al. The future of child and adolescent clinical psychopharmacology: a systematic review of phase 2, 3, or 4 randomized controlled trials of pharmacologic agents without regulatory approval or for unapproved indications. *Neurosci Biobehav Rev*. 2023;149:105149.
199. Parellada M, Andreu-Bernabeu Á, Burdeus M, et al. In search of biomarkers to guide interventions in autism spectrum disorder: a systematic review. *Am J Psychiatry*. 2023;180(1):23-40.
200. Ansel A, Posen Y, Ellis R, Deutsch L, Zisman PD, Gesundheit B. Biomarkers for autism spectrum disorders (ASD): a meta-analysis. *Rambam Maimonides Med J*. 2019;10(4):e0021.
201. Alharbi MG. Protein biomarkers in autistic children: a review. *Asian J Biochem Genet Mol Biol*. 2022;12(1):1-17.
202. Feng C, Chen Y, Pan J, et al. Redox proteomic identification of carbonylated proteins in autism plasma: insight into oxidative stress and its related biomarkers in autism. *Clin Proteomics*. 2017;14:2.
203. Cortelazzo A, De Felice C, Guerranti R, et al. Expression and oxidative modifications of plasma proteins in autism spectrum disorders: interplay between inflammatory response and lipid peroxidation. *Proteomics Clin Appl*. 2016;10(11):1103-1112.
204. Shen L, Zhao Y, Zhang H, et al. Advances in biomarker studies in autism spectrum disorders. *Adv Exp Med Biol*. 2019;1118:207-233.
205. Abraham J, Szoko N, Natowicz MR. Proteomic investigations of autism spectrum disorder: past findings, current challenges, and future prospects. *Adv Exp Med Biol*. 2019;1118:235-252.
206. Likhitweerawong N, Thonusin C, Boonchooduang N, et al. Profiles of urine and blood metabolomics in autism spectrum disorders. *Metab Brain Dis*. 2021;36(7):1641-1671.
207. Xu XJ, Cai XE, Meng FC, et al. Comparison of the metabolic profiles in the plasma and urine samples between autistic and typically developing boys: a preliminary study. *Front Psychiatry*. 2021;12:657105.
208. Arora A, Mastropasqua F, Bölte S, Tammimies K. Urine metabolomic profiles of autism and autistic traits—a twin study. *medRxiv*. 2023. 2023.04.24.23289030.
209. Al-Otaish H, Al-Ayadhi L, Bjørklund G, Chirumbolo S, Urbina MA, El-Ansary A. Relationship between absolute and relative ratios of glutamate, glutamine and GABA and severity of autism spectrum disorder. *Metab Brain Dis*. 2018;33(3):843-854.
210. El-Ansary A, Cannell JJ, Bjørklund G, et al. In the search for reliable biomarkers for the early diagnosis of autism spectrum disorder: the role of vitamin D. *Metab Brain Dis*. 2018;33(3):917-931.
211. Zhang H, Tang X, Feng C, et al. The use of data independent acquisition based proteomic analysis and machine learning to reveal potential biomarkers for autism spectrum disorder. *J Proteomics*. 2023;278:104872.
212. Cao X, Tang X, Feng C, et al. A systematic investigation of complement and coagulation-related protein in autism spectrum disorder using multiple reaction monitoring technology. *Neurosci Bull*. 2023;39(11):1623-1637.
213. Dai S, Lin J, Hou Y, Luo X, Shen Y, Ou J. Purine signaling pathway dysfunction in autism spectrum disorders: evidence from multiple omics data. *Front Mol Neurosci*. 2023;16:1089871.
214. Liu W, Li L, Xia X, et al. Integration of urine proteomic and metabolomic profiling reveals novel insights into neuroinflammation in autism spectrum disorder. *Front Psychiatry*. 2022;13:780747.
215. Tang X, Feng C, Zhao Y, et al. A study of genetic heterogeneity in autism spectrum disorders based on plasma proteomic and metabolomic analysis: multiomics study of autism heterogeneity. *Med Comm (2020)*. 2023;4(5):e380.
216. Kordulewska NK, Kostyra E, Piskorz-Ogórek K, et al. Serum cytokine levels in children with spectrum autism disorder: differences in pro- and anti-inflammatory balance. *J Neuroimmunol*. 2019;337:577066.
217. Hu CC, Xu X, Xiong GL, et al. Alterations in plasma cytokine levels in Chinese children with autism spectrum disorder. *Autism Res*. 2018;11(7):989-999.
218. Hoang N, Buchanan JA, Scherer SW. Heterogeneity in clinical sequencing tests marketed for autism spectrum disorders. *NPJ Genom Med*. 2018;3:27.
219. Wang T, Guo H, Xiong B, et al. De novo genic mutations among a Chinese autism spectrum disorder cohort. *Nat Commun*. 2016;7:13316.
220. Corbett BA, Kantor AB, Schulman H, et al. A proteomic study of serum from children with autism showing differential expression of apolipoproteins and complement proteins. *Mol Psychiatry*. 2007;12(3):292-306.
221. Magdalon J, Mansur F, Teles ESAL, de Goes VA, Reiner O, Sertié AL. Complement system in brain architecture and neurodevelopmental disorders. *Front Neurosci*. 2020;14:23.
222. Druart M, Le Magueresse C. Emerging roles of complement in psychiatric disorders. *Front Psychiatry*. 2019;10:573.
223. Garcia-Gutierrez E, Narbad A, Rodríguez JM. Autism spectrum disorder associated with gut microbiota at immune, metabolomic, and neuroactive level. *Front Neurosci*. 2020;14:578666.
224. West PR, Amaral DG, Bais P, et al. Metabolomics as a tool for discovery of biomarkers of autism spectrum disorder in the blood plasma of children. *PLoS One*. 2014;9(11):e112445.
225. Anwar A, Abruzzo PM, Pasha S, et al. Advanced glycation endproducts, dityrosine and arginine transporter dysfunction

in autism-a source of biomarkers for clinical diagnosis. *Mol Autism*. 2018;9(1):1-16.
226. Delaye J-B, Patin F, Lagrue E, et al. Post hoc analysis of plasma amino acid profiles: towards a specific pattern in autism spectrum disorder and intellectual disability. *Ann Clin Biochem*. 2018;55(5):543-552.
227. Lv Q-Q, You C, Zou X-B, Deng H-Z. Acyl-carnitine, C5DC, and C26 as potential biomarkers for diagnosis of autism spectrum disorder in children. *Psychiatry Res*. 2018;267:277-280.
228. Smith AM, King JJ, West PR, et al. Amino acid dysregulation metabotypes: potential biomarkers for diagnosis and individualized treatment for subtypes of autism spectrum disorder. *Biol Psychiatry*. 2019;85(4):345-354.
229. Brister D, Rose S, Delhey L, et al. Metabolomic signatures of autism spectrum disorder. *J Personalized Med*. 2022;12(10):1727.
230. Kaluzna-Czaplinska J, Socha E, Rynkowski J. Determination of homovanillic acid and vanillylmandelic acid in urine of autistic children by gas chromatography/mass spectrometry. *Med Sci Monit*. 2010;16(9):CR445-CR450.
231. Mavel S, Nadal-Desbarats L, Blasco H, et al. $^{1}H–^{13}C$ NMR-based urine metabolic profiling in autism spectrum disorders. *Talanta*. 2013;114:95-102.
232. Emond P, Mavel S, Aïdoud N, et al. GC–MS-based urine metabolic profiling of autism spectrum disorders. *Anal BioanalChem*. 2013;405:5291-5300.
233. Nadal-Desbarats L, Aïdoud N, Emond P, et al. Combined ^{1}H-NMR and $^{1}H–^{13}C$ HSQC-NMR to improve urinary screening in autism spectrum disorders. *Analyst*. 2014;139(13):3460-3468.
234. Liu A, Zhou W, Qu L, et al. Altered urinary amino acids in children with autism spectrum disorders. *Front Cell Neurosci*. 2019;13:7.
235. Delaye JB, Patin F, Lagrue E, et al. Post hoc analysis of plasma amino acid profiles: towards a specific pattern in autism spectrum disorder and intellectual disability. *Ann Clin Biochem*. 2018;55(5):543-552.
236. Smith AM, King JJ, West PR, et al. Amino acid dysregulation metabotypes: potential biomarkers for diagnosis and individualized treatment for subtypes of autism spectrum disorder. *Biol Psychiatry*. 2019;85(4):345-354.
237. Wang L, Christophersen CT, Sorich MJ, Gerber JP, Angley MT, Conlon MA. Elevated fecal short chain fatty acid and ammonia concentrations in children with autism spectrum disorder. *Dig Dis Sci*. 2012;57(8):2096-2102.
238. Brister D, Rose S, Delhey L, et al. Metabolomic signatures of autism spectrum disorder. *J Pers Med*. 2022;12(10):1727.
239. Liu A, Zhou W, Qu L, et al. Altered urinary amino acids in children with autism spectrum disorders. *Front Cell Neurosci*. 2019;13:7.
240. Nadal-Desbarats L, Aidoud N, Emond P, et al. Combined ^{1}H-NMR and ^{1}H-^{13}C HSQC-NMR to improve urinary screening in autism spectrum disorders. *Analyst*. 2014;139(13):3460-3468.
241. Olesova D, Galba J, Piestansky J, et al. A novel UHPLC-MS method targeting urinary metabolomic markers for autism spectrum disorder. *Metabolites*. 2020;10(11):443.
242. Mead J, Ashwood P. Evidence supporting an altered immune response in ASD. *Immunol Lett*. 2015;163(1):49-55.
243. Masi A, Quintana DS, Glozier N, Lloyd AR, Hickie IB, Guastella AJ. Cytokine aberrations in autism spectrum disorder: a systematic review and meta-analysis. *Mol Psychiatry*. 2015;20(4):440-446.
244. Saghazadeh A, Ataeinia B, Keynejad K, Abdolalizadeh A, Hirbod-Mobarakeh A, Rezaei N. A meta-analysis of pro-inflammatory cytokines in autism spectrum disorders: effects of age, gender, and latitude. *J Psychiatr Res*. 2019;115:90-102.
245. Saghazadeh A, Ataeinia B, Keynejad K, Abdolalizadeh A, Hirbod-Mobarakeh A, Rezaei N. Anti-inflammatory cytokines in autism spectrum disorders: a systematic review and meta-analysis. *Cytokine*. 2019;123:154740.
246. Zhao H, Zhang H, Liu S, Luo W, Jiang Y, Gao J. Association of peripheral blood levels of cytokines with autism spectrum disorder: a meta-analysis. *Front Psychiatry*. 2021;12:670200.
247. Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah IN, Van de Water J. Associations of impaired behaviors with elevated plasma chemokines in autism spectrum disorders. *J Neuroimmunol*. 2011;232(1-2):196-199.
248. Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah I, Van de Water J. Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. *Brain Behav Immun*. 2011;25(1):40-45.
249. Napolioni V, Ober-Reynolds B, Szelinger S, et al. Plasma cytokine profiling in sibling pairs discordant for autism spectrum disorder. *J Neuroinflammation*. 2013;10:38.
250. Li X, Chauhan A, Sheikh AM, et al. Elevated immune response in the brain of autistic patients. *J Neuroimmunol*. 2009;207(1–2):111-116.
251. Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. *Ann Neurol*. 2005;57(1):67-81.
252. Eftekharian MM, Ghafouri-Fard S, Noroozi R, et al. Cytokine profile in autistic patients. *Cytokine*. 2018;108:120-126.
253. Chen L, Shi XJ, Liu H, et al. Oxidative stress marker aberrations in children with autism spectrum disorder: a systematic review and meta-analysis of 87 studies ($N = 9109$). *Transl Psychiatry*. 2021;11(1):15.
254. Liu X, Lin J, Zhang H, et al. Oxidative stress in autism spectrum disorder—current progress of mechanisms and biomarkers. *Front Psychiatry*. 2022;13:813304.
255. Bala KA, Doğan M, Kaba S, Mutluer T, Aslan O, Doğan SZ. Hormone disorder and vitamin deficiency in attention deficit hyperactivity disorder (ADHD) and autism spectrum disorders (ASDs). *J Pediatr Endocrinol Metab*. 2016;29(9):1077-1082.
256. Fuentes-Albero M, Cauli O. Homocysteine levels in autism spectrum disorder: a clinical update. *Endocr Metab Immune Disord Drug Targets*. 2018;18(4):289-296.
257. Zhang Y, Hodgson NW, Trivedi MS, et al. Decreased brain levels of vitamin B12 in aging, autism and schizophrenia. *PLoS One*. 2016;11(1):e0146797.
258. Lou M, Cao A, Jin C, et al. Deviated and early unsustainable stunted development of gut microbiota in children with autism spectrum disorder. *Gut*. 2022;71(8):1588-1599.
259. De Angelis M, Piccolo M, Vannini L, et al. Fecal microbiota and metabolome of children with autism and pervasive developmental disorder not otherwise specified. *PLoS One*. 2013;8(10):e76993.

260. Gevi F, Zolla L, Gabriele S, Persico AM. Urinary metabolomics of young Italian autistic children supports abnormal tryptophan and purine metabolism. *Mol Autism*. 2016;7:47.
261. Chen Q, Qiao Y, Xu X-j, You X, Tao Y. Urine organic acids as potential biomarkers for autism-spectrum disorder in Chinese children. *Front Cellular Neurosci*. 2019;13:150.
262. Kang D-W, Adams JB, Vargason T, Santiago M, Hahn J, Krajmalnik-Brown R. Distinct fecal and plasma metabolites in children with autism spectrum disorders and their modulation after microbiota transfer therapy. *Msphere*. 2020;5(5):e00314-e00320.
263. Fattorusso A, Di Genova L, Dell'Isola GB, Mencaroni E, Esposito S. Autism spectrum disorders and the gut microbiota. *Nutrients*. 2019;11(3):521.
264. Mohamadkhani A. Gut microbiota and fecal metabolome perturbation in children with autism spectrum disorder. *Middle East J Dig Dis*. 2018;10(4):205-212.
265. Xu X, Zou X, Li T. Expert consensus on early identification, screening and early intervention of children with autism spectrum disorders. *Chin J Pediatr*. 2017;55(12):890-897.
266. Howes OD, Rogdaki M, Findon JL, et al. Autism spectrum disorder: consensus guidelines on assessment, treatment and research from the British Association for Psychopharmacology. *J Psychopharmacol*. 2018;32(1):3-29.
267. Sandbank M, Bottema-Beutel K, Crowley S, et al. Project AIM: autism intervention meta-analysis for studies of young children. *Psychol Bull*. 2020;146(1):1.
268. Hyman SL, Levy SE, Myers SM, Council on Children With Disabilities, Section on Developmental and Behavioral Pediatrics. Identification, evaluation, and management of children with autism spectrum disorder. *Pediatrics*. 2020;145(1):e20193447.
269. Gosling CJ, Cartigny A, Mellier BC, Solanes A, Radua J, Delorme R. Efficacy of psychosocial interventions for Autism spectrum disorder: an umbrella review. *Mol Psychiatry*. 2022;27(9):3647-3656.
270. Trembath D, Varcin K, Waddington H, et al. Non-pharmacological interventions for autistic children: an umbrella review. *Autism*. 2023;27(2):275-295.
271. French L, Kennedy EM. Annual research review: early intervention for infants and young children with, or at-risk of, autism spectrum disorder: a systematic review. *J Child Psychol Psychiatry*. 2018;59(4):444-456.
272. Gabriels RL, Pan Z, Dechant B, Agnew JA, Brim N, Mesibov G. Randomized controlled trial of therapeutic horseback riding in children and adolescents with autism spectrum disorder. *J Am Acad Child Adolesc Psychiatry*. 2015;54(7):541-549.
273. Bearss K, Johnson C, Smith T, et al. Effect of parent training vs parent education on behavioral problems in children with autism spectrum disorder: a randomized clinical trial. *JAMA*. 2015;313(15):1524-1533.
274. Bieleninik Ł, Geretsegger M, Mössler K, et al. Effects of improvisational music therapy vs enhanced standard care on symptom severity among children with autism spectrum disorder: the TIME—a randomized clinical trial. *JAMA*. 2017;318(6):525-535.
275. Sharda M, Tuerk C, Chowdhury R, et al. Music improves social communication and auditory–motor connectivity in children with autism. *Transl Psychiatry*. 2018;8(1):231.
276. Grimaldi R, Gibson GR, Vulevic J, et al. A prebiotic intervention study in children with autism spectrum disorders (ASDs). *Microbiome*. 2018;6(1):133.
277. DeVane CL, Charles JM, Abramson RK, et al. Pharmacotherapy of autism spectrum disorder: results from the randomized BAART clinical trial. *Pharmacother J Human Pharmacol Drug Therapy*. 2019;39(6):626-635.
278. Voss C, Schwartz J, Daniels J, et al. Effect of wearable digital intervention for improving socialization in children with autism spectrum disorder: a randomized clinical trial. *JAMA Pediatr*. 2019;173(5):446-454.
279. Malow BA, Findling RL, Schroder CM, et al. Sleep, growth, and puberty after 2 years of prolonged-release melatonin in children with autism spectrum disorder. *J Am Acad Child Adolesc Psychiatry*. 2021;60(2):252-261.e3.
280. Wood JJ, Kendall PC, Wood KS, et al. Cognitive behavioral treatments for anxiety in children with autism spectrum disorder: a randomized clinical trial. *JAMA Psychiatry*. 2020;77(5):474-483.
281. Sikich L, Kolevzon A, King BH, et al. Intranasal oxytocin in children and adolescents with autism spectrum disorder. *N Engl J Med*. 2021;385(16):1462-1473.
282. Aran A, Harel M, Cassuto H, et al. Cannabinoid treatment for autism: a proof-of-concept randomized trial. *Mol Autism*. 2021;12(1):6.
283. Scahill L, Shillingsburg MA, Ousley O, et al. A randomized trial of direct instruction language for learning in children with autism spectrum disorder. *J Am Acad Child Adolesc Psychiatry*. 2022;61(6):772-781.
284. Chu L, Shen L, Ma C, et al. Effects of a nonwearable digital therapeutic intervention on preschoolers with autism spectrum disorder in China: open-label randomized controlled trial. *J Med Internet Res*. 2023;25:e45836.
285. Lord C, Charman T, Havdahl A, et al. The Lancet Commission on the future of care and clinical research in autism. *Lancet North Am Ed*. 2022;399(10321):271-334.
286. Kux L. *Food and Drug Administration [Docket No. FDA-2012-D-0146]: Guidance for Industry on Irritable Bowel Syndrome-Clinical Evaluation of Drugs for Treatment; Availability; Correction*. Federal Register. Vol 77. Department of Health and Human Services. 2012:32124-32125.
287. Dawson G, Bernier R. A quarter century of progress on the early detection and treatment of autism spectrum disorder. *Dev Psychopathol*. 2013;25(4pt2):1455-1472.
288. Lovaas OI. Behavioral treatment and normal educational and intellectual functioning in young autistic children. *J Consult Clin Psychol*. 1987;55(1):3.
289. Smith T, Iadarola S. Evidence base update for autism spectrum disorder. *J Clin Child Adolesc Psychol*. 2015;44(6):897-922.
290. Cummings AR, Carr JE. Evaluating progress in behavioral programs for children with autism spectrum disorders via continuous and discontinuous measurement. *J Appl Behav Anal*. 2009;42(1):57-71.
291. Lerman DC, Dittlinger LH, Fentress G, Lanagan T. A comparison of methods for collecting data on performance during discrete trial teaching. *Behav Anal Pract*. 2011;4:53-62.
292. Myers SM, Johnson CP, American Academy of Pediatrics Council on Children With Disabilities. Management

of children with autism spectrum disorders. *Pediatrics*. 2007;120(5):1162-1182.
293. LeBlanc LA, Coates AM, Daneshvar S, Charlop-Christy MH, Morris C, Lancaster BM. Using video modeling and reinforcement to teach perspective-taking skills to children with autism. *J Appl Behav Anal*. 2003;36(2):253-257.
294. Koegel RL, O'dell MC, Koegel LK. A natural language teaching paradigm for nonverbal autistic children. *J Autism Dev Disord*. 1987;17(2):187-200.
295. Hardan AY, Gengoux GW, Berquist KL, et al. A randomized controlled trial of pivotal response treatment group for parents of children with autism. *J Child Psychol Psychiatry*. 2015;56(8):884-892.
296. Mohammadzaheri F, Koegel LK, Rezaee M, Rafiee SM. A randomized clinical trial comparison between pivotal response treatment (PRT) and structured applied behavior analysis (ABA) intervention for children with autism. *J Autism Dev Disord*. 2014;44:2769-2777.
297. Batey C, Missiuna C, Timmons B, Hay J, Faught B, Cairney J. Self-efficacy toward physical activity and the physical activity behavior of children with and without developmental coordination disorder. *Hum Mov Sci*. 2014;36:258-271.
298. Tan BW, Pooley JA, Speelman CP. A meta-analytic review of the efficacy of physical exercise interventions on cognition in individuals with autism spectrum disorder and ADHD. *J Autism Dev Disord*. 2016;46:3126-3143.
299. Bremer E, Crozier M, Lloyd M. A systematic review of the behavioural outcomes following exercise interventions for children and youth with autism spectrum disorder. *Autism*. 2016;20(8):899-915.
300. Oriel KN, George CL, Peckus R, Semon A. The effects of aerobic exercise on academic engagement in young children with autism spectrum disorder. *Pediatric Phys Ther*. 2011;23(2):187-193.
301. Liang X, Li R, Wong SH, et al. The effects of exercise interventions on executive functions in children and adolescents with autism spectrum disorder: a systematic review and meta-analysis. *Sports Med*. 2022;52(1):75-88.
302. Teh EJ, Vijayakumar R, Tan TXJ, Yap MJ. Effects of physical exercise interventions on stereotyped motor behaviours in children with ASD: a meta-analysis. *J Autism Dev Disord*. 2022;52(7):2934-2957.
303. Sam K-L, Chow B-C, Tong K-K. Effectiveness of exercise-based interventions for children with autism: a systematic review and meta-analysis. *Int J Learn Teach*. 2015;1(2):98-103.
304. Petrus C, Adamson SR, Block L, Einarson SJ, Sharifnejad M, Harris SR. Effects of exercise interventions on stereotypic behaviours in children with autism spectrum disorder. *Physiother Can*. 2008;60(2):134-145.
305. Toscano CV, Carvalho HM, Ferreira JP. Exercise effects for children with autism spectrum disorder: metabolic health, autistic traits, and quality of life. *Percept Mot Skills*. 2018;125(1):126-146.
306. Bahrami F, Movahedi A, Marandi SM, Sorensen C. The effect of karate techniques training on communication deficit of children with autism spectrum disorders. *J Autism Dev Disord*. 2016;46:978-986.
307. Gabriels RL, Agnew JA, Holt KD, et al. Pilot study measuring the effects of therapeutic horseback riding on school-age children and adolescents with autism spectrum disorders. *Autism Spectrum Disorders*. 2012;6(2):578-588.
308. Levinson LJ, Reid G. The effects of exercise intensity on the stereotypic behaviors of individuals with autism. *Adapt Phys Activity Quart*. 1993;10(3):255-268.
309. Rosenthal-Malek A, Mitchell S. Brief report: the effects of exercise on the self-stimulatory behaviors and positive responding of adolescents with autism. *J Autism Dev Disord*. 1997;27(2):193-202.
310. Pan C-Y. Effects of water exercise swimming program on aquatic skills and social behaviors in children with autism spectrum disorders. *Autism*. 2010;14(1):9-28.
311. Yilmaz I, Yanardağ M, Birkan B, Bumin G. Effects of swimming training on physical fitness and water orientation in autism. *Pediatr Int*. 2004;46(5):624-626.
312. Movahedi A, Bahrami F, Marandi SM, Abedi A. Improvement in social dysfunction of children with autism spectrum disorder following long term Kata techniques training. *Res Autism Spectrum Disorders*. 2013;7(9):1054-1061.
313. Todd T, Reid G, Butler-Kisber L. Cycling for students with ASD: self-regulation promotes sustained physical activity. *Adapt Phys Activity Quart*. 2010;27(3):226-241.
314. Koenig KP, Buckley-Reen A, Garg S. Efficacy of the get ready to learn yoga program among children with autism spectrum disorders: a pretest–posttest control group design. *Am J Occup Ther*. 2012;66(5):538-546.
315. Rosenblatt LE, Gorantla S, Torres JA, et al. Relaxation response–based yoga improves functioning in young children with autism: a pilot study. *J Alternative Complement Med*. 2011;17(11):1029-1035.
316. Toscano CV, Barros L, Lima AB, Nunes T, Carvalho HM, Gaspar JM. Neuroinflammation in autism spectrum disorders: exercise as a "pharmacological" tool. *Neurosci Biobehav Rev*. 2021;129:63-74.
317. Ferreira JP, Ghiarone T, Junior CRC, et al. Effects of physical exercise on the stereotyped behavior of children with autism spectrum disorders. *Medicina (Mex)*. 2019;55(10):685.
318. Olin SS, McFadden BA, Golem DL, et al. The effects of exercise dose on stereotypical behavior in children with autism. *Med Sci Sports Exercise*. 2017;49(5):983-990.
319. Colasanto M, Madigan S, Korczak DJ. Depression and inflammation among children and adolescents: a meta-analysis. *J Affect Disord*. 2020;277:940-948.
320. Liu JJ, Wei YB, Strawbridge R, et al. Peripheral cytokine levels and response to antidepressant treatment in depression: a systematic review and meta-analysis. *Mol Psychiatry*. 2020;25(2):339-350.
321. Reschke-Hernández AE. History of music therapy treatment interventions for children with autism. *J Music Ther*. 2011;48(2):169-207.
322. Brownell MD. Musically adapted social stories to modify behaviors in students with autism: four case studies. *J Music Ther*. 2002;39(2):117-144.
323. Fees BS, Kaff M, Holmberg T, Teagarden J, Delreal D. Children's responses to a social story song in three inclusive preschool classrooms: a pilot study. *Music Ther Perspect*. 2014;32(1):71-77.

324. Kern P, Wolery M, Aldridge D. Use of songs to promote independence in morning greeting routines for young children with autism. *J Autism Dev Disord*. 2007;37:1264-1271.
325. Kern P, Aldridge D. Using embedded music therapy interventions to support outdoor play of young children with autism in an inclusive community-based child care program. *J Music Ther*. 2006;43(4):270-294.
326. Kaplan RS, Steele AL. An analysis of music therapy program goals and outcomes for clients with diagnoses on the autism spectrum. *J Music Ther*. 2005;42(1):2-19.
327. Kim J, Wigram T, Gold C. The effects of improvisational music therapy on joint attention behaviors in autistic children: a randomized controlled study. *J Autism Dev Disord*. 2008;38:1758-1766.
328. LaGasse AB. Effects of a music therapy group intervention on enhancing social skills in children with autism. *J Music Ther*. 2014;51(3):250-275.
329. Ulfarsdottir LO, Erwin PG. The influence of music on social cognitive skills. *Arts Psychother*. 1999;26(2):81-84.
330. Lim HA. Effect of "developmental speech and language training through music" on speech production in children with autism spectrum disorders. *J Music Ther*. 2010;47(1):2-26.
331. Lim HA, Draper E. The effects of music therapy incorporated with applied behavior analysis verbal behavior approach for children with autism spectrum disorders. *J Music Ther*. 2011;48(4):532-550.
332. Vaiouli P, Grimmet K, Ruich LJ. "Bill is now singing": joint engagement and the emergence of social communication of three young children with autism. *Autism*. 2015;19(1):73-83.
333. Paul A, Sharda M, Menon S, et al. The effect of sung speech on socio-communicative responsiveness in children with autism spectrum disorders. *Front Human Neurosci*. 2015;9:555.
334. Gee BM, Thompson K, St John H. Efficacy of a sound-based intervention with a child with an autism spectrum disorder and auditory sensory over-responsivity. *Occup Ther Int*. 2014;21(1):12-20.
335. Hillier A, Greher G, Poto N, Dougherty M. Positive outcomes following participation in a music intervention for adolescents and young adults on the autism spectrum. *Psychol Music*. 2012;40(2):201-215.
336. Finnigan E, Starr E. Increasing social responsiveness in a child with autism: a comparison of music and non-music interventions. *Autism*. 2010;14(4):321-348.
337. Thompson G, McFerran K, Gold C. Family-centred music therapy to promote social engagement in young children with severe autism spectrum disorder: a randomized controlled study. *Child Care Health Dev*. 2014;40(6):840-852.
338. Schwartzberg ET, Silverman MJ. Effects of music-based social stories on comprehension and generalization of social skills in children with autism spectrum disorders: a randomized effectiveness study. *Arts Psychother*. 2013;40(3):331-337.
339. Corbett BA, Shickman K, Ferrer E. Brief report: the effects of Tomatis sound therapy on language in children with autism. *J Autism Dev Disord*. 2008;38:562-566.
340. Rapp JT, Vollmer TR. Stereotype I: a review of behavioral assessment and treatment. *Res Dev Disabil*. 2005;26(6):527-547.
341. Vivanti G, Zhong HN. Naturalistic developmental behavioral interventions for children with autism. *Clinical Guide to Early Interventions for Children With Autism*. 2020:93-130.
342. Snyder TD, De Brey C, Dillow SA. Digest of Education Statistics 2014, NCES 2016-006. *National Center for Education Statistics*. 2016.
343. Murphy MA, Ruble LA. A comparative study of rurality and urbanicity on access to and satisfaction with services for children with autism spectrum disorders. *Rural Special Educ Quart*. 2012;31(3):3-11.
344. Burrell TL. Parents' involvement in ASD treatment: what is their role? *Cognit Behav Pract*. 2012;19(3):423-432.
345. Downer JT, Pianta RC. Academic and cognitive functioning in first grade: associations with earlier home and child care predictors and with concurrent home and classroom experiences. *School Psychol Rev*. 2006;35(1):11-30.
346. Webster-Stratton C, Herman KC. The impact of parent behavior-management training on child depressive symptoms. *J Counsel Psychol*. 2008;55(4):473.
347. Menting AT, de Castro BO, Matthys W. Effectiveness of the incredible years parent training to modify disruptive and prosocial child behavior: a meta-analytic review. *Clin Psychol Rev*. 2013;33(8):901-913.
348. Neitzel C, Stright A. Relations between mothers' scaffolding and children's academic self-regulation: establishing a foundation of self-regulatory competence. *J Fam Psychol*. 2003;17(1):147-159.
349. Cline KD, Edwards CP. The instructional and emotional quality of parent–child book reading and early head start children's learning outcomes. *Early Educ Dev*. 2013;24(8):1214-1231.
350. Ingersoll B, Wainer A. Initial efficacy of Project ImPACT: a parent-mediated social communication intervention for young children with ASD. *J Autism Dev Disord*. 2013;43:2943-2952.
351. Matson ML, Mahan S, Matson JL. Parent training: a review of methods for children with autism spectrum disorders. *Res Autism Spectrum Disorders*. 2009;3(4):868-875.
352. McConachie H, Diggle T. Parent implemented early intervention for young children with autism spectrum disorder: a systematic review. *J Eval Clin Pract*. 2007;13(1):120-129.
353. Wong C, Odom SL, Hume KA, et al. Evidence-based practices for children, youth, and young adults with autism spectrum disorder: a comprehensive review. *J Autism Dev Disord*. 2015;45:1951-1966.
354. Kim EM, Sheridan SM. Foundational aspects of family–school connections: definitions, conceptual frameworks, and research needs. *Foundational Aspects of Family–School Partnership Research*. 2015:1-14.
355. Sheridan SM, Bovaird JA, Glover TA, Andrew Garbacz S, Witte A, Kwon K. A randomized trial examining the effects of conjoint behavioral consultation and the mediating role of the parent–teacher relationship. *School Psychol Rev*. 2012;41(1):23-46.
356. Rispoli MJ, Franco JH, van der Meer L, Lang R, Camargo SPH. The use of speech generating devices in communication interventions for individuals with developmental disabilities: a review of the literature. *Dev Neurorehab*. 2010;13(4):276-293.
357. Mirenda P. Toward functional augmentative and alternative communication for students with autism: manual signs, graphic symbols, and voice output communication aids. *Lang Speech Hear Serv Sch*. 2003;34(3):203-216.
358. Lorah ER, Parnell A, Whitby PS, Hantula D. A systematic review of tablet computers and portable media players as

speech generating devices for individuals with autism spectrum disorder. *J Autism Dev Disord*. 2015;45:3792-3804.
359. Lorah ER, Tincani M, Dodge J, Gilroy S, Hickey A, Hantula D. Evaluating picture exchange and the iPad™ as a speech generating device to teach communication to young children with autism. *J Dev Phys Disab*. 2013;25:637-649.
360. Schlosser R. Roles of speech output in augmentative and alternative communication: narrative review. *Augment Altern Commun*. 2003;19(1):5-27.
361. Ganz JB, Earles-Vollrath TL, Heath AK, Parker RI, Rispoli MJ, Duran JB. A meta-analysis of single case research studies on aided augmentative and alternative communication systems with individuals with autism spectrum disorders. *J Autism Dev Disord*. 2012;42:60-74.
362. Schlosser RW, Koul RK. Speech output technologies in interventions for individuals with autism spectrum disorders: a scoping review. *Augment Altern Commun*. 2015;31(4):285-309.
363. Mineo BA, Ziegler W, Gill S, Salkin D. Engagement with electronic screen media among students with autism spectrum disorders. *J Autism Dev Disord*. 2009;39:172-187.
364. Schmidt M, Laffey JM, Schmidt CT, Wang X, Stichter J. Developing methods for understanding social behavior in a 3D virtual learning environment. *Comput Hum Behav*. 2012;28(2):405-413.
365. Bailenson J, Patel K, Nielsen A, Bajscy R, Jung S-H, Kurillo G. The effect of interactivity on learning physical actions in virtual reality. *Media Psychol*. 2008;11(3):354-376.
366. Blascovich J, Loomis J, Beall AC, Swinth KR, Hoyt CL, Bailenson JN. Immersive virtual environment technology as a methodological tool for social psychology. *Psychol Inquiry*. 2002;13(2):103-124.
367. Wallace S, Coleman M, Bailey A. An investigation of basic facial expression recognition in autism spectrum disorders. *Cogn Emotion*. 2008;22(7):1353-1380.
368. Kandalaft MR, Didehbani N, Krawczyk DC, Allen TT, Chapman SB. Virtual reality social cognition training for young adults with high-functioning autism. *J Autism Dev Disord*. 2013;43:34-44.
369. Pennisi P, Tonacci A, Tartarisco G, et al. Autism and social robotics: a systematic review. *Autism Res*. 2016;9(2):165-183.
370. Diehl JJ, Crowell CR, Villano M, Wier K, Tang K, Riek LD. Clinical applications of robots in autism spectrum disorder diagnosis and treatment. *Comprehensive Guide to Autism*. 2014:411-422.
371. Diehl JJ, Schmitt LM, Villano M, Crowell CR. The clinical use of robots for individuals with autism spectrum disorders: a critical review. *Res Autism Spectrum Disord*. 2012;6(1):249-262.
372. Ricks DJ, Colton MB. *Trends and Considerations in Robot-Assisted Autism Therapy*. IEEE; 2010:4354-4359.
373. Scassellati B, Admoni H, Matarić M. Robots for use in autism research. *Annu Rev Biomed Eng*. 2012;14:275-294.
374. Feil-Seifer D, Matarić MJ. *Toward Socially Assistive Robotics for Augmenting Interventions for Children With Autism Spectrum Disorders*. Springer; 2009:201-210.
375. Scassellati B. *How Social Robots Will Help Us to Diagnose, Treat, and Understand Autism*. Springer; 2007:552-563.
376. Cabibihan J-J, Javed H, Ang M, Aljunied SM. Why robots? A survey on the roles and benefits of social robots in the therapy of children with autism. *Int J Social Robot*. 2013;5:593-618.
377. Kumazaki H, Muramatsu T, Yoshikawa Y, et al. Role-play-based guidance for job interviews using an android robot for individuals with autism spectrum disorders. *Front Psychiatry*. 2019;10:239.
378. Anzalone SM, Tilmont E, Boucenna S, et al. How children with autism spectrum disorder behave and explore the 4-dimensional (spatial 3D+ time) environment during a joint attention induction task with a robot. *Res Autism Spectrum Disord*. 2014;8(7):814-826.
379. Robins B, Dautenhahn K, Boekhorst RT, Billard A. Robotic assistants in therapy and education of children with autism: can a small humanoid robot help encourage social interaction skills? *Universal Access Inform Soc*. 2005;4:105-120.
380. Kozima H, Nakagawa C, Kawai N, Kosugi D, Yano Y. *A Humanoid in Company With Children*. IEEE; 2004:470-477.
381. Kozima H, Nakagawa C, Yasuda Y. *Interactive Robots for Communication-Care: A Case-Study in Autism Therapy*. IEEE; 2005:341-346.
382. Maniram J, Karrim SB, Oosthuizen F, Wiafe E. Pharmacological management of core symptoms and comorbidities of autism spectrum disorder in children and adolescents: a systematic review. *Neuropsychiatr Disease Treat*. 2022;18:1629-1644.
383. Yu Y, Chaulagain A, Pedersen SA, et al. Pharmacotherapy of restricted/repetitive behavior in autism spectrum disorder: a systematic review and meta-analysis. *BMC Psychiatry*. 2020;20(1):1-11.
384. Lim JJ, Anagnostou E. Biomedical interventions for autism spectrum disorder. *Neurodevelopmental Pediatrics: Genetic and Environmental Influences*. Springer; 2023:327-335.
385. Deb S, Roy M, Limbu B, Bertelli M. Anti-anxiety medications and novel treatments for autism. *Handbook of Autism and Pervasive Developmental Disorder: Assessment, Diagnosis, and Treatment*. Springer; 2022:1157-1172.
386. Hurwitz R, Blackmore R, Hazell P, Williams K, Woolfenden S. Tricyclic antidepressants for autism spectrum disorders (ASD) in children and adolescents. *Cochrane Database System Rev*. 2012:CD008372.
387. Mathew S, Bichenapally S, Khachatryan V, et al. Role of serotoninergic antidepressants in the development of autism spectrum disorders: a systematic review. *Cureus*. 2022;14(8):e28505.
388. Häge A, Banaschewski T, Buitelaar JK, et al. Glutamatergic medication in the treatment of obsessive compulsive disorder (OCD) and autism spectrum disorder (ASD)—study protocol for a randomised controlled trial. *Trials*. 2016;17(1):1-16.
389. Jobski K, Höfer J, Hoffmann F, Bachmann C. Use of psychotropic drugs in patients with autism spectrum disorders: a systematic review. *Acta Psychiatr Scand*. 2017;135(1):8-28.
390. Rodrigues R, Lai MC, Beswick A, et al. Practitioner review: pharmacological treatment of attention-deficit/hyperactivity disorder symptoms in children and youth with autism spectrum disorder: a systematic review and meta-analysis. *J Child Psychol Psychiatr*. 2021;62(6):680-700.
391. Farmer CA, Aman MG. Aripiprazole for the treatment of irritability associated with autism. *Expert Opin Pharmacother*. 2011;12(4):635-640.
392. Hesapcioglu ST, Ceylan MF, Kasak M, Sen CP. Olanzapine, risperidone, and aripiprazole use in children and adolescents with autism spectrum disorders. *Autism Spectrum Disorders*. 2020;72:101520.

393. Reiersen AM, Handen B. Commentary on 'Selective serotonin reuptake inhibitors (SSRIs) for autism spectrum disorders (ASD)'. *Evidence-Based Child Health Cochrane Rev J*. 2011;6(4):1082-1085.
394. Williams K, Wheeler DM, Silove N, Hazell P. Cochrane review: selective serotonin reuptake inhibitors (SSRIs) for autism spectrum disorders (ASD). *Evidence-Based Child Health Cochrane Rev J*. 2011;6(4):1044-1078.
395. Reddihough DS, Marraffa C, Mouti A, et al. Effect of fluoxetine on obsessive-compulsive behaviors in children and adolescents with autism spectrum disorders: a randomized clinical trial. *JAMA*. 2019;322(16):1561-1569.
396. Green J, Garg S. Annual research review: the state of autism intervention science: progress, target psychological and biological mechanisms and future prospects. *J Child Psychol Psychiatry*. 2018;59(4):424-443.
397. Murari K, Abushaibah A, Rho JM, Turner RW, Cheng N. A clinically relevant selective ERK-pathway inhibitor reverses core deficits in a mouse model of autism. *EBioMedicine*. 2023;91:104565.
398. Pacheva I, Ivanov I. Targeted biomedical treatment for autism spectrum disorders. *Curr Pharm Des*. 2019;25(41):4430-4453.
399. Majhi S, Kumar S, Singh L. A review on autism spectrum disorder: pathogenesis, biomarkers, pharmacological and non-pharmacological interventions. *CNS Neurol Disord Drug Targets*. 2023;22(5):659-677.
400. Liu Y, Yang Z, Du Y, Shi S, Cheng Y. Antioxidant interventions in autism spectrum disorders: a meta-analysis. *Prog Neuropsychopharmacol Biol Psychiatry*. 2022;113:110476.
401. Bent S, Lawton B, Warren T, et al. Identification of urinary metabolites that correlate with clinical improvements in children with autism treated with sulforaphane from broccoli. *Mol Autism*. 2018;9:1-12.
402. Hendouei F, Sanjari Moghaddam H, Mohammadi MR, Taslimi N, Rezaei F, Akhondzadeh S. Resveratrol as adjunctive therapy in treatment of irritability in children with autism: a double-blind and placebo-controlled randomized trial. *J Clin Pharm Ther*. 2020;45(2):324-334.
403. Mousavinejad E, Ghaffari MA, Riahi F, Hajmohammadi M, Tiznobeyk Z, Mousavinejad M. Coenzyme Q10 supplementation reduces oxidative stress and decreases antioxidant enzyme activity in children with autism spectrum disorders. *Psychiatry Res*. 2018;265:62-69.
404. Nikoo M, Radnia H, Farokhnia M, Mohammadi M-R, Akhondzadeh S. N-acetylcysteine as an adjunctive therapy to risperidone for treatment of irritability in autism: a randomized, double-blind, placebo-controlled clinical trial of efficacy and safety. *Clin Neuropharmacol*. 2015;38(1):11-17.
405. Bent S, Hendren RL, Zandi T, et al. Internet-based, randomized, controlled trial of omega-3 fatty acids for hyperactivity in autism. *J Am Acad Child Adolesc Psychiatry*. 2014;53(6):658-666.
406. Yui K, Koshiba M, Nakamura S, Kobayashi Y. Effects of large doses of arachidonic acid added to docosahexaenoic acid on social impairment in individuals with autism spectrum disorders: a double-blind, placebo-controlled, randomized trial. *J Clin Psychopharmacol*. 2012;32(2):200-206.
407. Hendren RL, James SJ, Widjaja F, Lawton B, Rosenblatt A, Bent S. Randomized, placebo-controlled trial of methyl B12 for children with autism. *J Child Adolesc Psychopharmacol*. 2016;26(9):774-783.
408. Lynch R, Diggins EL, Connors SL, et al. Sulforaphane from broccoli reduces symptoms of autism: a follow-up case series from a randomized double-blind study. *Global Adv Health Med*. 2017;6:2164957×17735826.
409. Al-Ayadhi LY, Elamin NE. Camel milk as a potential therapy as an antioxidant in autism spectrum disorder (ASD). *Evid-Based Complement Altern Med*. 2013;2013:602834.
410. Sporn MB, Liby KT. NRF2 and cancer: the good, the bad and the importance of context. *Nat Rev Cancer*. 2012;12(8):564-571.
411. Yang J, Fu X, Liao X, Li Y. Nrf2 activators as dietary phytochemicals against oxidative stress, inflammation, and mitochondrial dysfunction in autism spectrum disorders: a systematic review. *Front Psychiatr*. 2020;11:561998.
412. Liu X, Lin J, Zhang H, et al. Oxidative stress in autism spectrum disorder—current progress of mechanisms and biomarkers. *Front Psychiatry*. 2022;13:813304.
413. Frye RE, Sequeira J, Quadros E, James S, Rossignol D. Cerebral folate receptor autoantibodies in autism spectrum disorder. *Mol Psychiatry*. 2013;18(3):369-381.
414. James SJ, Melnyk S, Fuchs G, et al. Efficacy of methylcobalamin and folinic acid treatment on glutathione redox status in children with autism. *Am J Clin Nutr*. 2009;89(1):425-430.
415. Frye RE, Melnyk S, Fuchs G, et al. Effectiveness of methylcobalamin and folinic acid treatment on adaptive behavior in children with autistic disorder is related to glutathione redox status. *Autism Res Treat*. 2013;2013:609705.
416. Naviaux RK, Curtis B, Li K, et al. Low-dose suramin in autism spectrum disorder: a small, phase I/II, randomized clinical trial. *Ann Clin Transl Neurol*. 2017;4(7):491-505.
417. Pangrazzi L, Balasco L, Bozzi Y. Natural antioxidants: a novel therapeutic approach to autism spectrum disorders? *Antioxidants*. 2020;9(12):1186.
418. Al-Amin MM, Rahman MM, Khan FR, Zaman F, Reza HM. Astaxanthin improves behavioral disorder and oxidative stress in prenatal valproic acid-induced mice model of autism. *Behav Brain Res*. 2015;286:112-121.
419. Ajami M, Pazoki-Toroudi H, Amani H, et al. Therapeutic role of sirtuins in neurodegenerative disease and their modulation by polyphenols. *Neurosci Biobehav Rev*. 2017;73:39-47.
420. Bertolino B, Crupi R, Impellizzeri D, et al. Beneficial effects of co-ultramicronized palmitoylethanolamide/luteolin in a mouse model of autism and in a case report of autism. *CNS Neurosci Ther*. 2017;23(1):87-98.
421. Bozzatello P, Brignolo E, De Grandi E, Bellino S. Supplementation with omega-3 fatty acids in psychiatric disorders: a review of literature data. *J Clin Med*. 2016;5(8):67.
422. Niederhofer H. First preliminary results of an observation of Ginkgo Biloba treating patients with autistic disorder. *Phytother Res*. 2009;23(11):1645-1646.
423. Singh R, Kisku A, Kungumaraj H, et al. Autism spectrum disorders: a recent update on targeting inflammatory pathways with natural anti-inflammatory agents. *Biomedicines*. 2023;11(1):115.
424. Liao X, Li Y. Nuclear factor kappa B in autism spectrum disorder: a systematic review. *Pharmacol Res*. 2020;159:104918.
425. Xin P, Xu X, Deng C, et al. The role of JAK/STAT signaling pathway and its inhibitors in diseases. *Int Immunopharmacol*. 2020;80:106210.

426. Parker-Athill E, Luo D, Bailey A, et al. Flavonoids, a prenatal prophylaxis via targeting JAK2/STAT3 signaling to oppose IL-6/MIA associated autism. *J Neuroimmunol*. 2009;217(1–2):20-27.
427. Zawadzka A, Cieślik M, Adamczyk A. The role of maternal immune activation in the pathogenesis of autism: a review of the evidence, proposed mechanisms and implications for treatment. *Int J Mol Sci*. 2021;22(21):11516.
428. Choi GB, Yim YS, Wong H, et al. The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring. *Science*. 2016;351(6276):933-939.
429. Marchezan J, Winkler dos Santos EGA, Deckmann I, Dos Santos Riesgo R. Immunological dysfunction in autism spectrum disorder: a potential target for therapy. *NeuroImmunoModulation*. 2019;25(5–6):300-319.
430. Arteaga-Henríquez G, Gisbert L, Ramos-Quiroga JA. Immunoregulatory and/or anti-inflammatory agents for the management of core and associated symptoms in individuals with autism spectrum disorder: a narrative review of randomized, placebo-controlled trials. *CNS Drugs*. 2023;37(3):215-229.
431. Siafis S, Çıray O, Wu H, et al. Pharmacological and dietary-supplement treatments for autism spectrum disorder: a systematic review and network meta-analysis. *Mol Autism*. 2022;13(1):1-17.
432. Hafizi S, Tabatabaei D, Lai M-C. Review of clinical studies targeting inflammatory pathways for individuals with autism. *Front Psychiatr*. 2019;10:849.
433. Liu J, Gao Z, Liu C, et al. Alteration of gut microbiota: new strategy for treating autism spectrum disorder. *Front Cell Dev Biol*. 2022;10:792490.
434. Rao AV, Bested AC, Beaulne TM, et al. A randomized, double-blind, placebo-controlled pilot study of a probiotic in emotional symptoms of chronic fatigue syndrome. *Gut Pathogens*. 2009;1(1):1-6.
435. Silk D, Davis A, Vulevic J, Tzortzis G, Gibson G. Clinical trial: the effects of a trans-galactooligosaccharide prebiotic on faecal microbiota and symptoms in irritable bowel syndrome. *Aliment Pharmacol Ther*. 2009;29(5):508-518.
436. Liu G, Yuan L. Analysis on the etiology, pathogenesis and syndrome differentiation of children with autism in traditional Chinese medicine. *Liaoning J Trad Chin Med*. 2007;9:1226-1227.
437. Jiang X, Cai Z, Zhang Z, Li A, Cheng Y, Lyu Y. Combined treatment of children with autism with modified yinhuo decoction and therapeutic interventions. *Chin J Trad Chin Med Pharm*. 2016;31(10):4322-4324.
438. Zhou N, Li Y, Jiang X, Lu Y. Clinical observation of supplemented Lizhong decoction in treating children autism. *J New Chin Med*. 2015;47(6):200-202.
439. Wu H, Wu Z. Trinity" traditional chinese medicine treatment of autism. *Chin Med Herald*. 2006;11:116-117.
440. Kang D-W, Adams JB, Gregory AC, et al. Microbiota transfer therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. *Microbiome*. 2017;5(1):1-16.
441. Zhang S, Chen Q, Kelly CR, et al. Donor screening for fecal microbiota transplantation in China: evaluation of 8483 candidates. *Gastroenterology*. 2022;162(3):966-968.e3.
442. Wang J, Cao Y, Hou W, et al. Fecal microbiota transplantation improves VPA-induced ASD mice by modulating the serotonergic and glutamatergic synapse signaling pathways. *Transl Psychiatry*. 2023;13(1):17.
443. Kang D-W, Adams JB, Coleman DM, et al. Long-term benefit of microbiota transfer therapy on autism symptoms and gut microbiota. *Sci Rep*. 2019;9(1):5821.
444. Saurman V, Margolis KG, Luna RA. Autism spectrum disorder as a brain–gut–microbiome axis disorder. *Dig Dis Sci*. 2020;65(3):818-828.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Zhuang H, Liang Z, Ma G, et al. Autism spectrum disorder: pathogenesis, biomarker, and intervention therapy. *MedComm*. 2024;5:e497. https://doi.org/10.1002/mco2.497

Biochemical Pharmacology 205 (2022) 115261

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Biosynthesis and metabolism of endocannabinoids and their congeners from the monoacylglycerol and *N*-acyl-ethanolamine families

Mélissa Simard [a,b,1], Anne-Sophie Archambault [a,b,1,2], Jean-Philippe C. Lavoie [a,b], Élizabeth Dumais [a,b], Vincenzo Di Marzo [a,b,c,d,e], Nicolas Flamand [a,b,*]

[a] *Centre de Recherche de l'Institut Universitaire de Cardiologie et de Pneumologie de Québec, Département of Médecine, Faculté de Médecine, Université Laval, Québec City, QC G1V 4G5, Canada*

[b] *Canada Excellence Research Chair on the Microbiome-Endocannabinoidome Axis in Metabolic Health (CERC-MEND), Université Laval, Québec City, QC G1V 0A6, Canada*

[c] *Endocannabinoid Research Group, Institute of Biomolecular Chemistry, Consiglio Nazionale Delle Ricerche (CNR), 80078 Pozzuoli, Italy*

[d] *Institut sur la Nutrition et les Aliments Fonctionnels, Centre NUTRISS, École de Nutrition, Faculté des Sciences de l'agriculture et de l'alimentation, Université Laval, Québec City, QC G1V 0A6, Canada*

[e] *Joint International Unit between the Consiglio Nazionale delle Ricerche (Italy) and Université Laval (Canada) on Chemical and Biomolecular Research on the Microbiome and Its Impact on Metabolic Health and Nutrition (UMI-MicroMeNu)*

A R T I C L E I N F O

Keywords:
Endocannabinoid
Cyclooxygenase
Lipoxygenase
Cytrochrome P450
Hydrolase

A B S T R A C T

The endocannabinoids 2-arachidonoyl-glycerol (2-AG) and *N*-arachidonoyl-ethanolamine (AEA) are eicosanoids implicated in numerous physiological processes like appetite, adipogenesis, inflammatory pain and inflammation. They mediate most of their physiological effects by activating the cannabinoid (CB) receptors 1 and 2. Other than directly binding to the CB receptors, 2-AG and AEA are also metabolized by most eicosanoid biosynthetic enzymes, yielding many metabolites that are part of the oxyendocannabinoidome. Some of these metabolites have been found *in vivo*, have the ability to modulate specific receptors and thus potentially influence physiological processes. In this review, we discuss the biosynthesis and metabolism of 2-AG and AEA, as well as their congeners from the monoacyl-glycerol and *N*-acyl-ethanolamine families, with a special focus on the metabolism by oxygenases involved in arachidonic acid metabolism. We highlight the knowledge gaps in our understanding of the regulation and roles the oxyendocannabinoidome mediators.

1. Introduction

Cannabinoid- and endocannabinoid-related research truly jumpstarted with the structure elucidation of (−)-Δ^9-tetrahydrocannabinol, the main psychoactive substance found in cannabis, and the chemical synthesis of its structural analog CP 55,940 [1–4]. These two

Abbreviations: **12-HTTre**, 12(S)-hydroxyheptadeca-5*Z*,8*E*,10*E*-trienoic acid; **AA**, arachidonic acid; **ABHD**, α/β Hydrolase Domain-Containing Protein; **AEA**, *N*-arachidonoyl-ethanolamine; **2-AG**, 2-arachidonoyl-glycerol; **CB_1**, cannabinoid receptor 1; **CB_2**, cannabinoid receptor 2; **CES**, carboxylesterase; **COX**, cyclooxygenase; **CYP**, Cytochrome; **DAG**, diacylglycerol; **DHEA**, *N*-docosahexaenoyl-ethanolamine; **DHG**, docosahexaenoyl-glycerol; **DiHET**, Dihydroxy-eicosatrienic acid; **DPEA**, *N*-docosapentaenoyl-ethanolamine; **DPG**, docosapentaenoyl-glycerol; **-EA**, ethanolamine; **EDP-EA**, epoxydocosapentaenoic acid-ethanolamide; **EET**, epoxyeicosatrienoic acid; **EEQ-EA**, epoxyeicosatetraenoic acid-ethanolamide; **EPEA**, *N*-eicosapentaenoyl-ethanolamine; **EPG**, eicosapentaenoyl-glycerol; **EX**, eoxin; **FAAH**, Fatty Acid Amide Hydrolase; **G**, glycerol; **GDP1**, glycerophosphodiesterase 1; **HDHEA**, hydroxy-DHEA; **HEET-EA**, hydroxy-epoxyeicosatrienoyl-ethanolamides; **HDPA**, Hydroxy-docosapentaenoic acid; **HETE**, hydroxyeicosatetraenoic acid; **LEA**, *N*-linoleoyl-ethanolamine; **LG**, linoleoyl-glycerol; **LO**, lipoxygenase; **LT**, leukotriene; **$LYPLA_2$**, lysophospholipase 2; **MAG**, monoacyl-glycerol; **NAAA**, *N*-acylethanolamine acid amidase; **NAE**, *N*-acyl-ethanolamine; **NAPE**, *N*-acyl-phosphaditylethanolamines; **NAPE-PLD**, NAPE-specific phospholipase D; **PA**, phosphatidic acid; **PC**, Phosphatidyl-choline; **PG**, prostaglandin; **PI**, Phosphatidyl-inositol; **PL**, phospholipase; **PPAR**, Peroxisome proliferator-activated receptor; **PPT1**, palmitoyl-protein thioesterase 1; PTPN22, Protein Tyrosine Phosphatase Non-Receptor Type 22; **TRPV**, transient receptor potential vanilloid; **TX**, Thromboxane.

* Corresponding author at: Centre de recherche de l'IUCPQ – Université Laval, 2725 chemin Sainte-Foy, Room A3140, Québec City, QC G1V 4G5, Canada.
E-mail address: Nicolas.Flamand@criucpq.ulaval.ca (N. Flamand).

[1] These authors contributed equally.

[2] Present address: Department of Pathology and Laboratory Medicine, University of British Columbia / BC Children's Hospital Research Institute, Vancouver, British Colombia, Canada.

https://doi.org/10.1016/j.bcp.2022.115261
Received 31 July 2022; Received in revised form 14 September 2022; Accepted 15 September 2022
Available online 21 September 2022

key compounds allowed the pharmacological characterization and the cloning of a specific receptor localized in the brain, now referred to as the cannabinoid receptor 1 or CB_1 [5–7]. Another cannabinoid receptor, the CB_2 receptor, was next cloned from HL-60 cells, a human promyelocytic cell line [8]. The CB_1 and CB_2 receptors are G protein-coupled receptors and their activation triggers signaling events such as adenylyl cyclase inhibition and mitogen activated protein kinases activation [9]. These receptors are involved in the regulation of numerous functions, notably appetite, adipogenesis, inflammatory pain and inflammation [10–12].

Soon after the cloning of the CB_1 and CB_2 receptors, their endogenous ligands were identified. The first endocannabinoid, *N*-arachidonoyl-ethanolamine (AEA), was identified by Devane and colleagues in porcine brain extracts and named anandamide, which is an amalgam of the Sanskrit term Ananda (meaning bliss and denoting its psychoactive effects) and amide, referring to the molecular structure of AEA [13]. The subsequent cloning of the CB_2 receptor by Munro and colleagues consolidated AEA as a potent endocannabinoid that activates both the CB_1 and CB_2 receptors [8]. 2-Arachidonoyl-glycerol (2-AG) was next identified by two independent groups as a ligand for both the CB_1 and CB_2 receptors [14,15]. Interestingly, 2-AG and AEA are structurally unrelated to (−)-Δ^9-tetrahydrocannabinol [16]. AEA and 2-AG, together with enzymes for their biosynthesis and degradation and the CB receptors, were proposed to form a new signaling system, known as the endocannabinoidome [12].

AEA and 2-AG are eicosanoids containing a molecule of arachidonic acid (AA) in their structure. They are usually regarded as the only two endocannabinoids, despite the fact that other lipid-related molecules,

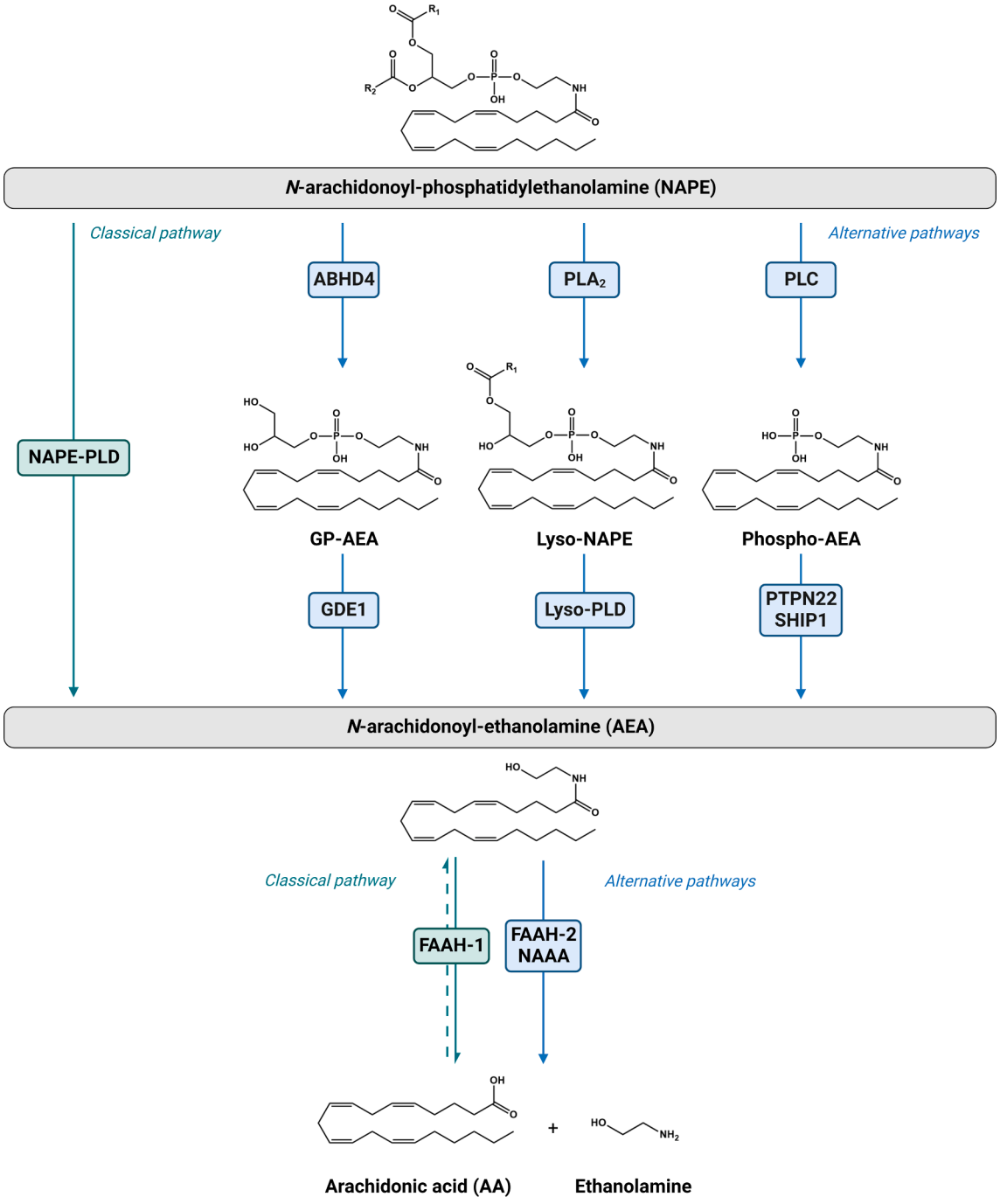


Fig. 1. Biosynthesis and hydrolysis of the endocannabinoid AEA. Created with BioRender.com.

such as noladin ether, can also activate the CB_1 and CB_2 receptors to a different extent and that most long chain *N*-acyl-ethanolamines (NAEs) can weakly activate the CB_2 receptor [17–20]. In order to fully comprehend and appreciate the endocannabinoid system, a new term has emerged: the endocannabinoidome. It comprises AEA and 2-AG, their congeners from the NAE and monoacylglycerol (MAG) families, *N*-acyl-amines, their oxidized metabolites, anabolic and catabolic enzymes, as well as the receptors they activate [12].

As mentioned above, AEA and 2-AG are eicosanoids. As such, they are metabolized by eicosanoid biosynthetic enzymes, notably cyclooxygenase (COX)-2, cytochrome P450 enzymes, and some lipoxygenases (LOs). Herein, we will review the biosynthesis and enzymatic hydrolysis of AEA and 2-AG, as well as their metabolism by eicosanoid biosynthetic enzymes.

2. Biosynthesis and hydrolysis of AEA and its congeners

AEA (Fig. 1) arises from the metabolism of AA-containing *N*-acyl-phosphatidylethanolamines (NAPEs). The current knowledge indicates that NAPEs are obtained following the acylation of the amine group of phosphatidyl-ethanolamines by Ca^{2+}-dependent and -independent *N*-acyl-transferases including the cytosolic phospholipase $A_2\varepsilon$ [21–24]. The biosynthesis of AEA (and other NAEs) then occurs by four biosynthetic routes (Fig. 1). The first and most straightforward biosynthetic pathway consists in a one-step hydrolysis of NAPEs by the NAPE-specific phospholipase D (NAPE-PLD) [25–28]. A second pathway has also been described in which the hydrolysis of NAPEs by a PLC occurs, generating a phospho-AEA that is dephosphorylated by phosphatases such as Protein Tyrosine Phosphatase Non-Receptor Type 22 (PTPN22) or SHIP1 [29,30]. A third biosynthetic pathway involves a PLA_2 activity generating a lyso-NAPE that is next hydrolyzed by a lyso-PLD [31]. The fourth AEA biosynthetic pathway involves the generation of a lyso-NAPE by a PLA_2, its hydrolysis into glycerophospho-AEA by the α/β Hydrolase Domain-Containing Protein 4 (ABHD4), followed by the action of glycerophosphodiesterase 1 (GDP1) [32]. In mice, the deletion of NAPE-PLD leads to decreased levels of AEA and other NAEs, indicating that this one-step biosynthetic route is very important but not necessarily the predominant one [28,33–36].

There is a limited number of enzymes involved in the hydrolysis of AEA and other NAEs. The first and main enzyme responsible for the hydrolysis of AEA is the Fatty Acid Amide Hydrolase (FAAH)-1, which was shown to hydrolyze AEA into AA and ethanolamine as well as allowing the biosynthesis of AEA in presence of high (supra-physiological) concentrations of AA and ethanolamine by allowing the condensation of ethanolamine and AA, at least in rats [37–40]. However, AEA levels are increased by ~ 15 fold in FAAH-deficient mice [41], supporting the concept that FAAH is mostly involved in the hydrolysis of AEA and other NAEs rather than heavily participating in AEA biosynthesis [40,42,43]. FAAH-2, which is expressed in humans but not in mice, also participates in the hydrolysis of AEA and other NAEs but, in contrast to FAAH-1, it preferentially hydrolyzes *N*-oleoyl-ethanolamine and does not hydrolyze *N*-acyl-taurines [44]. Finally, the *N*-acylethanolamine acid amidase (NAAA) can also hydrolyze AEA and other NAEs, although its activity is much better toward saturated NAEs such as *N*-palmitoyl-ethanolamine than for unsaturated NAEs such as AEA [45–47].

3. Biosynthesis and hydrolysis of 2-AG and its congeners

The biosynthesis of 2-AG has been largely documented as arising from the breakdown of AA-containing diacylglycerols (DAGs). DAGs can be obtained from the hydrolysis of glyceryl-phosphatidyl-inositol by PLCs [48,49] or from the breakdown of phosphatidyl-cholines by the concerted action of a PLD and a phosphatidic acid phosphatase [50]. 2-AG is next released when AA-containing DAG species are hydrolyzed by DAG lipases α or β [51,52]. 2-AG can also be obtained from the dephosphorylation of arachidonoyl-lysophosphatidic acid by a lysophosphatidic acid phosphatase [53] (Fig. 2A). While several groups documented that leukocytes biosynthesized 2-AG in response to Ca^{2+} ionophores such as A23187 or G-protein-coupled receptor agonists activating the PLC-DAG lipase pathway (reviewed in [54]), a recent study showed that this biosynthetic route is not very efficient in human leukocytes [55]. Instead, a novel 2-AG biosynthetic pathway (Fig. 2B) involving the acylation of exogenous AA into phospholipids, followed by the release of 2-AG, led to 2-AG levels that were up to 700-fold greater than those obtained with PLC-activating agonists such as platelet-activating factor [55]. Of note, this novel biosynthetic pathway was not inhibited by PLC or DAG lipase inhibitors and coincided with a lysophosphatidic acid intermediate although phosphatase inhibitors did not prevent 2-AG biosynthesis. Importantly, this novel pathway led to the biosynthesis of other unsaturated MAGs when their fatty acid precursors were used as stimuli, notably when 2-AG/MAG hydrolysis was prevented [55]. Whether this novel 2-AG/MAG biosynthetic pathway is also functional in other cells and tissues remain to be explored.

2-AG is very labile and isomerizes into 1(3)-AG in complete RPMI medium within minutes [56]. Furthermore, 1(3)- and 2-AG, as well as other MAGs are rapidly hydrolyzed into fatty acids and glycerol by several enzymes, notably the serine hydrolase MAG lipase [57–60]. In the brain of mice, 85 % of 2-AG hydrolysis is the consequence of MAG lipase and its deletion can increase 2-AG levels up to tenfold [61,62]. Other enzymes also contribute to the hydrolysis of 2-AG. In the mouse brain, ABHD12 and ABHD6 are respectively responsible for 9 % and 4 % of 2-AG hydrolysis [61]. Thus, together with MAG lipase, these three serine hydrolases contribute to 98 % of 2-AG hydrolysis in the mouse brain [61]. ABHD16A can also hydrolyze 2-AG and other MAGs, 1-linoleoyl-glycerol being its preferred substrate [63]. Carboxylesterase (CES) 1 and 2, as well as palmitoyl-protein thioesterase 1 (PPT1), can also participate in the hydrolysis of 2-AG and other MAGs, although their involvement remains to be fully elucidated [64–66].

As stated above, the hydrolysis of 2-AG and other MAGs leads to the production of fatty acids (e.g. AA), raising the possibility that 2-AG and other MAGs such as 2-docosahexaenoyl-glycerol could serve as precursors for the biosynthesis of eicosanoids and docosanoids. Accordingly, the treatment of human leukocytes with 2-AG leads to its rapid hydrolysis (within seconds to minutes) and a subsequent biosynthesis of eicosanoids such as leukotrienes and eoxins [67–70]. The involvement of 2-AG as a source of AA and/or eicosanoids (mainly prostaglandins (PGs)) has also been observed in MAG lipase deficient mice [71–73].

4. Metabolism of endocannabinoids by the cyclooxygenase pathway

Both 2-AG (Fig. 4) and AEA (Fig. 3) can be metabolized via the COX-2 pathway. The first evidence of this was provided by Yu and colleagues, who showed that human recombinant COX-2, but not COX-1, could metabolize AEA into PGH_2-EA [74]. This was followed three years later by the work of Kozak and colleagues showing that 2-AG was also metabolized by COX-2, but not COX-1 [75]. While COX-2 metabolizes 2-AG and AA to a comparable extent, the metabolism of AEA by COX-2 is less efficient, being ~ 18 % that of AA, based on kcat/Km determinations [74,75]. Interestingly, a COX-2 inhibitor-sensitive pathway in *Candida albicans* was found to convert AEA into 3-hydroxy-AEA, a compound inactive at CB receptors but still capable of activating TRPV1 channels [76]. In this section, we provide the key studies that documented the metabolism of AEA and 2-AG to the corresponding PGs by the COX pathway.

4.1. Biosynthesis and metabolism of prostaglandin-ethanolamides (prostamides)

In 1997, Yu *et al.* reported that AEA is a substrate for COX-2, leading to the biosynthesis of PG-ethanolamides (EA) also known as prostamides

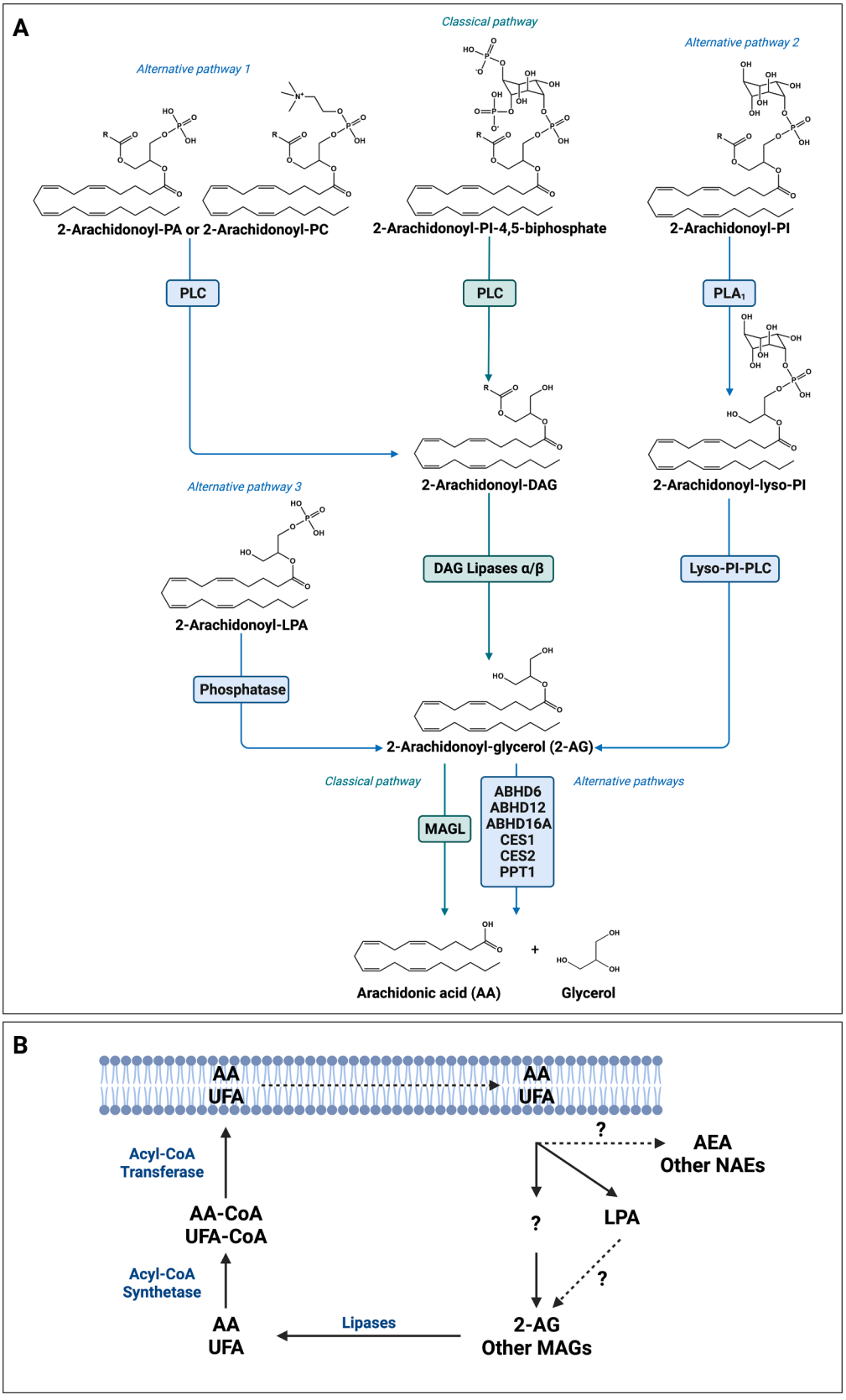


Fig. 2. Biosynthesis of the endocannabinoid 2-AG. A) Classical and alternatives pathways by which the biosynthesis of 2-AG is recognized to occur. **B)** Biosynthetic pathway by which 2-AG and other MAG biosynthesis is occurring in human myeloid leukocytes. Created with BioRender.com.

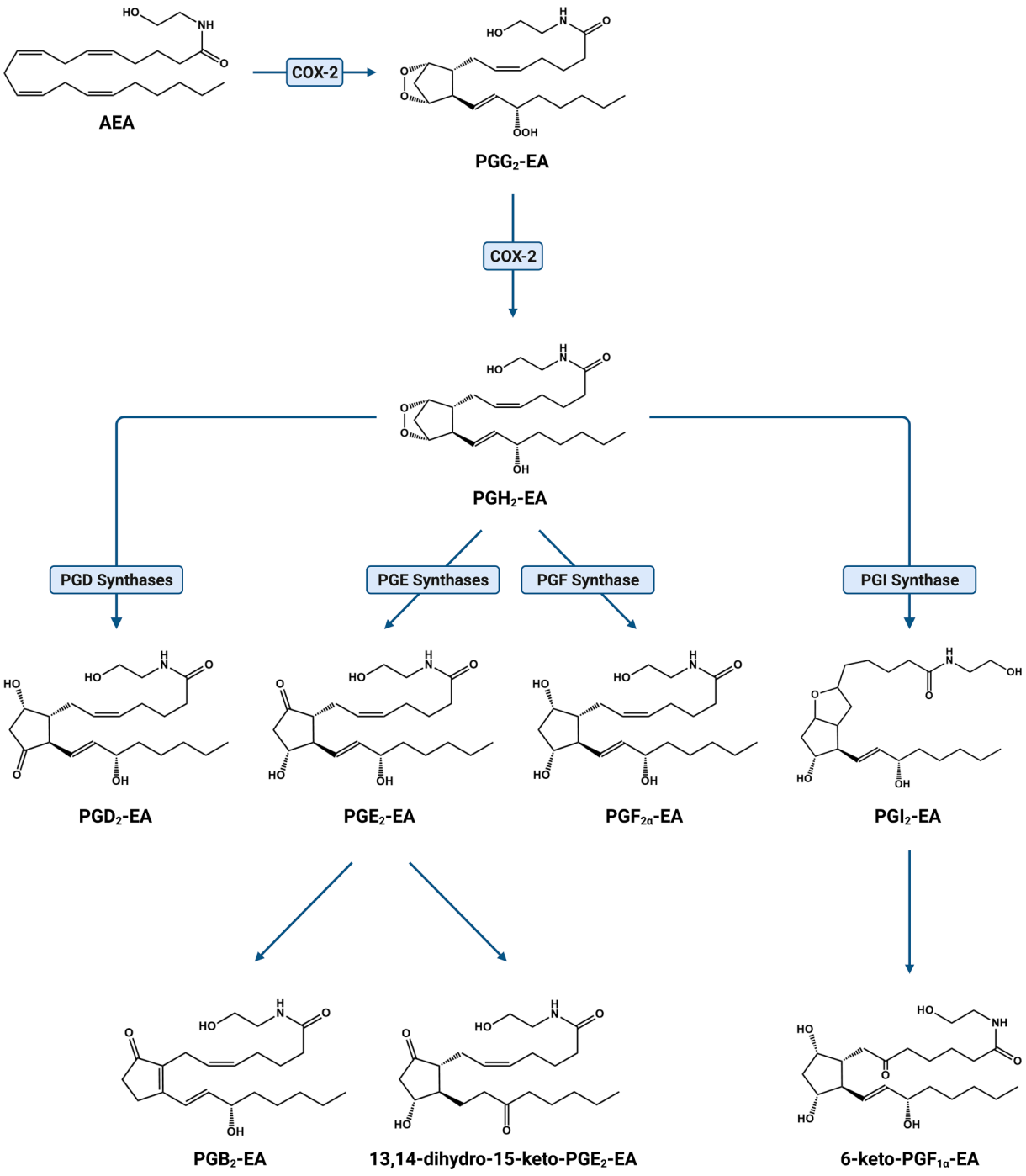


Fig. 3. Biosynthesis and degradation of prostamides. Created with BioRender.com.

[74]. The biosynthesis of prostamides from AEA occurs through identical biosynthetic steps as for PGs from AA, thus involving the generation of an endoperoxide intermediate (prostamide H_2, PGH_2-EA). The latter is next converted by PG synthases into PG congeners. Indeed, PGD_2-EA, PGE_2-EA, $PGF_{2\alpha}$-EA and PGI_2-EA are obtained from the enzymatic conversion of PGH_2 by PG synthases [77,78]. Of note, and despite displaying similar molecular features of PGH_2, PGH_2-EA is not efficiently converted into TXA_2-EA [77]. Finally, AEA can also be converted into 11- and 15-hydroxy-eicosatetraenoyl-ethanolamide (HETE-EA), at least by murine COX-2 [79].

The first evidence of prostamide biosynthesis *in cellulo* were obtained from a human foreskin cell line expressing COX-2, which can biosynthesize PGE_2-EA [74]. The biosynthesis of prostamides was also observed in other cells [77,78,80,81]. The detection of prostamides *in vivo* remains limited and initially seemed to necessitate facilitating conditions. For instance, in FAAH deficient mice, $PGF_{2\alpha}$-EA was found in the liver, the lungs, the kidneys and the small intestine [82]. However, $PGF_{2\alpha}$-EA was detected in mouse spinal cord and adipose tissue, where it plays a facilitatory role in pain transduction and an inhibitory role in adipogenesis [83,84].

In contrast to AEA, prostamides are not efficiently hydrolyzed by FAAHs and NAAA [37,47,85]. However, PGE_2-EA can be metabolized into 13,14-dihydro-15-keto-PGE_2-EA by the combination of the 15-hydroxyprostaglandin dehydrogenase and Δ^{13}-15-keto-prostaglandin reductase [77]. A slow non-enzymatic dehydration/isomerization of PGE_2-EA yielding PGB_2-EA was also observed [86].

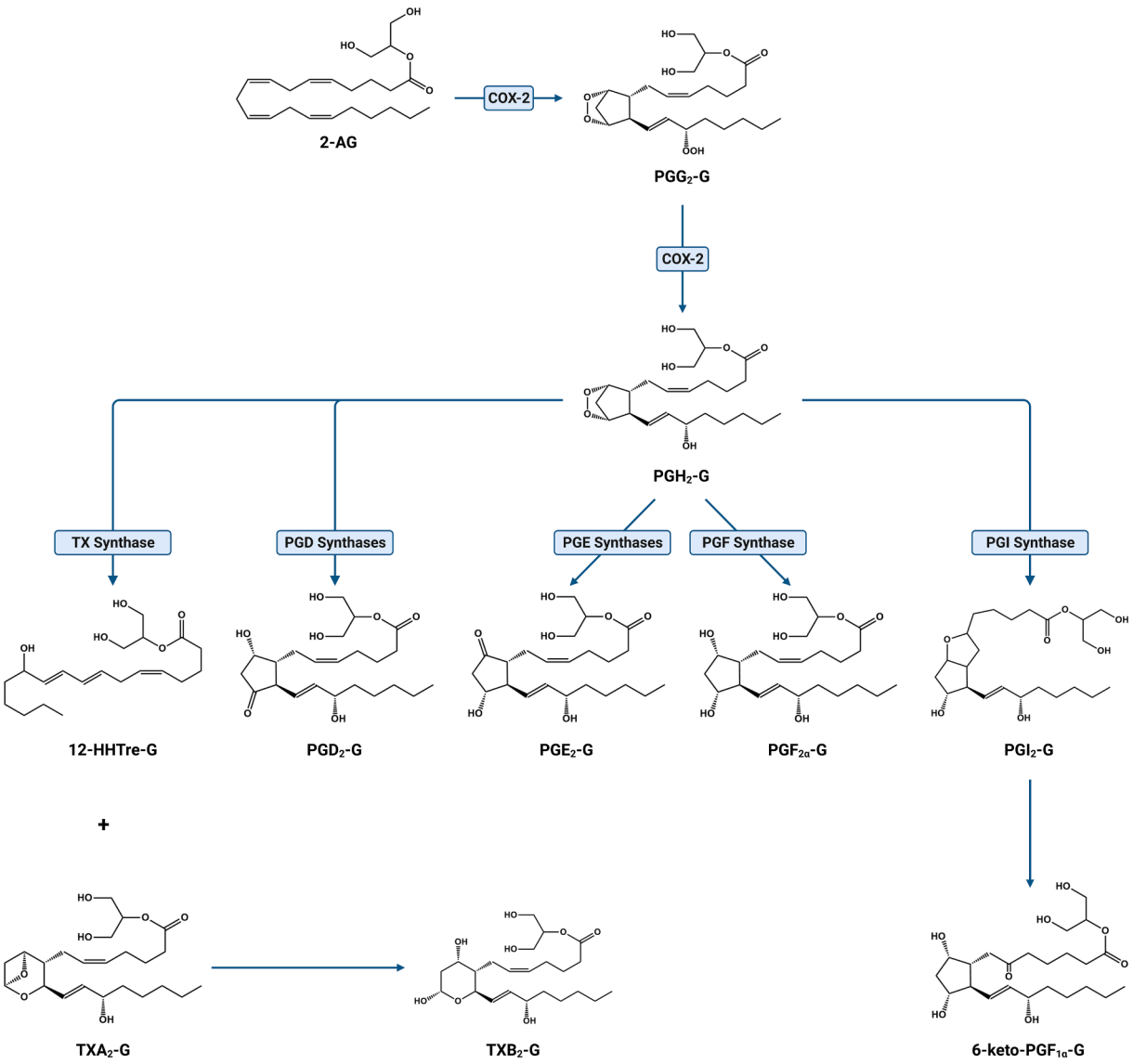


Fig. 4. Biosynthesis and degradation of Prostaglandins-Glycerol. Created with BioRender.com.

Aside from AEA, other NAEs are also metabolized by COX-2. Indeed, the metabolism of *N*-eicosapentaenoyl-ethanolamine by human recombinant COX-2 results in the formation of PGD_3-EA, PGE_3-EA, 11-hydroxy-eicosapentaenoyl-ethanolamide (HEPE-EA) and possibly 14- and 18-HEPE-EA as well [87]. Additionally, human recombinant COX-2 metabolizes *N*-docosahexaenoyl-ethanolamine (DHEA) into 13- and 16-hydroxy-DHEA [87]. These DHEA metabolites could be detected in LPS-stimulated RAW264.7 macrophages [87].

4.2. Biosynthesis and metabolism of prostaglandins-glycerol

2-AG is also a COX-2 substrate, and its metabolism by the latter leads to the biosynthesis of PGs-Glycerol (G) [77]. The metabolism of 2-AG by COX-2 leads to the common intermediate PGH_2-G, which is then converted to PGD_2-G, PGE_2-G, $PGF_{2\alpha}$-G or PGI_2-G [77]. In contrast to PGH_2, PGH_2-G is a poor substrate for the thromboxane synthase but TXA_2-G (assessed by quantitating TXB_2-G) can nonetheless be observed, raising the possibility that the 2-AG metabolites TXA_2-G and 12(S)-hydroxyheptadeca-5*Z*,8*E*,10*E*-trienoic acid (12-HHTre)-G might be detected *in vivo* [77].

Unlike prostamides, PGs-G are short-lived and rapidly hydrolyzed into PGs. Indeed, PGE_2-G half-life is ~ 15 s in rat plasma and ~ 10 min in human plasma [86]. PG-Gs, notably PGE_2-G, are hydrolyzed into PG and glycerol by several hydrolases. Among them are the MAG lipase, CES1, CES2, lysophospholipase 2 (LYPLA2) and PPT1 [64,65,88,89]. The involvement of MAG lipase in the hydrolysis of PG-Gs was first questioned. Indeed, rat recombinant MAG lipase hydrolyzes PG-Gs much less efficiently (2 orders of magnitude) than 2-AG [90]. In addition, the MAG lipase inhibitor URB602 led to an unexplained decrease in PGE_2-G in rats, which was inconsistent with the increased levels of its precursor 2-AG and the unaffected levels of its hydrolysis product PGE_2 [91]. However, Xie *et al.* next showed that human recombinant MAG lipase hydrolyzed PG-Gs up to 25 times the rate of its rat homolog [64]. This was further confirmed by Savinainen et al. who showed that human recombinant MAG lipase hydrolyzed PGs-G with the following efficacy: 15-deoxy-PGJ_2-G > PGD_2-G > PGE_2-G ~ $PGF_{2\alpha}$-G > 1-AG [88]. Subsequently, human recombinant CES1 and CES2 were shown to also hydrolyze PGE_2-G and $PGF_{2\alpha}$-G, CES2 hydrolyzing $PGF_{2\alpha}$-G 6.5-fold faster than PGE_2-G [64]. This latter study concluded that hydrolysis rates for PG-Gs by human recombinant hydrolases was as follows: MAG lipase > CES1 ≫ FAAH [64]. *In cellulo*, THP1 cells expressing CES1 (but not CES2) mainly hydrolyzed exogenously provided PG-Gs into PGs [64], the remaining hydrolase activity being the consequence of PPT1 [65]. ABHD6 and ABHD12 can also hydrolyze PG-Gs [88]. Human

recombinant ABHD6 preferentially hydrolyzes PGD_2-G over the other PGs-G (1-AG > PGD_2-G > 15-deoxy-PGJ_2-G > PGE_2-G = $PGF_{2\alpha}$-G), while ABHD12 weakly hydrolyzes PGs-G compared to 1-AG (1-AG ≫ PGE_2-G > 15-deoxy-PGJ_2-G ~ PGD_2-G ~ $PGF_{2\alpha}$-G [88]. Last but not least, LYPLA2 and LYPLA2-expressing cells (the breast cancer cell lines MCF7 and MDA-MB-231 and the prostate cancer cell lines PC3 and LNCaP) also hydrolyze PGE_2-G [89]. Knockdown of LYPLA2 in these cell lines resulted in 60–80 % decrease of PGE_2-G hydrolysis [89]. Interestingly, LYPLA2 does not hydrolyze 2-AG or AEA [89].

4.3. Receptors and bioactivity of COX-derived metabolites of NAEs

Identifying the receptors through which prostamides and PG-Gs exert their biological activities has been challenging (Table 1). Prostamides were shown to be weak activators of the CB_1 and CB_2 receptors as well as weak agonists of the PG receptors [85,92,93]. The most studied prostamide is $PGF_{2\alpha}$-EA because of its important regulatory role in the eye [94]. Indeed, $PGF_{2\alpha}$-EA and its analog bimatoprost are among the most efficient treatments against glaucoma [95,96]. Great effort was thus put into characterizing the receptor behind $PGF_{2\alpha}$-EA and bimatoprost biological activities, which led to the identification of the FP receptor/FPalt4 splicing variant heterodimers [84,97]. The identification of PG-G receptors has proven to be even more challenging due to the rapid hydrolysis of PG-Gs [90]. Indeed, PGs-G likely act at the vicinity of their biosynthesis then are rapidly cleared from the tissues [98,99]. Moreover, it underscores the possibility that some biological effects attributed to PGs-G may in fact be the consequence of their hydrolysis products, as recently documented [98,100]. Thus, it is not surprising that effects of PGE_2-G were neither attributed to CB_1 nor PG receptors [98,101]. Interestingly, the UDP receptor $P2Y_6$ was identified as a specific target of PGE_2-G, using subtractive screening approach based on transcriptome-wide RNA sequencing analysis in PGE_2-G response-positive and -negative cell lines [102,103].

5. Metabolism of endocannabinoids by lipoxygenases

Humans express six LOs encoded by the genes ALOXE3, ALOX5, ALOX12, ALOX12B, ALOX15, and ALOX15B. With the exception of the protein encoded by the ALOXE3 gene (which is an hydroperoxide isomerase), LOs catalyze the peroxidation of unsaturated fatty acids possessing a 1*Z*,4*Z*-pentadiene motif into a 1-hydroperoxy-2*E*-4*Z*-pentadiene. LOs can metabolize several fatty acids but have been named according to the carbon on which they add molecular oxygen on AA. Endocannabinoids and some of their congeners were also identified as substrates for the different LOs.

Table 1
Prostamides, PGs-G, and their documented receptors.

Substrate	Metabolite	Receptors	Reference
AEA	PGD_2-EA	$EP_{1\text{-}4}$ (no)	[85]
		DP_1(yes)	[85]
		FP/IP/TP (no)	[85]
		CB_1 / CB_2 (no)	[85,94]
	PGE_2-EA	$EP_{1\text{-}4}$ (no)	[85,141]
		DP/FP/IP/TP (no)	[85,141]
		TRPV1 (no)	[85]
		CB_1/CB_2 (no)	[85,93]
	$PGF_{2\alpha}$-EA	$EP_{1\text{-}4}$ (no)	[85]
		DP/IP/TP (no)	[85]
		FP (no)	[85]
		FP (yes)	[84,97,142]
		TRPV1 (no)	[85]
		CB_1/CB_2 (no)	[93]
2-AG	PGD_2-G	CB_1 (no)	[98]
	PGE_2-G	$EP_{1\text{-}2}$ (no)	[143]
		$P2Y_6$ (yes)	[102]
		CB_1 (no)	[98,101]
		TRPV1 (no)	[143]
	$PGF_{2\alpha}$-G	CB_1 (no)	[98]

5.1. Metabolism of AEA by lipoxygenases

The first reports of AEA metabolism by LOs came in 1995, when it was shown that soybean LO could convert AEA into its 15-hydroxy derivative 15-HETE-EA [104–110]. The metabolism of AEA was then reported in human recombinant 15-LO-1 [111,112] and in 15-LO-1-expressing human cells [106,113,114]. While 15-HETE-EA was the main metabolite from AEA, many reported 12-HETE-EA as a minor product [106,111,112]. Human 15-LO-2 can also metabolize AEA into 15-HETE-EA exclusively, in both recombinant 15-LO-2 [112,115] and, possibly in human neutrophils [114]. Of note, AEA is a better substrate for both 15-LO-1 and 15-LO-2 compared to AA [112]. 15-hydroxylation of AEA is not exclusive to humans and was reported with recombinant enzymes from orangutans [111], rabbits [104,112] and rats [116].

AEA is also a substrate for 12-LO, which leads to the biosynthesis of 12-HETE-EA in porcine 12-LO [104,106], human platelets 12-LO [104,106], rat pineal gland 12-LO [105], human and mouse recombinant 12-LO [112]. It is also a substrate for barley 5-LO and tomato 9-LO, mainly yielding 11-HETE-EA with 5-HETE-EA as a minor product [107,109]. While tomato and barley 5-LO can metabolize AEA, the porcine, murine or human enzyme cannot [104,107,112], probably because the carboxylic end of AA enters the catalytic site of 5-LO first [117] and that the ethanolamine group of AEA generates sufficient hindrance to prevent that entry.

Other LO-derived metabolites from AEA were also described. For instance, AEA is metabolized into the dihydroxylated metabolites 5,15-diHETE-EA and 8,15-diHETE-EA by soybean LO [107,108]. Finally, AEA is metabolized into the eoxamides EXC_4-EA, EXD_4-EA and EXE_4-EA by the combined activity of 15-LO-1 and LTC_4 synthase [113].

5.2. Metabolism of 2-AG by lipoxygenases

2-AG is a substrate for some LOs but the amount of evidence is limited, most likely because of the challenges associated with its stability, this endocannabinoid (and its metabolites) being hydrolyzed very rapidly *in cellulo* (section 3). Moody *et al* were the first to report that 2-AG was metabolized into 12-HETE-G by partially purified porcine leukocyte 12-LO but not by partially purified human platelet 12-LO [118]. In that study, the efficiency of porcine leukocyte 12-LO toward 2-AG was ~ 40 % that of AA. In contrast, both human and mouse recombinant 12-LO could metabolize 2-AG into 12-HETE-G as efficiently as AA [112]. This is intriguing and the discrepancy between the two studies remains somewhat difficult to explain as no study reported the ability/inability of platelets to biosynthesize 12-HETE-G in response to 2-AG.

2-AG can also be metabolized by 15-LOs, mainly into 15-HETE-G. This was observed with soybean LO, rabbit reticulocyte 15-LO, human 15-LO-1 and -2 [108,112,115]. Importantly, 12-HETE-G was also observed as a minor product for rabbit reticulocyte 15-LO and human 15-LO-1 [115]. Noteworthy, soybean LO and rabbit reticulocyte 15-LO were less capable of metabolizing 2-AG, respectively reaching 80 % and 40 % yield compared to AA [115]. As for 2-AG, 15-HETE-G is very unstable and is rapidly hydrolyzed into 15-HETE, notably by human leukocytes [70]. As such, the metabolism of 2-AG into 15-HETE-G in human neutrophils and eosinophils can only be detected when cells are treated with the serine hydrolase inhibitor MAFP [114]. As for eoxamides, 2-AG can be transformed into the eoxins-glycerol EXC_4-G, EXD_4-G and EXE_4-G by the combination of 15-LO and LTC_4 synthase. However, the levels of eoxins-glycerol that were reported remained modest, possibly due to the hydrolysis of these metabolites [113].

While both 12- and 15-LO can metabolize 2-AG, potato 5-LO and human recombinant 5-LO cannot convert 2-AG into 5-HETE-G [112,115]. However, mouse recombinant 5-LO generated a modest but detectable amount of 5-HETE-G when incubated with 2-AG [112].

5.3. Metabolism of other MAGs and NAEs by lipoxygenases

AEA and 2-AG are not the only NAEs or MAGs that are metabolized by lipoxygenases. The first report of endocannabinoid congeners being metabolized by lipoxygenases was published by van der Stelt *et al* in 1997 in which the authors described the metabolism of *N*-linoleoyl-ethanolamine (LEA) into 13-hydroxyoctadecadienoyl(HODE)-EA by soybean LO [119]. This was confirmed by others, either with soybean LO, human 15-LO-1 and -2, as well as with human leukocytes such as eosinophils and neutrophils [108,119–122]. Similarly, to LEA, a recent study demonstrated that 1-linoleoyl-glycerol (1-LG) was metabolized into 13-HODE-G by soybean LO, recombinant 15-LO-1 and -2, human eosinophils and neutrophils [122]. DHEA is also a substrate for 15-LO and can be transformed into 17-hydroxy-DHEA (HDHEA), 4,17-diHDHEA, and 10,17-DiHDHEA by human neutrophils [123]. This raises the possibility that other MAGs and NAEs, notably those containing fatty acids that are metabolized by either the 12- or 15-lipoxygenases are also metabolized by these enzymes, yielding additional oxy-endocannabinoidome metabolites that are yet to be documented (Fig. 5).

5.4. Receptors and bioactivity of LO-derived mediators

The bioactivity of LO-derived endocannabinoid-related mediators remains ill-defined. While some have reported possible binding to peroxisome proliferator-activated receptors (PPARs), CB receptors or TRP channels, the biological roles of these metabolites and the identification of specific receptors are limited. As shown in Table 2, some have reported the possible modulation by LO-derived mediators of endocannabinoidome-linked receptors, notably CB_1, CB_2 and TRPV1. However, the activity of the metabolites at these receptors is often modest compared to AEA or 2-AG. Nonetheless, 15-HETE-EA enhanced the neuroprotective effect of AEA in the context of brain inflammation [116], reduced the AEA-induced platelet activation [124], and inhibited the electrically-induced contraction of the vas deferens from mice [104]. Moreover, in human whole blood, 10,17-DiHDHEA could prevent the formation of platelet-leukocyte aggregates [123]. It will thus be of critical interest to better define the receptors, signaling and biological effects of these novel LO-derived lipid mediators.

Table 2
LO-derived metabolites of endocannabinoids and their congeners and their documented receptors.

Substrates	Metabolite	Receptors	Reference
AEA	5-HETE-EA	CB_1/CB_2 (no)	[108]
		TRPV1 (yes)	[144]
	11-HETE-EA*	CB_1/CB_2 (no)	[108]
		CB_1 (yes)	[105,106]
	12-HETE-EA	CB_1(yes)	[105,106,108]
	15-HETE-EA	CB_1 (yes)	[105,106,108]
		CB_2 (no)	[108]
		TRPV1 (yes)	[145]
	5,15-diHETE-EA	CB_1 (no)	[108]
		CB_2 (no)	[108]
	8,15-diHETE-EA	CB_1 (no)	[108]
		CB_2 (no)	[108]
2-AG	15-HETE-G	PPARγ(no)	[115]
		PPARδ(no)	[115]
		PPARα(yes)	[115]
		CB_2 (yes)	[108]
LEA	13-HODE-EA	CB_1 (no)	[108,121,146]
		CB_2 (yes)	[108]
		CB_2 (no)	[121]
		TRPV1 (yes)	[121]
		$PPAR_\alpha$ (no)	[121]
1-LG	13-HODE-G	$PPAR_\alpha$ (no)	[122]
		PPARγ (no)	[122]
		CB_1/CB_2 (no)	[122]
		TRPV1 (no)	[122]
DHEA	10,17-DiHDHEA	CB_2 (yes)	[123]

* This metabolite mainly arises from COX-2 activity.

6. Metabolism of endocannabinoids by the cytochrome P450 pathway

The conversion of AEA into at least 20 metabolites by various CYP members was reported by Bornheim and colleagues in 1995 [125,126]. In contrast, 2-AG is the substrate of CYP2J2 only [127,128]. In this section, we provide the key studies regarding the metabolism of AEA and 2-AG through the CYP pathways (Fig. 6).

6.1. Metabolism of AEA through the cytochrome P450 pathway

As for AA, AEA can be epoxygenated by CYPs at position 5,6, 8,9, 11,12 and 14,15 to form epoxyeicosatrienoyl-ethanolamides (EET-EA) or metabolized into 20-HETE-EA [129,130]. This metabolism of AEA into various metabolites differs depending on the CYP involved, and thus the location in the human body (Table 3). The metabolism of AEA by human liver microsomes results in the biosynthesis of all four EET-EAs through CYP3A4 metabolism, as well as 20-HETE-EA, catalyzed by the CYP4F2 [130]. Human kidney microsomes convert AEA into 20-HETE-EA also through CYP4F2 [130]. In human brain microsomes and mitochondria, AEA is converted into EETs-EA and 20-HETE-EA, involving both CYP4F2 and CYP3A4, in addition to major CYPs in the brain, namely CYP2D6 and CYP2B6 [131,132]. Human purified CYP2D6 produced primarily high levels of 20-HETE-EA (20-HETE-EA > 14,15-EET-EA > 8,9-EET-EA > 11,12-EET-EA > 5,6-EET-EA), while the main metabolite of the CYP2B6 is 11,12-EET-EA (11,12-EET-EA > 8,9-EET-EA > 14,15-EET-EA > 20-HETE-EA > 5,6-EET-EA) [132]. Interestingly, CYP2D6 also generates several hydroxy-epoxyeicosatrienoyl-ethanolamides (HEET-EA) through the ω-hydroxylation of the EET-EA at the ω-1, ω-2 and ω-3 positions [133,134]. CYP4X1, which is found in amygdala, prostate and skin, metabolize AEA into a single product, namely 14,15-EET-EA [135,136]. Human intestinal microsomes (containing CYP2J2) and purified human CYP2J2 metabolize AEA into 20-HETE-EA, as well as 5,6-, 8,9-, and 11,12-EET-EA [137]. However, specific P450 inhibitors suggest that CYP2J2 would not be the primary CYP responsible for AEA metabolism in human intestines [137]. Some

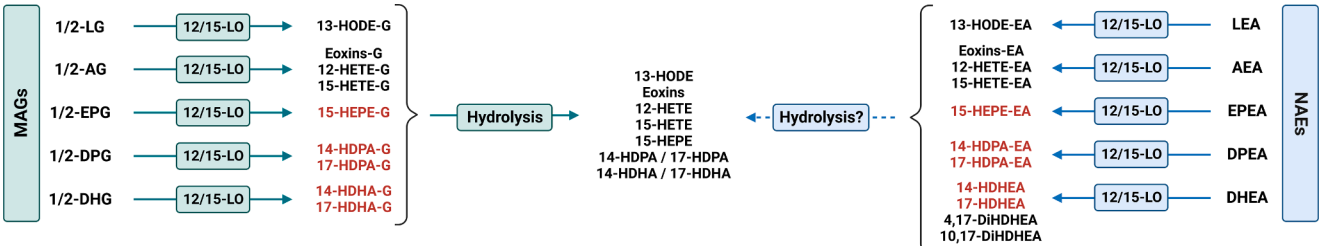


Fig. 5. Documented and putative lipoxygenase metabolites of 2-AG, AEA and their congeners from the MAG and NAE families. Documented 12/15-LO metabolites are in black while putative metabolites are in red. Created with BioRender.com.

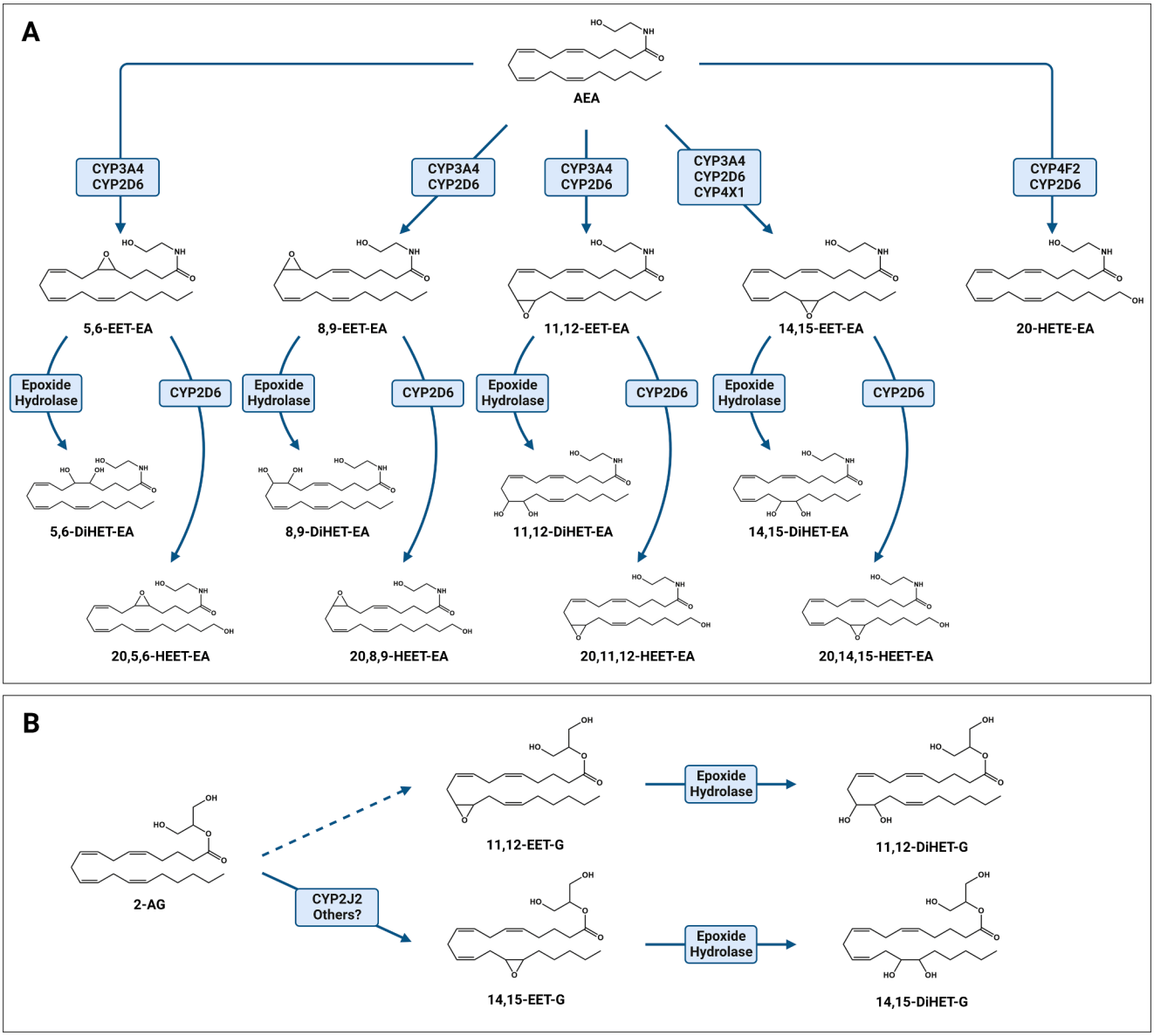


Fig. 6. Documented and putative CYP450 metabolites of AEA and 2-AG. Created with BioRender.com.

EETs-EA can be hydrolyzed by FAAH to form EETs [132,138].

6.2. Metabolism of 2-AG through the cytochrome P450 pathway

CYP450-derived metabolites have not been thoroughly investigated yet but one 2008 study reported the investigation of two CYP450-derived metabolites, namely 11,12-EET-G and 14,15-EET-G [127]. The authors found that 11,12-EET-G and 14,15-EET-G were both found in rat kidney and spleen, while 11,12-EET-G was detected in rat brain. While the dihydroxy-eicosatrienoyl (DiHET) derivatives of those compounds were not investigated per se, recombinant soluble epoxide hydrolase abrogated their activity, indicating that the DiHET metabolites of 11,12-EET-G and 14,15-EET-G were inactive in the authors' experimental model. Of note, the authors could not pinpoint which CYP450 enzyme could generate the EET-G metabolites, as recombinant CYP2C8, CYP2C11, or CYP2C23 could not biosynthesize them. Consequently, they postulated that their biosynthesis might arise from the esterification of their EET counterparts, followed by their subsequent release involving enzymes from the classical 2-AG biosynthetic pathway (Fig. 2), which rather seems unlikely. In 2014, McDougle and colleagues showed using bovine and porcine heart microsomes that 2-AG was a potential substrate for the CYP2J2, mainly generating 14,15-EET-G [128]. Ligand-protein interactions were confirmed using spectral titrations, stopped flow small-molecule ligand egress and molecular modeling.

6.3. Metabolism of endocannabinoid congeners by the cytochrome P450 pathway

Besides AEA and 2-AG, the endocannabinoid congeners EPEA and DHEA were also shown to be epoxygenated by rat brain microsomes and human CYP2J2 incorporated into nanodiscs, generating epoxyeicosatetraenoic acid-ethanolamide (EEQ-EA) and epoxydocosapentaenoic acid-ethanolamide (EDP-EA) respectively [139]. These metabolites were detected in the rat brain and LPS-stimulated BV-2 microglial cells, with 17,18-EEQ-EA and 19,20-EDP-EA being the predominant species [139]. The 17,18-EEQ-EA and 19,20-EDP-EA are both hydrolyzed efficiently by FAAH and epoxyde hydrolase (17,18-DiHET-EA and 19,20-DiHDPA-EA) [139].

Table 3
Endocannabinoid metabolism through the cytochrome P450 pathway.

Substrate	Enzyme	Metabolites	Experimental models	Reference
AEA	CYP2B6	20-HETE-EA	Purified human CYP2B6	[132]
AEA	CYP2D6	20-HETE-EA 5,6-EET-EA 8,9-EET-EA 11,12-EET-EA 14,15-EET-EA	Human recombinant CYP2D6, human brain microsomes and mitochondria and human purified CYP2D6	[131,132]
AEA	CYP2J2	20-HETE-EA 5,6-EET-EA 8,9-EET-EA 11,12-EET-EA 14,15-EET-EA	Purified human CYP2J2 and human intestine microsomes	[137]
AEA	CYP2J2	20-HETE-EA 19-HETE-EA 5,6-EET-EA 8,9-EET-EA 11,12-EET-EA 14,15-EET-EA	Recombinant CYP2J2 on lipid bilayered nanomeric disque	[128]
AEA	CYP3A4	5,6-EET-EA 8,9-EET-EA 11,12-EET-EA 14,15-EET-EA	Human liver microsomes and recombinant CYP3A4	[130]
		19-HETE-EA	Human recombinant variant CYP3A4.4 (compared to wild type CYP3A4)	[147]
AEA	CYP4F2	20-HETE-EA	Human liver microsomes and human kidney microsomes	[130]
AEA	CYP4X1	14,15-EET-EA	Recombinant CYP4X1 proten, co-expressed with human NADPH-P450 reductase in E. coli	[135]
DHEA	CYP2J2	19,20-EDP-EA	Human recombinant CYP2J2	[139]
EPEA	CYP2J2	17,18-EEQ-EA	Human recombinant CYP2J2	[139]
5,6-EET-EA	CYP2D6	20,5,6-HEET-EA	Human recombinant CYP2D6	[133]
8,9-EET-EA	CYP2D6	20,8,9-HEET-EA	Human recombinant CYP2D6	[133]
11,12-EET-EA	CYP2D6	20,11,12-HEET-EA	Human recombinant CYP2D6	[133]
14,15-EET-EA	CYP2D6	20,14,15-HEET-EA	Human recombinant CYP2D6	[133]
2-AG	CYP2J2	11,12-EET-G 14,15-EET-G	Recombinant CYP2J2 on lipid bilayered nanomeric disque	[128]

6.4. Receptors and bioactivity of CYP-derived mediators

The molecular targets and bioactivity of the CYP-derived oxy-endocannabinoidome mediators has also been so far poorly investigated and only by very few research groups. These have reported that 5,6-EET-EA selectively binds the CB_2 receptor with a 300-fold greater affinity than the CB_1 receptor [138]. Moreover, it seems to be a more stable CB_2 receptor ligand (1000-fold) than AEA itself [138]. 5,6-EET-EA also activates TRPV4 [133]. 20-HETE-EA and 14,15-EET-EA have less affinity for the CB_1 receptor than AEA [132]. Both 11,12-EET-G and 14,15-EET-G bind to the CB_1 and CB_2 receptors with similar affinity than 2-AG [127,140].

7. Conclusion

While the biosynthesis and hydrolytic pathways of endocannabinoids and their congeners is well documented, some gaps remain regarding their metabolism by eicosanoid biosynthetic enzymes. The metabolism of endocannabinoids by the cyclooxygenase pathway has been thoroughly documented, but our understanding of the resulting metabolites remains fragile. Aside from the COX-2 pathway, endocannabinoids can be oxygenated by other enzymes, notably CYP450 and 12/15-LOs. While some of the obtained metabolites have documented functions, we still do not completely understand the purpose, time and the location of their biosynthesis. Furthermore, congeners of 2-AG and AEA, notably the unsaturated ones, are very likely susceptible to the action of CYP450 enzymes and different LOs, despite the fact that the resulting metabolites have not been thoroughly investigated. It will be a challenging task to identify all metabolites of the oxy-endocannabinoidome, notably those arising from the metabolism of MAGs, as they seem more susceptible to hydrolysis than their NAE counterparts. Whether the new and/or putative metabolites have important roles in health and disease remains to be elucidated. In particular, it will be interesting to understand whether or not these so many mediators are produced concomitantly and in the same cell type, starting from fatty acid or endocannabinoidome mediator precursors, to convey coordinated and simultaneous signals at many receptors in a manner that could be interpreted only using systems biology approaches.

Funding

This work was supported by a grant to NF from The Natural Sciences and Engineering Research Council of Canada (RGPIN-2021-03777) and the Canada Excellence Research Chair on the Gut Microbiome-Endocannabinoidome Axis in Metabolic Health (CERC-MEND) at Université Laval, attributed to VD and funded by the Federal Tri-Agency of Canada.

CRediT authorship contribution statement

Mélissa Simard: Conceptualization, Writing – original draft, Writing – review & editing. **Anne-Sophie Archambault:** Conceptualization, Writing – original draft, Writing – review & editing. **Jean-Philippe C. Lavoie:** Writing – original draft, Visualization. **Élizabeth Dumais:** Writing – original draft, Visualization, Writing – original draft, Visualization. **Vincenzo Di Marzo:** Writing – review & editing. **Nicolas Flamand:** Conceptualization, Project administration, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

References

[1] Y. Gaoni, R. Mechoulam, Isolation, structure elucidation and partial synthesis of an active constituent of hashish, J. Am. Chem. Soc. 27 (1) (1964) 67–71.
[2] M.R. Johnson, L.S. Melvin, G.M. Milne, Prototype cannabinoid analgetics, prostaglandins and opiates–a search for points of mechanistic interaction, Life Sci. 31 (16–17) (1982) 1703–1706.
[3] L.S. Melvin, M.R. Johnson, C.A. Harbert, G.M. Milne, A. Weissman, A cannabinoid derived prototypical analgesic, J. Med. Chem. 27 (1) (1984) 67–71.

[4] L.S. Melvin, M.R. Johnson, Structure-activity relationships of tricyclic and nonclassical bicyclic cannabinoids, NIDA Res. Monogr. 79 (1987) 31–47.
[5] W.A. Devane, F.A. Dysarz 3rd, M.R. Johnson, L.S. Melvin, A.C. Howlett, Determination and characterization of a cannabinoid receptor in rat brain, Mol. Pharmacol. 34 (5) (1988) 605–613.
[6] L.A. Matsuda, S.J. Lolait, M.J. Brownstein, A.C. Young, T.I. Bonner, Structure of a cannabinoid receptor and functional expression of the cloned cDNA, Nature 346 (6284) (1990) 561–564.
[7] C.M. Gerard, C. Mollereau, G. Vassart, M. Parmentier, Molecular cloning of a human cannabinoid receptor which is also expressed in testis, Biochem. J. 279 (Pt 1) (1991) 129–134.
[8] S. Munro, K.L. Thomas, M. Abu-Shaar, Molecular characterization of a peripheral receptor for cannabinoids, Nature 365 (6441) (1993) 61–65.
[9] A.C. Howlett, F. Barth, T.I. Bonner, G. Cabral, P. Casellas, W.A. Devane, C. C. Felder, M. Herkenham, K. Mackie, B.R. Martin, R. Mechoulam, R.G. Pertwee, International Union of Pharmacology. XXVII. Classification of cannabinoid receptors, Pharmacol. Rev. 54 (2) (2002) 161–202.
[10] C. Turcotte, M.R. Blanchet, M. Laviolette, N. Flamand, The CB2 receptor and its role as a regulator of inflammation, Cell. Mol. Life Sci. 73 (23) (2016) 4449–4470.
[11] M. Simard, V. Rakotoarivelo, V. Di Marzo, N. Flamand, Expression and Functions of the CB2 Receptor in Human Leukocytes, Front. Pharmacol. 13 (2022), 826400.
[12] V. Di Marzo, New approaches and challenges to targeting the endocannabinoid system, Nat. Rev. Drug Discov. 17 (9) (2018) 623–639.
[13] W.A. Devane, L. Hanus, A. Breuer, R.G. Pertwee, L.A. Stevenson, G. Griffin, D. Gibson, A. Mandelbaum, A. Etinger, R. Mechoulam, Isolation and structure of a brain constituent that binds to the cannabinoid receptor, Science 258 (5090) (1992) 1946–1949.
[14] R. Mechoulam, S. Ben-Shabat, L. Hanus, M. Ligumsky, N.E. Kaminski, A. R. Schatz, A. Gopher, S. Almog, B.R. Martin, D.R. Compton, et al., Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors, Biochem. Pharmacol. 50 (1) (1995) 83–90.
[15] T. Sugiura, S. Kondo, A. Sukagawa, S. Nakane, A. Shinoda, K. Itoh, A. Yamashita, K. Waku, 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain, Biochem. Biophys. Res. Commun. 215 (1) (1995) 89–97.
[16] R. Mechoulam, A Delightful Trip Along the Pathway of Cannabinoid and Endocannabinoid Chemistry and Pharmacology, Annu. Rev. Pharmacol. Toxicol. (2022).
[17] L. Hanus, S. Abu-Lafi, E. Fride, A. Breuer, Z. Vogel, D.E. Shalev, I. Kustanovich, R. Mechoulam, 2-arachidonyl glyceryl ether, an endogenous agonist of the cannabinoid CB1 receptor, Proc. Natl. Acad. Sci. USA 98 (7) (2001) 3662–3665.
[18] M.G. Balvers, K.C. Verhoeckx, P. Plastina, H.M. Wortelboer, J. Meijerink, R. F. Witkamp, Docosahexaenoic acid and eicosapentaenoic acid are converted by 3T3-L1 adipocytes to N-acyl ethanolamines with anti-inflammatory properties, BBA 1801 (10) (2010) 1107–1114.
[19] I. Brown, M.G. Cascio, K.W. Wahle, R. Smoum, R. Mechoulam, R.A. Ross, R. G. Pertwee, S.D. Heys, Cannabinoid receptor-dependent and -independent anti-proliferative effects of omega-3 ethanolamides in androgen receptor-positive and -negative prostate cancer cell lines, Carcinogenesis 31 (9) (2010) 1584–1591.
[20] N. Alharthi, P. Christensen, W. Hourani, C. Ortori, D.A. Barrett, A.J. Bennett, V. Chapman, S.P.H. Alexander, n-3 polyunsaturated N-acylethanolamines are CB2 cannabinoid receptor-preferring endocannabinoids, Biochim. Biophys. Acta, Mol. Cell. Biol. Lipids 1863 (11) (2018) 1433–1440.
[21] V. Natarajan, P.V. Reddy, P.C. Schmid, H.H. Schmid, N-Acylation of ethanolamine phospholipids in canine myocardium, BBA 712 (2) (1982) 342–355.
[22] X.H. Jin, Y. Okamoto, J. Morishita, K. Tsuboi, T. Tonai, N. Ueda, Discovery and characterization of a Ca2+-independent phosphatidylethanolamine N-acyltransferase generating the anandamide precursor and its congeners, J. Biol. Chem. 282 (6) (2007) 3614–3623.
[23] S.S. Binte Mustafiz, T. Uyama, K. Morito, N. Takahashi, K. Kawai, Z. Hussain, K. Tsuboi, N. Araki, K. Yamamoto, T. Tanaka, N. Ueda, Intracellular Ca(2+)-dependent formation of N-acyl-phosphatidylethanolamines by human cytosolic phospholipase A2epsilon, Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1864 (12) (2019), 158515.
[24] S.M.K. Rahman, Z. Hussain, K. Morito, N. Takahashi, M.M. Sikder, T. Tanaka, K. I. Ohta, M. Ueno, H. Takahashi, T. Yamamoto, M. Murakami, T. Uyama, N. Ueda, Formation of N-acyl-phosphatidylethanolamines by cytosolic phospholipase A2epsilon in an ex vivo murine model of brain ischemia, Biochim. Biophys. Acta Mol. Cell Biol. Lipids (2022), 159222.
[25] V. Di Marzo, A. Fontana, H. Cadas, S. Schinelli, G. Cimino, J.C. Schwartz, D. Piomelli, Formation and inactivation of endogenous cannabinoid anandamide in central neurons, Nature 372 (6507) (1994) 686–691.
[26] Y. Okamoto, J. Morishita, K. Tsuboi, T. Tonai, N. Ueda, Molecular characterization of a phospholipase D generating anandamide and its congeners, J. Biol. Chem. 279 (7) (2004) 5298–5305.
[27] H.S. Hansen, L. Lauritzen, A.M. Strand, B. Moesgaard, A. Frandsen, Glutamate stimulates the formation of N-acylphosphatidylethanolamine and N-acylethanolamine in cortical neurons in culture, BBA 1258 (3) (1995) 303–308.
[28] D. Leung, A. Saghatelian, G.M. Simon, B.F. Cravatt, Inactivation of N-acyl phosphatidylethanolamine phospholipase D reveals multiple mechanisms for the biosynthesis of endocannabinoids, Biochemistry 45 (15) (2006) 4720–4726.
[29] J. Liu, L. Wang, J. Harvey-White, D. Osei-Hyiaman, R. Razdan, Q. Gong, A. C. Chan, Z. Zhou, B.X. Huang, H.Y. Kim, G. Kunos, A biosynthetic pathway for anandamide, Proc. Natl. Acad. Sci. USA 103 (36) (2006) 13345–13350.
[30] J. Liu, L. Wang, J. Harvey-White, B.X. Huang, H.Y. Kim, S. Luquet, R.D. Palmiter, G. Krystal, R. Rai, A. Mahadevan, R.K. Razdan, G. Kunos, Multiple pathways involved in the biosynthesis of anandamide, Neuropharmacology 54 (1) (2008) 1–7.
[31] Y.X. Sun, K. Tsuboi, Y. Okamoto, T. Tonai, M. Murakami, I. Kudo, N. Ueda, Biosynthesis of anandamide and N-palmitoylethanolamine by sequential actions of phospholipase A2 and lysophospholipase D, Biochem. J. 380 (Pt 3) (2004) 749–756.
[32] G.M. Simon, B.F. Cravatt, Endocannabinoid biosynthesis proceeding through glycerophospho-N-acyl ethanolamine and a role for alpha/beta-hydrolase 4 in this pathway, J. Biol. Chem. 281 (36) (2006) 26465–26472.
[33] E. Leishman, K. Mackie, S. Luquet, H.B. Bradshaw, Lipidomics profile of a NAPE-PLD KO mouse provides evidence of a broader role of this enzyme in lipid metabolism in the brain, BBA 1861 (6) (2016) 491–500.
[34] C. Lefort, M. Roumain, M. Van Hul, M. Rastelli, R. Manco, I. Leclercq, N. M. Delzenne, V.D. Marzo, N. Flamand, S. Luquet, C. Silvestri, G.G. Muccioli, P. D. Cani, Hepatic NAPE-PLD Is a Key Regulator of Liver Lipid Metabolism, Cells 9 (5) (2020).
[35] L. Lin, A.H. Metherel, A.P. Kitson, S.M. Alashmali, K.E. Hopperton, M. O. Trepanier, P.J. Jones, R.P. Bazinet, Dietary fatty acids augment tissue levels of n-acylethanolamines in n-acylphosphatidylethanolamine phospholipase D (NAPE-PLD) knockout mice, J. Nutr. Biochem. 62 (2018) 134–142.
[36] A. Everard, H. Plovier, M. Rastelli, M. Van Hul, A. de Wouters d'Oplinter, L. Geurts, C. Druart, S. Robine, N.M. Delzenne, G.G. Muccioli, W.M. de Vos, S. Luquet, N. Flamand, V. Di Marzo, P.D. Cani, Intestinal epithelial N-acylphosphatidylethanolamine phospholipase D links dietary fat to metabolic adaptations in obesity and steatosis, Nat. Commun. 10 (1) (2019) 457.
[37] D.G. Deutsch, S.A. Chin, Enzymatic synthesis and degradation of anandamide, a cannabinoid receptor agonist, Biochem. Pharmacol. 46 (5) (1993) 791–796.
[38] B.F. Cravatt, D.K. Giang, S.P. Mayfield, D.L. Boger, R.A. Lerner, N.B. Gilula, Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides, Nature 384 (6604) (1996) 83–87.
[39] M.K. McKinney, B.F. Cravatt, Structure and function of fatty acid amide hydrolase, Annu. Rev. Biochem. 74 (2005) 411–432.
[40] Y. Kurahashi, N. Ueda, H. Suzuki, M. Suzuki, S. Yamamoto, Reversible hydrolysis and synthesis of anandamide demonstrated by recombinant rat fatty-acid amide hydrolase, Biochem. Biophys. Res. Commun. 237 (3) (1997) 512–515.
[41] B.F. Cravatt, K. Demarest, M.P. Patricelli, M.H. Bracey, D.K. Giang, B.R. Martin, A.H. Lichtman, Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase, Proc. Natl. Acad. Sci. USA 98 (16) (2001) 9371–9376.
[42] L. Lin, A.H. Metherel, P.J. Jones, R.P. Bazinet, Fatty acid amide hydrolase (FAAH) regulates hypercapnia/ischemia-induced increases in n-acylethanolamines in mouse brain, J. Neurochem. 142 (5) (2017) 662–671.
[43] N. Ueda, R.A. Puffenbarger, S. Yamamoto, D.G. Deutsch, The fatty acid amide hydrolase (FAAH), Chem. Phys. Lipids 108 (1–2) (2000) 107–121.
[44] B.Q. Wei, T.S. Mikkelsen, M.K. McKinney, E.S. Lander, B.F. Cravatt, A second fatty acid amide hydrolase with variable distribution among placental mammals, J. Biol. Chem. 281 (48) (2006) 36569–36578.
[45] N. Ueda, K. Yamanaka, Y. Terasawa, S. Yamamoto, An acid amidase hydrolyzing anandamide as an endogenous ligand for cannabinoid receptors, FEBS Lett. 454 (3) (1999) 267–270.
[46] K. Tsuboi, Y.X. Sun, Y. Okamoto, N. Araki, T. Tonai, N. Ueda, Molecular characterization of N-acylethanolamine-hydrolyzing acid amidase, a novel member of the choloylglycine hydrolase family with structural and functional similarity to acid ceramidase, J. Biol. Chem. 280 (12) (2005) 11082–11092.
[47] X. Xie, Y. Li, S. Xu, P. Zhou, L. Yang, Y. Xu, Y. Qiu, Y. Yang, Y. Li, Genetic Blockade of NAAA Cell-specifically Regulates Fatty Acid Ethanolamides (FAEs) Metabolism and Inflammatory Responses, Front. Pharmacol. 12 (2021), 817603.
[48] S.M. Prescott, P.W. Majerus, Characterization of 1,2-diacylglycerol hydrolysis in human platelets. Demonstration of an arachidonoyl-monoacylglycerol intermediate, J. Biol. Chem. 258 (2) (1983) 764–769.
[49] S. Kondo, H. Kondo, S. Nakane, T. Kodaka, A. Tokumura, K. Waku, T. Sugiura, 2-Arachidonoylglycerol, an endogenous cannabinoid receptor agonist: identification as one of the major species of monoacylglycerols in various rat tissues, and evidence for its generation through CA2+-dependent and -independent mechanisms, FEBS Lett. 429 (2) (1998) 152–156.
[50] T. Bisogno, D. Melck, L. De Petrocellis, V. Di Marzo, Phosphatidic acid as the biosynthetic precursor of the endocannabinoid 2-arachidonoylglycerol in intact mouse neuroblastoma cells stimulated with ionomycin, J. Neurochem. 72 (5) (1999) 2113–2119.
[51] T. Bisogno, F. Howell, G. Williams, A. Minassi, M.G. Cascio, A. Ligresti, I. Matias, A. Schiano-Moriello, P. Paul, E.J. Williams, U. Gangadharan, C. Hobbs, V. Di Marzo, P. Doherty, Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain, J. Cell Biol. 163 (3) (2003) 463–468.
[52] Y. Gao, D.V. Vasilyev, M.B. Goncalves, F.V. Howell, C. Hobbs, M. Reisenberg, R. Shen, M.Y. Zhang, B.W. Strassle, P. Lu, L. Mark, M.J. Piesla, K. Deng, E. V. Kouranova, R.H. Ring, G.T. Whiteside, B. Bates, F.S. Walsh, G. Williams, M. N. Pangalos, T.A. Samad, P. Doherty, Loss of retrograde endocannabinoid signaling and reduced adult neurogenesis in diacylglycerol lipase knock-out mice, J. Neurosci. 30 (6) (2010) 2017–2024.
[53] S. Nakane, S. Oka, S. Arai, K. Waku, Y. Ishima, A. Tokumura, T. Sugiura, 2-Arachidonoyl-sn-glycero-3-phosphate, an arachidonic acid-containing lysophosphatidic acid: occurrence and rapid enzymatic conversion to 2-arachidonoyl-sn-glycerol, a cannabinoid receptor ligand, in rat brain, Arch. Biochem. Biophys. 402 (1) (2002) 51–58.

[54] C. Turcotte, F. Chouinard, J.S. Lefebvre, N. Flamand, Regulation of inflammation by cannabinoids, the endocannabinoids 2-arachidonoyl-glycerol and arachidonoyl-ethanolamide, and their metabolites, J. Leukoc. Biol. 97 (6) (2015) 1049–1070.
[55] C. Turcotte, A.S. Archambault, E. Dumais, C. Martin, M.R. Blanchet, E. Bissonnette, N. Ohashi, K. Yamamoto, T. Itoh, M. Laviolette, A. Veilleux, L. P. Boulet, V. Di Marzo, N. Flamand, Endocannabinoid hydrolysis inhibition unmasks that unsaturated fatty acids induce a robust biosynthesis of 2-arachidonoyl-glycerol and its congeners in human myeloid leukocytes, FASEB J. 34 (3) (2020) 4253–4265.
[56] C.A. Rouzer, K. Ghebreselasie, L.J. Marnett, Chemical stability of 2-arachidonylglycerol under biological conditions, Chem. Phys. Lipids 119 (1–2) (2002) 69–82.
[57] H. Tornqvist, P. Belfrage, Purification and some properties of a monoacylglycerol-hydrolyzing enzyme of rat adipose tissue, J. Biol. Chem. 251 (3) (1976) 813–819.
[58] F.P. Kupiecki, Partial purification of monoglyceride lipase from adipose tissue, J. Lipid Res. 7 (2) (1966) 230–235.
[59] T.P. Dinh, D. Carpenter, F.M. Leslie, T.F. Freund, I. Katona, S.L. Sensi, S. Kathuria, D. Piomelli, Brain monoglyceride lipase participating in endocannabinoid inactivation, Proc. Natl. Acad. Sci. USA 99 (16) (2002) 10819–10824.
[60] M. Karlsson, J.A. Contreras, U. Hellman, H. Tornqvist, C. Holm, cDNA cloning, tissue distribution, and identification of the catalytic triad of monoglyceride lipase. Evolutionary relationship to esterases, lysophospholipases, and haloperoxidases, J. Biol. Chem. 272 (43) (1997) 27218–27223.
[61] J.L. Blankman, G.M. Simon, B.F. Cravatt, A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol, Chem. Biol. 14 (12) (2007) 1347–1356.
[62] J.E. Schlosburg, J.L. Blankman, J.Z. Long, D.K. Nomura, B. Pan, S.G. Kinsey, P. T. Nguyen, D. Ramesh, L. Booker, J.J. Burston, E.A. Thomas, D.E. Selley, L.J. Sim-Selley, Q.S. Liu, A.H. Lichtman, B.F. Cravatt, Chronic monoacylglycerol lipase blockade causes functional antagonism of the endocannabinoid system, Nat. Neurosci. 13 (9) (2010) 1113–1119.
[63] J.R. Savinainen, J.Z. Patel, T. Parkkari, D. Navia-Paldanius, J.J. Marjamaa, T. Laitinen, T. Nevalainen, J.T. Laitinen, Biochemical and pharmacological characterization of the human lymphocyte antigen B-associated transcript 5 (BAT5/ABHD16A), PLoS ONE 9 (10) (2014) e109869.
[64] S. Xie, A. Borazjani, M.J. Hatfield, C.C. Edwards, P.M. Potter, M.K. Ross, Inactivation of lipid glyceryl ester metabolism in human THP1 monocytes/macrophages by activated organophosphorus insecticides: role of carboxylesterases 1 and 2, Chem. Res. Toxicol. 23 (12) (2010) 1890–1904.
[65] R. Wang, A. Borazjani, A.T. Matthews, L.C. Mangum, M.J. Edelmann, M.K. Ross, Identification of palmitoyl protein thioesterase 1 in human THP1 monocytes and macrophages and characterization of unique biochemical activities for this enzyme, Biochemistry 52 (43) (2013) 7559–7574.
[66] G. Chalhoub, S. Kolleritsch, L.K. Maresch, U. Taschler, L. Pajed, A. Tilp, H. Eisner, P. Rosina, B. Kien, F.P.W. Radner, R. Schicho, M. Oberer, G. Schoiswohl, G. Haemmerle, Carboxylesterase 2 proteins are efficient diglyceride and monoglyceride lipases possibly implicated in metabolic disease, J. Lipid Res. 62 (2021), 100075.
[67] F. Chouinard, J.S. Lefebvre, P. Navarro, L. Bouchard, C. Ferland, M. Lalancette-Hebert, D. Marsolais, M. Laviolette, N. Flamand, The endocannabinoid 2-arachidonoyl-glycerol activates human neutrophils: critical role of its hydrolysis and de novo leukotriene B4 biosynthesis, J. Immunol. 186 (5) (2011) 3188–3196.
[68] F. Chouinard, C. Turcotte, X. Guan, M.C. Larose, S. Poirier, L. Bouchard, V. Provost, L. Flamand, N. Grandvaux, N. Flamand, 2-Arachidonoyl-glycerol- and arachidonic acid-stimulated neutrophils release antimicrobial effectors against E. coli, S. aureus, HSV-1, and RSV, J. Leukoc. Biol. 93 (2) (2013) 267–276.
[69] M.C. Larose, C. Turcotte, F. Chouinard, C. Ferland, C. Martin, V. Provost, M. Laviolette, N. Flamand, Mechanisms of human eosinophil migration induced by the combination of IL-5 and the endocannabinoid 2-arachidonoyl-glycerol, J. Allergy Clin. Immunol. 133 (5) (2014) 1480-2, 1482 e1-3.
[70] C. Turcotte, E. Dumais, A.S. Archambault, C. Martin, M.R. Blanchet, E. Bissonnette, L.P. Boulet, M. Laviolette, V. Di Marzo, N. Flamand, Human leukocytes differentially express endocannabinoid-glycerol lipases and hydrolyze 2-arachidonoyl-glycerol and its metabolites from the 15-lipoxygenase and cyclooxygenase pathways, J. Leukoc. Biol. 106 (6) (2019) 1337–1347.
[71] D.K. Nomura, D.P. Lombardi, J.W. Chang, S. Niessen, A.M. Ward, J.Z. Long, H. H. Hoover, B.F. Cravatt, Monoacylglycerol lipase exerts dual control over endocannabinoid and fatty acid pathways to support prostate cancer, Chem. Biol. 18 (7) (2011) 846–856.
[72] D.K. Nomura, B.E. Morrison, J.L. Blankman, J.Z. Long, S.G. Kinsey, M. C. Marcondes, A.M. Ward, Y.K. Hahn, A.H. Lichtman, B. Conti, B.F. Cravatt, Endocannabinoid hydrolysis generates brain prostaglandins that promote neuroinflammation, Science 334 (6057) (2011) 809–813.
[73] Z. Cao, M.M. Mulvihill, P. Mukhopadhyay, H. Xu, K. Erdelyi, E. Hao, E. Holovac, G. Hasko, B.F. Cravatt, D.K. Nomura, P. Pacher, Monoacylglycerol lipase controls endocannabinoid and eicosanoid signaling and hepatic injury in mice, Gastroenterology 144 (4) (2013) 808–817, e15.
[74] M. Yu, D. Ives, C.S. Ramesha, Synthesis of prostaglandin E2 ethanolamide from anandamide by cyclooxygenase-2, J. Biol. Chem. 272 (34) (1997) 21181–21186.
[75] K.R. Kozak, S.W. Rowlinson, L.J. Marnett, Oxygenation of the endocannabinoid, 2-arachidonylglycerol, to glyceryl prostaglandins by cyclooxygenase-2, J. Biol. Chem. 275 (43) (2000) 33744–33749.
[76] L. De Petrocellis, R. Deva, F. Mainieri, M. Schaefer, T. Bisogno, R. Ciccoli, A. Ligresti, K. Hill, S. Nigam, G. Appendino, V. Di Marzo, Chemical synthesis, pharmacological characterization, and possible formation in unicellular fungi of 3-hydroxy-anandamide, J. Lipid Res. 50 (4) (2009) 658–666.
[77] K.R. Kozak, B.C. Crews, J.D. Morrow, L.H. Wang, Y.H. Ma, R. Weinander, P. J. Jakobsson, L.J. Marnett, Metabolism of the endocannabinoids, 2-arachidonylglycerol and anandamide, into prostaglandin, thromboxane, and prostacyclin glycerol esters and ethanolamides, J. Biol. Chem. 277 (47) (2002) 44877–44885.
[78] W. Yang, J. Ni, D.F. Woodward, D.D. Tang-Liu, K.H. Ling, Enzymatic formation of prostamide F2alpha from anandamide involves a newly identified intermediate metabolite, prostamide H2, J. Lipid Res. 46 (12) (2005) 2745–2751.
[79] K.R. Kozak, J.J. Prusakiewicz, S.W. Rowlinson, D.R. Prudhomme, L.J. Marnett, Amino acid determinants in cyclooxygenase-2 oxygenation of the endocannabinoid anandamide, Biochemistry 42 (30) (2003) 9041–9049.
[80] N. Koda, Y. Tsutsui, H. Niwa, S. Ito, D.F. Woodward, K. Watanabe, Synthesis of prostaglandin F ethanolamide by prostaglandin F synthase and identification of Bimatoprost as a potent inhibitor of the enzyme: new enzyme assay method using LC/ESI/MS, Arch. Biochem. Biophys. 424 (2) (2004) 128–136.
[81] P. Urquhart, A. Nicolaou, D.F. Woodward, Endocannabinoids and their oxygenation by cyclo-oxygenases, lipoxygenases and other oxygenases, BBA 1851 (4) (2015) 366–376.
[82] A. Weber, J. Ni, K.H. Ling, A. Acheampong, D.D. Tang-Liu, R. Burk, B.F. Cravatt, D. Woodward, Formation of prostamides from anandamide in FAAH knockout mice analyzed by HPLC with tandem mass spectrometry, J. Lipid Res. 45 (4) (2004) 757–763.
[83] L. Gatta, F. Piscitelli, C. Giordano, S. Boccella, A. Lichtman, S. Maione, V. Di Marzo, Discovery of prostamide F2alpha and its role in inflammatory pain and dorsal horn nociceptive neuron hyperexcitability, PLoS ONE 7 (2) (2012) e31111.
[84] C. Silvestri, A. Martella, N.J. Poloso, F. Piscitelli, R. Capasso, A. Izzo, D. F. Woodward, V. Di Marzo, Anandamide-derived prostamide F2alpha negatively regulates adipogenesis, J. Biol. Chem. 288 (32) (2013) 23307–23321.
[85] I. Matias, J. Chen, L. De Petrocellis, T. Bisogno, A. Ligresti, F. Fezza, A.H. Krauss, L. Shi, C.E. Protzman, C. Li, Y. Liang, A.L. Nieves, K.M. Kedzie, R.M. Burk, V. Di Marzo, D.F. Woodward, Prostaglandin ethanolamides (prostamides): in vitro pharmacology and metabolism, J. Pharmacol. Exp. Ther. 309 (2) (2004) 745–757.
[86] K.R. Kozak, B.C. Crews, J.L. Ray, H.H. Tai, J.D. Morrow, L.J. Marnett, Metabolism of prostaglandin glycerol esters and prostaglandin ethanolamides in vitro and in vivo, J. Biol. Chem. 276 (40) (2001) 36993–36998.
[87] I. de Bus, H. Zuilhof, R. Witkamp, M. Balvers, B. Albada, Novel COX-2 products of n-3 polyunsaturated fatty acid-ethanolamine-conjugates identified in RAW264.7 macrophages, J. Lipid Res. 60 (11) (2019) 1829–1840.
[88] J.R. Savinainen, E. Kansanen, T. Pantsar, D. Navia-Paldanius, T. Parkkari, M. Lehtonen, T. Laitinen, T. Nevalainen, A. Poso, A.L. Levonen, J.T. Laitinen, Robust hydrolysis of prostaglandin glycerol esters by human monoacylglycerol lipase (MAGL), Mol. Pharmacol. 86 (5) (2014) 522–535.
[89] J.D. Manna, J.A. Wepy, K.L. Hsu, J.W. Chang, B.F. Cravatt, L.J. Marnett, Identification of the major prostaglandin glycerol ester hydrolase in human cancer cells, J. Biol. Chem. 289 (49) (2014) 33741–33753.
[90] A. Vila, A. Rosengarth, D. Piomelli, B. Cravatt, L.J. Marnett, Hydrolysis of prostaglandin glycerol esters by the endocannabinoid-hydrolyzing enzymes, monoacylglycerol lipase and fatty acid amide hydrolase, Biochemistry 46 (33) (2007) 9578–9585.
[91] S.S. Hu, H.B. Bradshaw, J.S. Chen, B. Tan, J.M. Walker, Prostaglandin E2 glycerol ester, an endogenous COX-2 metabolite of 2-arachidonoylglycerol, induces hyperalgesia and modulates NFkappaB activity, Br. J. Pharmacol. 153 (7) (2008) 1538–1549.
[92] D.F. Woodward, Y. Liang, A.H. Krauss, Prostamides (prostaglandin-ethanolamides) and their pharmacology, Br. J. Pharmacol. 153 (3) (2008) 410–419.
[93] B.A. Berglund, D.L. Boring, A.C. Howlett, Investigation of structural analogs of prostaglandin amides for binding to and activation of CB1 and CB2 cannabinoid receptors in rat brain and human tonsils, Adv. Exp. Med. Biol. 469 (1999) 527–533.
[94] J.A. Bertrand, D.F. Woodward, J.M. Sherwood, A. Spenlehauer, C. Silvestri, F. Piscitelli, V.D. Marzo, M. Yamazaki, K. Sakimura, Y. Inoue, K. Watanabe, D. R. Overby, Deletion of the gene encoding prostamide/prostaglandin F synthase reveals an important role in regulating intraocular pressure, Prostaglandins Leukot. Essent. Fatty Acids 165 (2021), 102235.
[95] D.F. Woodward, A.H.P. Krauss, J. Chen, R.K. Lai, C.S. Spada, R.M. Burk, S. W. Andrews, L. Shi, Y. Liang, K.M. Kedzie, R. Chen, D.W. Gil, A. Kharlamb, A. Archeampong, J. Ling, C. Madhu, J. Ni, P. Rix, J. Usansky, H. Usansky, A. Weber, D. Welty, W. Yang, D.D.S. Tang-Liu, M.E. Garst, B. Brar, L.A. Wheeler, L.J. Kaplan, The Pharmacology of Bimatoprost (Lumigan™), Surv. Ophthalmol. 45 (2001) S337–S345.
[96] H. DuBiner, D. Cooke, M. Dirks, W.C. Stewart, A.M. VanDenburgh, C. Felix, Efficacy and Safety of Bimatoprost in Patients with Elevated Intraocular Pressure, Surv. Ophthalmol. 45 (2001) S353–S360.
[97] Y. Liang, D.F. Woodward, V.M. Guzman, C. Li, D.F. Scott, J.W. Wang, L. A. Wheeler, M.E. Garst, K. Landsverk, G. Sachs, A.H. Krauss, C. Cornell, J. Martos, S. Pettit, H. Fliri, Identification and pharmacological characterization of the prostaglandin FP receptor and FP receptor variant complexes, Br. J. Pharmacol. 154 (5) (2008) 1079–1093.
[98] N. Sang, J. Zhang, C. Chen, PGE2 glycerol ester, a COX-2 oxidative metabolite of 2-arachidonoyl glycerol, modulates inhibitory synaptic transmission in mouse hippocampal neurons, J. Physiol. 572 (Pt 3) (2006) 735–745.
[99] M. Alhouayek, J. Masquelier, P.D. Cani, D.M. Lambert, G.G. Muccioli, Implication of the anti-inflammatory bioactive lipid prostaglandin D2-glycerol ester in the control of macrophage activation and inflammation by ABHD6, Proc. Natl. Acad. Sci. USA 110 (43) (2013) 17558–17563.

[100] C. Turcotte, S. Zarini, S. Jean, C. Martin, R.C. Murphy, D. Marsolais, M. Laviolette, M.R. Blanchet, N. Flamand, The Endocannabinoid Metabolite Prostaglandin E2 (PGE2)-Glycerol Inhibits Human Neutrophil Functions: Involvement of Its Hydrolysis into PGE2 and EP Receptors, J. Immunol. 198 (8) (2017) 3255–3263.
[101] N. Sang, J. Zhang, C. Chen, COX-2 oxidative metabolite of endocannabinoid 2-AG enhances excitatory glutamatergic synaptic transmission and induces neurotoxicity, J. Neurochem. 102 (6) (2007) 1966–1977.
[102] A. Bruser, A. Zimmermann, B.C. Crews, G. Sliwoski, J. Meiler, G.M. Konig, E. Kostenis, V. Lede, L.J. Marnett, T. Schoneberg, Prostaglandin E2 glyceryl ester is an endogenous agonist of the nucleotide receptor P2Y6, Sci. Rep. 7 (1) (2017) 2380.
[103] A. Zimmermann, O. Vu, A. Bruser, G. Sliwoski, L.J. Marnett, J. Meiler, T. Schoneberg, Mapping the Binding Sites of UDP and Prostaglandin E2 Glyceryl Ester in the Nucleotide Receptor P2Y6, ChemMedChem 17 (7) (2022) e202100683.
[104] N. Ueda, K. Yamamoto, S. Yamamoto, T. Tokunaga, E. Shirakawa, H. Shinkai, M. Ogawa, T. Sato, I. Kudo, K. Inoue, et al., Lipoxygenase-catalyzed oxygenation of arachidonylethanolamide, a cannabinoid receptor agonist, BBA 1254 (2) (1995) 127–134.
[105] A.J. Hampson, W.A. Hill, M. Zan-Phillips, A. Makriyannis, E. Leung, R.M. Eglen, L.M. Bornheim, Anandamide hydroxylation by brain lipoxygenase:metabolite structures and potencies at the cannabinoid receptor, BBA 1259 (2) (1995) 173–179.
[106] W.S. Edgemond, C.J. Hillard, J.R. Falck, C.S. Kearn, W.B. Campbell, Human platelets and polymorphonuclear leukocytes synthesize oxygenated derivatives of arachidonylethanolamide (anandamide): their affinities for cannabinoid receptors and pathways of inactivation, Mol. Pharmacol. 54 (1) (1998) 180–188.
[107] G. van Zadelhoff, G.A. Veldink, J.F. Vliegenthart, With anandamide as substrate plant 5-lipoxygenases behave like 11-lipoxygenases, Biochem. Biophys. Res. Commun. 248 (1) (1998) 33–38.
[108] M. van der Stelt, J.A. van Kuik, M. Bari, G. van Zadelhoff, B.R. Leeflang, G. A. Veldink, A. Finazzi-Agro, J.F. Vliegenthart, M. Maccarrone, Oxygenated metabolites of anandamide and 2-arachidonoylglycerol: conformational analysis and interaction with cannabinoid receptors, membrane transporter, and fatty acid amide hydrolase, J. Med. Chem. 45 (17) (2002) 3709–3720.
[109] W.E. Boeglin, A. Itoh, Y. Zheng, G. Coffa, G.A. Howe, A.R. Brash, Investigation of substrate binding and product stereochemistry issues in two linoleate 9-lipoxygenases, Lipids 43 (11) (2008) 979–987.
[110] E. Dainese, A. Sabatucci, C.B. Angelucci, D. Barsacchi, M. Chiarini, M. Maccarrone, Impact of embedded endocannabinoids and their oxygenation by lipoxygenase on membrane properties, ACS Chem. Neurosci. 3 (5) (2012) 386–392.
[111] M. Johannesson, L. Backman, H.E. Claesson, P.K. Forsell, Cloning, purification and characterization of non-human primate 12/15-lipoxygenases, Prostaglandins Leukot. Essent. Fatty Acids 82 (2–3) (2010) 121–129.
[112] I. Ivanov, K.R. Kakularam, E.V. Shmendel, M. Rothe, P. Aparoy, D. Heydeck, H. Kuhn, Oxygenation of endocannabinoids by mammalian lipoxygenase isoforms, Biochim. Biophys. Acta, Mol. Cell. Biol. Lipids 1866 (6) (2021), 158918.
[113] P.K. Forsell, A. Brunnstrom, M. Johannesson, H.E. Claesson, Metabolism of anandamide into eoxamides by 15-lipoxygenase-1 and glutathione transferases, Lipids 47 (8) (2012) 781–791.
[114] A.S. Archambault, C. Turcotte, C. Martin, V. Provost, M.C. Larose, C. Laprise, J. Chakir, E. Bissonnette, M. Laviolette, Y. Bosse, N. Flamand, Comparison of eight 15-lipoxygenase (LO) inhibitors on the biosynthesis of 15-LO metabolites by human neutrophils and eosinophils, PLoS ONE 13 (8) (2018) e0202424.
[115] K.R. Kozak, R.A. Gupta, J.S. Moody, C. Ji, W.E. Boeglin, R.N. DuBois, A.R. Brash, L.J. Marnett, 15-Lipoxygenase metabolism of 2-arachidonylglycerol. Generation of a peroxisome proliferator-activated receptor alpha agonist, J. Biol. Chem. 277 (26) (2002) 23278–23286.
[116] W.B. Veldhuis, M. van der Stelt, M.W. Wadman, G. van Zadelhoff, M. Maccarrone, F. Fezza, G.A. Veldink, J.F. Vliegenthart, P.R. Bar, K. Nicolay, V. Di Marzo, Neuroprotection by the endogenous cannabinoid anandamide and arvanil against in vivo excitotoxicity in the rat: role of vanilloid receptors and lipoxygenases, J. Neurosci. 23 (10) (2003) 4127–4133.
[117] N.C. Gilbert, S.G. Bartlett, M.T. Waight, D.B. Neau, W.E. Boeglin, A.R. Brash, M. E. Newcomer, The structure of human 5-lipoxygenase, Science 331 (6014) (2011) 217–219.
[118] J.S. Moody, K.R. Kozak, C. Ji, L.J. Marnett, Selective oxygenation of the endocannabinoid 2-arachidonylglycerol by leukocyte-type 12-lipoxygenase, Biochemistry 40 (4) (2001) 861–866.
[119] M. van der Stelt, W.F. Nieuwenhuizen, G.A. Veldink, J.F. Vliegenthart, Dioxygenation of N-linoleoyl amides by soybean lipoxygenase-1, FEBS Lett. 411 (2–3) (1997) 287–290.
[120] M. Van Der Stelt, M.A. Noordermeer, T. Kiss, G. Van Zadelhoff, B. Merghart, G. A. Veldink, J.F. Vliegenthart, Formation of a new class of oxylipins from N-acyl (ethanol)amines by the lipoxygenase pathway, Eur. J. Biochem. 267 (7) (2000) 2000–2007.
[121] F. Tinto, A.S. Archambault, E. Dumais, V. Rakotoarivelo, M. Kostrzewa, C. Martin, P.L. Plante, Y. Desjardins, M. Simard, R. Pouliot, L. De Petrocellis, A. Ligresti, V. Di Marzo, N. Flamand, Synthesis and molecular targets of N-13-hydroxy-octadienoyl-ethanolamine, a novel endogenous bioactive 15-lipoxygenase-derived metabolite of N-linoleoyl-ethanolamine found in the skin and saliva, Biochim. Biophys. Acta, Mol. Cell. Biol. Lipids 1866 (8) (2021), 158954.
[122] A.S. Archambault, F. Tinto, E. Dumais, V. Rakotoarivelo, M. Kostrzewa, P. L. Plante, C. Martin, M. Simard, C. Silvestri, R. Pouliot, M. Laviolette, L.P. Boulet, R.M. Vitale, A. Ligresti, V. Di Marzo, N. Flamand, Biosynthesis of the Novel Endogenous 15-Lipoxygenase Metabolites N-13-Hydroxy-octodecadienoyl-ethanolamine and 13-Hydroxy-octodecadienoyl-glycerol by Human Neutrophils and Eosinophils, Cells 10 (9) (2021).
[123] R. Yang, G. Fredman, S. Krishnamoorthy, N. Agrawal, D. Irimia, D. Piomelli, C. N. Serhan, Decoding functional metabolomics with docosahexaenoyl ethanolamide (DHEA) identifies novel bioactive signals, J. Biol. Chem. 286 (36) (2011) 31532–31541.
[124] M. Maccarrone, M. Bari, A. Menichelli, D. Del Principe, A.F. Agro, Anandamide activates human platelets through a pathway independent of the arachidonate cascade, FEBS Lett. 447 (2–3) (1999) 277–282.
[125] L.M. Bornheim, K.Y. Kim, B. Chen, M.A. Correia, The effect of cannabidiol on mouse hepatic microsomal cytochrome P450-dependent anandamide metabolism, Biochem. Biophys. Res. Commun. 197 (2) (1993) 740–746.
[126] L.M. Bornheim, K.Y. Kim, B. Chen, M.A. Correia, Microsomal cytochrome P450-mediated liver and brain anandamide metabolism, Biochem. Pharmacol. 50 (5) (1995) 677–686.
[127] J.K. Chen, J. Chen, J.D. Imig, S. Wei, D.L. Hachey, J.S. Guthi, J.R. Falck, J. H. Capdevila, R.C. Harris, Identification of novel endogenous cytochrome p450 arachidonate metabolites with high affinity for cannabinoid receptors, J. Biol. Chem. 283 (36) (2008) 24514–24524.
[128] D.R. McDougle, A. Kambalyal, D.D. Meling, A. Das, Endocannabinoids anandamide and 2-arachidonoylglycerol are substrates for human CYP2J2 epoxygenase, J. Pharmacol. Exp. Ther. 351 (3) (2014) 616–627.
[129] J.H. Capdevila, J.R. Falck, The CYP P450 arachidonic acid monooxygenases: from cell signaling to blood pressure regulation, Biochem. Biophys. Res. Commun. 285 (3) (2001) 571–576.
[130] N.T. Snider, A.M. Kornilov, U.M. Kent, P.F. Hollenberg, Anandamide metabolism by human liver and kidney microsomal cytochrome p450 enzymes to form hydroxyeicosatetraenoic and epoxyeicosatrienoic acid ethanolamides, J. Pharmacol. Exp. Ther. 321 (2) (2007) 590–597.
[131] N.T. Snider, M.J. Sikora, C. Sridar, T.J. Feuerstein, J.M. Rae, P.F. Hollenberg, The endocannabinoid anandamide is a substrate for the human polymorphic cytochrome P450 2D6, J. Pharmacol. Exp. Ther. 327 (2) (2008) 538–545.
[132] C. Sridar, N.T. Snider, P.F. Hollenberg, Anandamide oxidation by wild-type and polymorphically expressed CYP2B6 and CYP2D6, Drug Metab. Dispos. 39 (5) (2011) 782–788.
[133] N.T. Snider, V.J. Walker, P.F. Hollenberg, Oxidation of the endogenous cannabinoid arachidonoyl ethanolamide by the cytochrome P450 monooxygenases: physiological and pharmacological implications, Pharmacol. Rev. 62 (1) (2010) 136–154.
[134] N.T. Snider, V.J. Walker, P.F. Hollenberg, Assay of Endocannabinoid Oxidation by Cytochrome P450, Methods Mol. Biol. 1412 (2016) 227–236.
[135] K. Stark, M. Dostalek, F.P. Guengerich, Expression and purification of orphan cytochrome P450 4X1 and oxidation of anandamide, FEBS J. 275 (14) (2008) 3706–3717.
[136] S. Kumar, Computational identification and binding analysis of orphan human cytochrome P450 4X1 enzyme with substrates, BMC Res. Notes 8 (2015) 9.
[137] V.J. Walker, A.P. Griffin, D.K. Hammar, P.F. Hollenberg, Metabolism of Anandamide by Human Cytochrome P450 2J2 in the Reconstituted System and Human Intestinal Microsomes, J. Pharmacol. Exp. Ther. 357 (3) (2016) 537–544.
[138] N.T. Snider, J.A. Nast, L.A. Tesmer, P.F. Hollenberg, A cytochrome P450-derived epoxygenated metabolite of anandamide is a potent cannabinoid receptor 2-selective agonist, Mol. Pharmacol. 75 (4) (2009) 965–972.
[139] D.R. McDougle, J.E. Watson, A.A. Abdeen, R. Adili, M.P. Caputo, J.E. Krapf, R. W. Johnson, K.A. Kilian, M. Holinstat, A. Das, Anti-inflammatory omega-3 endocannabinoid epoxides, Proc. Natl. Acad. Sci. USA 114 (30) (2017) E6034–E6043.
[140] E.M. Awumey, S.K. Hill, D.I. Diz, R.D. Bukoski, Cytochrome P-450 metabolites of 2-arachidonoylglycerol play a role in Ca2+-induced relaxation of rat mesenteric arteries, Am. J. Physiol. Heart Circ. Physiol. 294 (5) (2008) H2363–H2370.
[141] D.F. Finnegan, E.L. Shelnut, S.P. Nikas, N. Chiang, C.N. Serhan, A. Makriyannis, Novel tail and head group prostamide probes, Bioorg. Med. Chem. Lett. 25 (6) (2015) 1228–1231.
[142] K. Iwasa, S. Yamamoto, M. Takahashi, S. Suzuki, S. Yagishita, T. Awaji, K. Maruyama, K. Yoshikawa, Prostaglandin F2alpha FP receptor inhibitor reduces demyelination and motor dysfunction in a cuprizone-induced multiple sclerosis mouse model, Prostaglandins Leukot. Essent. Fatty Acids 91 (5) (2014) 175–182.
[143] C.A. Lindgren, Z.L. Newman, J.J. Morford, S.B. Ryan, K.A. Battani, Z. Su, Cyclooxygenase-2, prostaglandin E2 glycerol ester and nitric oxide are involved in muscarine-induced presynaptic enhancement at the vertebrate neuromuscular junction, J. Physiol. 591 (19) (2013) 4749–4764.
[144] D. Amadio, F. Fezza, G. Catanzaro, O. Incani, G. van Zadelhoff, A. Finazzi Agro, M. Maccarrone, Methylation and acetylation of 15-hydroxyanandamide modulate its interaction with the endocannabinoid system, Biochimie 92 (4) (2010) 378–387.
[145] K. Starowicz, W. Makuch, M. Korostynski, N. Malek, M. Slezak, M. Zychowska, S. Petrosino, L. De Petrocellis, L. Cristino, B. Przewlocka, V. Di Marzo, Full inhibition of spinal FAAH leads to TRPV1-mediated analgesic effects in

neuropathic rats and possible lipoxygenase-mediated remodeling of anandamide metabolism, PLoS ONE 8 (4) (2013) e60040.
[146] M. van der Stelt, A.M. Paoletti, M. Maccarrone, W.F. Nieuwenhuizen, G. Bagetta, G.A. Veldink, A. Finazzi Agro, J.F. Vliegenthart, The effect of hydroxylation of linoleoyl amides on their cannabinomimetic properties, FEBS Lett. 415 (3) (1997) 313–316.
[147] M. Pratt-Hyatt, H. Zhang, N.T. Snider, P.F. Hollenberg, Effects of a commonly occurring genetic polymorphism of human CYP3A4 (I118V) on the metabolism of anandamide, Drug Metab. Dispos. 38 (11) (2010) 2075–2082.

Handbook of Cannabis for Clinicians

Principles and Practice

Dustin Sulak, D.O.

Note to Readers: Standards of clinical practice and protocol change over time, and no technique or recommendation is guaranteed to be safe or effective in all circumstances. This volume is intended as a general information resource for professionals practicing in the fields of medicine and mental health; it is not a substitute for appropriate training, peer review, and/or clinical supervision. Neither the publisher nor the author(s) can guarantee the complete accuracy, efficacy, or appropriateness of any particular recommendation in every respect. As of press time, the URLs displayed in this book link or refer to existing sites. The publisher and author are not responsible for any content that appears on third-party websites.

Printed in the United States of America
First Edition

For information about permission to reproduce selections from this book, write to
Permissions, W. W. Norton & Company, Inc., 500 Fifth Avenue, New York, NY 10110
For information about special discounts for bulk purchases, please contact
W. W. Norton Special Sales at specialsales@wwnorton.com or 800-233-4830

Manufacturing by Versa Press
Book design by Daniel Lagin
Production manager: Katelyn MacKenzie

Library of Congress Cataloging-in-Publication Data

Names: Sulak, Dustin, author.
Title: Handbook of cannabis for clinicians : principles and practice / Dustin Sulak.
Description: First edition. | New York : W.W. Norton & Company, [2021] | "A Norton professional book." | Includes bibliographical references and index.
Identifiers: LCCN 2020034469 | ISBN 9780393714180 (cloth) | ISBN 9780393714197 (epub)
Subjects: LCSH: Marijuana—Therapeutic use. | Clinicians
Classification: LCC RM666.C266 S85 2021 | DDC 615.3/23648—dc23
LC record available at https://lccn.loc.gov/2020034469

W. W. Norton & Company, Inc., 500 Fifth Avenue, New York, N.Y. 10110
www.wwnorton.com

W. W. Norton & Company Ltd., 15 Carlisle Street, London W1D 3BS

1 2 3 4 5 6 7 8 9 0

Chapter 17

Cannabis Dosing

CANNABIS IS UNLIKE any other medicine I've encountered due to its wide range of effective dosages, impressive safety profile, broad physiological mechanisms of action, and versatility in treating a wide range of symptoms and diseases. I've found that using the correct dose of cannabis is the single most important factor in minimizing potential harms and maximizing potential benefits.

Unlike most medications, cannabis cannot be prescribed at a certain quantity and frequency based on body weight, age, and medical condition. Due to the complexity of the endocannabinoid system and interindividual variability, as well as the wide range of cannabis chemovars and formulations, patients typically require an individualized dosing plan that includes titration and self-awareness. With an understanding of the dosing range of cannabis, dose–response effects, therapeutic window, and phytoconstituent synergy, you can provide your patients with an individualized plan to achieve maximal benefit and minimal harm.

Broad Safe and Effective Dosing Range

When I started seeing medical cannabis users in my practice, I was surprised to find that some patients were using a very low dose (e.g., one inhalation), while other patients required much higher doses (e.g., an entire large joint or a potent edible) to achieve therapeutic benefits. Over the years, I have seen this range grow even wider, with some patients safely and effectively using ultrahigh doses and others using ultralow doses.

While I typically calculate dosing per body weight for pediatric patients only, the oral dosing range I have observed in my practice, including patients of all ages, extends

from 0.01 to 50 $mg \cdot kg^{-1} \cdot day^{-1}$ total cannabinoids. Interestingly, human use in this range is reported in the peer-reviewed literature, with Δ^9-tetrahydrocannabinol (THC) reported to be effective for pediatric spasticity at doses as low as 0.08 $mg \cdot kg^{-1} \cdot day^{-1}$,[1] and safety trials conducted on cannabidiol (CBD) for seizures at doses up to 50 $mg \cdot kg^{-1} \cdot day^{-1}$.[2]

Despite this 5,000-fold dosing range, even the highest clinically observed doses are well below the acute toxic dose found in primates, with single doses of THC up to 9,000 mg/kg in rhesus monkeys proving nonlethal and producing no histopathological changes;[3] daily doses up to 250 mg/kg were nonlethal over 28 days.[4]

The 0.01 and 50 $mg \cdot kg^{-1} \cdot day^{-1}$ responders are clearly outliers in my practice, with most patients experiencing efficacy and tolerability in the 0.1–2 $mg \cdot kg^{-1} \cdot day^{-1}$ range of total cannabinoids. A general rule for cannabis dosing and titration is to start low, go slow, and do not be afraid to go all the way. Some patients do very well with high doses.

Multiphasic and Bidirectional Dose–Response Effects

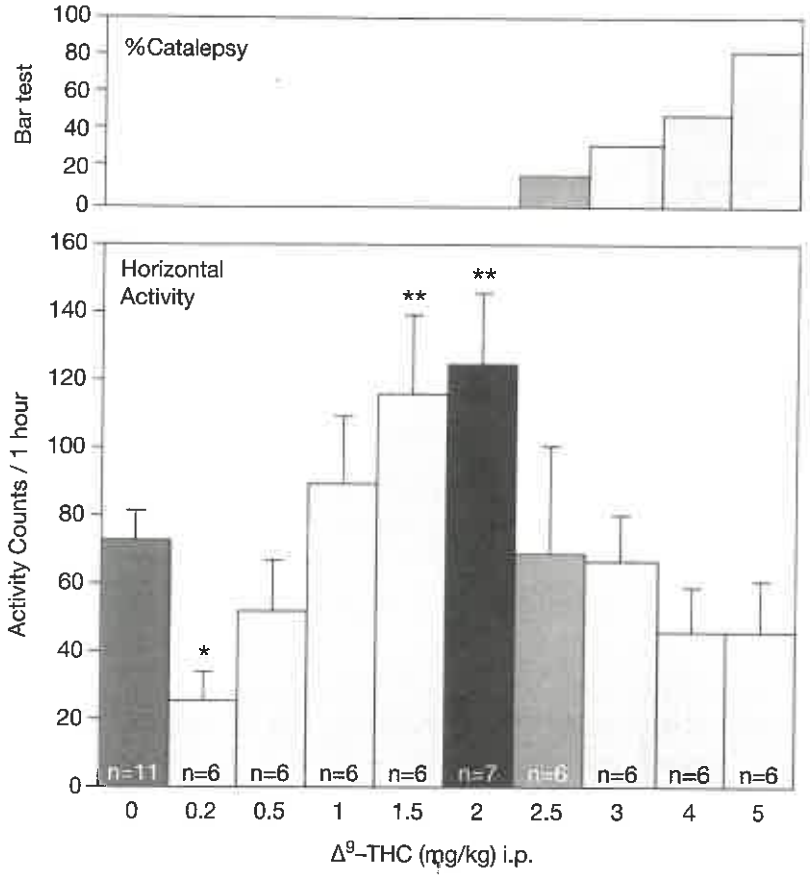
Within the broad safe dosing range, cannabinoids are notorious for producing multiphasic and bidirectional dose–response effects.* If a certain dose of a cannabinoid causes a particular effect, one cannot assume that a higher dose will produce a stronger effect or that a lower dose will cause a weaker effect. Patients and clinicians are often surprised to find that reducing the dose of cannabis can increase therapeutic effects, and that different doses of cannabis could produce opposite effects in the same individual.

A straightforward example of multiphasic and bidirectional dose–response effects can be seen in the activity of rats exposed to various doses of THC (Figure 17.1). A triphasic effect was demonstrated: low doses of THC (0.2 mg/kg) suppressed locomotor activity, higher doses (1–2 mg/kg) dose-dependently stimulated movement greater than baseline, until catalepsy emerged at even higher doses (>2.5 mg/kg), accompanied by decreases in activity. Interestingly, the suppression of activity at 0.2 mg/kg was more profound than at 5 mg/kg.[5]

* A multiphasic dose–response effect indicates that the magnitude of effect undergoes different phases of direct and indirect relationship with increasing dose. Bidirectional dose–response effects are opposite effects from different doses of the same agent.

FIGURE 17.1

Dose–Response Curve Showing Effects of Systemic Administration of THC on Horizontal Activity in Rats



Note. *significantly different from the rest of the groups except those receiving 4 or 5 mg/kg, $p<0.05$; **significantly different from the rest of the groups except the one receiving 1mg/kg, $p<0.05$

Adapted from "Activational role of cannabinoids on movement," by M. C. Sañudo-Peña, J. Romero, G. E. Seale, J. J. Fernandez-Ruiz, and J. M. Walker, 2000, *European Journal of Pharmacology, 391*(3), pp. 269–274 (https://doi.org/10.1016/S0014-2999(00)00044-3). Copyright © 2000 by Elsevier. Reprinted with permission.

Cerebral metabolism in rodents has also been shown to exhibit a biphasic dose–response relationship to THC: very low doses increased cerebral metabolism, measured by 2-deoxyglucose uptake, while higher doses of THC decreased cerebral metabolism. Limbic regions, particularly the hippocampus, were more sensitive to THC, suggesting a selective regional action of THC at lower doses.[6] This highlights the complexity of

cannabinoid-related biphasic dose–response patterns, with varying responses in one organ or responses in different tissues potentially shifting phase at different doses.

THC is well-known for its biphasic and bidirectional effects on anxiety in animals and humans. Several studies have shown that acute administration of low doses of THC and CB1 agonists can reduce anxiety behaviors in a variety of animal models, while higher doses or administration under stressful environmental conditions can increase anxiety behaviors and neuroendocrine responses to acute stress. Promoting relaxation and reducing tension are some of the most commonly reported benefits in cannabis users; paradoxically, the most consistently documented adverse effects of cannabis intoxication are anxiety and panic-like reactions. The complex biphasic dose–response curve of cannabinoids on anxiety appears to undergo a leftward shift under stressful environmental conditions, may undergo a rightward shift under socially permissive situations, and could also be shifted by personality factors or comorbid anxiety disorders.[7]

Biphasic psychological effects are common in domains other than anxiety. For example, in human subjects with some history of cannabis use, oral THC was shown to dampen negative emotional responses without impairing performance at 7.5 mg but increased negative affect and impaired performance at 12.5 mg.[8] This suggests that mood benefits of THC can be achieved with doses lower than those causing impairment.

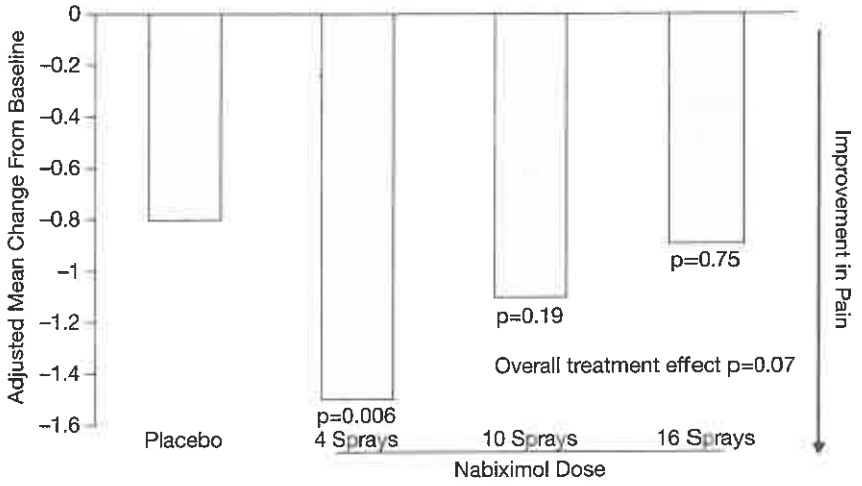
CBD has also been shown to exhibit biphasic effects on anxiety in several animal models and two human clinical experiments. In one study, using a simulated public speaking test model of anxiety in 57 healthy male volunteers, pretreatment with 300 mg of CBD resulted in significant reductions in anxiety compared to placebo, while doses of 150 and 600 mg were not effective.[9] Another study evaluated 60 healthy subjects of both sexes in a real-life public speaking test; 300 mg of CBD, but neither 100 nor 900 mg, reduced anxiety in the postspeech phase.[10]

Similar to its effects in models of anxiety, CBD has also demonstrated biphasic dose–response relationships in animal models of depression,[11,12] compulsive behavior,[13] and pain.[14] This reminds us that the optimal dose of CBD likely depends on the condition and the individual, and it highlights the importance of testing different doses in experimental studies as well as in the clinic.

THC and CBD can have biphasic effects on pain, demonstrated in a placebo-controlled study of opioid-treated cancer patients with poorly controlled chronic pain treated with nabiximols (THC/CBD oromucosal spray) at three doses or placebo (Figure 17.2). The low-dose group, receiving 4 sprays/day (20.8 mg of THC + CBD), had an overall 26% reduction in pain, while the medium-dose group (10 sprays/day) had 19% reduction and the high-dose group (16 sprays/day) was no different than placebo, ~14% reduction.[15]

FIGURE 17.2

Analysis of Change from Baseline in Average Pain Score



Note. From "Nabiximols for opioid-treated cancer patients with poorly-controlled chronic pain: A randomized, placebo-controlled, graded-dose trial," by R. K. Portenoy, E. D. Ganae-Motan, S. Allende, R. Yanagihara, L. Shaiova, S. Weinstein, R. McQuade, S. Wright, and M. T. Fallon, 2012, *The Journal of Pain, 13*(5), pp. 438–449 (https://doi.org/10.1016/j.jpain.2012.01.003). Copyright 2012 by American Pain Society. Reprinted with permission.

THC can have bidirectional effects on pain depending on dose, experience with cannabis, and the nature of the pain. For example, THC has been known to increase the intensity of acute pain, especially at higher doses and in the cannabis-naïve,[16,17] though THC is well-known to ameliorate chronic pain.

As mentioned above, bidirectional effects are not always dose-dependent but are related to factors inherent in set (external environmental conditions) and setting (internal mindset and expectations of the participant): the same dose of THC in the same individual may cause anxiolytic effects in a serene environment or anxiogenic effects in a stressful environment; anxious individuals may be more likely to experience anxiolytic effects, while nonanxious individuals may be more likely to experience anxiogenic effects.[18] Different chemovars at the same dose have also been reported to produce bidirectional effects; patients often prefer certain chemovars for daytime use, due to awakening effects, and others for evening use, due to sedating effects.

Perhaps the clearest example of bidirectional effects of cannabinoids is the acute effects of a THC overdose, which produces a symptom constellation characterized by many of the same symptoms often ameliorated by appropriately dosed THC: anorexia, nausea, vomiting, diarrhea, anxiety or panic, dyskinesia, spasticity, and pain.

Why do we see so many multiphasic and bidirectional dose–response effects in cannabinoid medicine? There are likely several reasons, including tolerance building via receptor downregulation, complex heterogenous effects on the endocannabinoid system and the other physiological systems it modulates, and interindividual variability, including the influence of set and setting.

Ultralow Doses

On the basis of surprising clinical observations, I have been keenly interested in the ultra-low-dose range of cannabinoids, and I suspect that more research in this area will yield additional therapeutic applications, including health promotion and disease prevention.

Ultralow doses of cannabinoids have been shown to be physiologically active in several preclinical models. A single intraperitoneal (ip) injection of 0.002 mg/kg THC to mice induced long-lasting activation of protective signaling molecules in the brain, including the transcription factor CREB and brain-derived neurotrophic factor (BDNF), and provided protection against a variety of neuronal insults.[19] Incredibly, another study found that the same single dose induced mild but long-term cognitive deficits in young mice that lasted for at least 5 months.[20] Yet another study showed that the same ultralow single dose in female older mice improved performance in six different behavioral assays of memory and learning, with 24-month-old treated mice scoring similarly to naïve 2-month-old mice; remarkably, the beneficial effect lasted for at least 7 weeks.[21] This suggests a bidirectional effect of ultra-low-dose THC based on one's age and baseline physiological activity.

Other preclinical studies have reported that ip injection of 0.002 mg/kg THC reduced damage and preserved cardiac function when administered to mice 2 hr before myocardial infarction[22] and also reduced apoptotic, oxidative, and inflammatory injury in mice with hepatic ischemia and reperfusion.[23] Keep in mind that 0.002 mg/kg is extraordinarily low for mice, with an allometrically scaled dose in humans around 0.00016 mg/kg or 0.16 µg/kg.

I have also observed acidic cannabinoids at ultralow doses to be effective in my practice, supported by preclinical literature. In a rat model of nausea, Δ^9-tetrahydrocannabinolic acid demonstrated antiemetic effects at 0.05 mg/kg, $\frac{1}{10}$ the minimal effective dose of THC.[24] Subsequent work on cannabidiolic acid (CBDA) found an effective dose as low as 0.5 µg/kg, approximately 2,000 times lower than that of CBD (1–5 mg/kg).[25] Interestingly, the dose–response curve for the antiemetic effect of CBDA was not biphasic in the ranges tested, as has been reported for CBD, which potentiated vomiting at 20–40 mg/kg.[26]

Ultra-low-dose synergistic effects have also been described among the cannabinoids. For example, in a rodent model of hyperalgesia and inflammation, doses of THC (100 µg/kg, orally) or CBDA (0.1 µg/kg, orally) that were ineffective alone effectively reduced symptoms when combined.[27] Cannabis users are frequently exposed to dozens of minor cannabinoids in this dosing range, perhaps contributing to chemovar-specific entourage effects.

Hormesis, a dose–response phenomenon characterized by low-dose stimulation and high-dose inhibition, explains some of these surprising results and is far from unique to cannabinoids. A simple example of hormesis is the ability of ultralow doses of disinfectants to stimulate the growth of *Candida*, while higher doses inhibit growth and even higher doses are fungicidal. A number of mechanisms have been described, commonly involving the activation of protective and regenerative cellular pathways upon low-dose exposure. An understanding of the paradigm of hormesis, recently reviewed in relation to the effects of THC on memory and cognition,[28] is important for the cannabis clinician.

Therapeutic Window for Δ^9-Tetrahydrocannabinol

The therapeutic window, which is the range between the effective dose and a dose that causes intolerable adverse effects, can be most narrow in cannabis-naïve patients, who are more likely to experience adverse effects. This can be managed with appropriate dosing strategies.

Compared to the cannabis-naïve patient, frequent users have shown blunted responses to the psychotomimetic, perception-altering, cognition-impairing, anxiogenic, and cortisol-modulating effects of THC but not to the euphoric effects.[29] This heterogenous tolerance-building to various effects may be due to cannabinoid receptor downregulation and desensitization occurring at varying rates and magnitudes in different brain regions. For example, CB1 downregulation upon exposure to THC occurs faster in the hippocampus, an area that contributes to the effects of THC on memory, than in the basal ganglia, an area that contributes to the euphoric effects of THC.[30]

In general, the therapeutic effects of THC, especially those produced by lower doses, are more resistant to tolerance development than adverse effects or effects produced by higher doses. For example, rodents build tolerance to high-dose behavioral effects of THC faster than moderate-dose hypothermic effects, and they are slowest to build tolerance to low-dose analgesic effects.[31]

Thus, when titrated appropriately, THC has the ability to widen its own therapeutic window, a strategy that can be intentionally utilized with patients who have not recently or ever been exposed to THC. Cannabis-naïve patients tend to have a narrow therapeutic

window at first but are typically able to tolerate higher and more therapeutic doses with fewer adverse effects after a period of regular administration, which in my experience ranges from a few days to 2 weeks.

Strategically, I start my cannabis-naïve patients on a subtherapeutic dose of THC that is unlikely to produce any noticeable effects, titrate up to the minimum dose needed to produce mild effects, hold at that dose for 3 days, and then resume slow titration. I believe the therapeutic window widens during the 3-day pause at the minimal noticeable dose, increasing the likelihood of success with subsequent titration. In those who can more patiently wait for satisfactory results, I typically titrate every 3–5 days, which accomplishes a similar goal.

Δ^9-Tetrahydrocannabinol and Cannabidiol Synergy

Among the synergistic effects of cannabis constituents, the interaction of THC and CBD is the most well-studied. Preclinical and clinical literature demonstrate that adding CBD can help mitigate many of the adverse effects of THC, such as intoxication, sedation, and tachycardia, while potentiating many of the benefits of THC, such as analgesia and antiemesis. While the reduction in adverse effects is likely related to the activity of CBD as a negative allosteric modulator of the CB1 receptor, pharmacodynamic interactions and pharmacokinetic interactions (such as decreased 11-hydroxylation of THC) that are not mediated by cannabinoid receptors likely also play a role.[32]

Though CBD certainly offers therapeutic effects on its own, its most useful clinical property may be its role as an adjunct to THC, widening the therapeutic window of THC while providing additional benefits. The large body of clinical trial evidence and long history of effective clinical use of nabiximols THC/CBD oromucosal spray support this strategy.

For example, in a clinical trial of 177 patients with chronic cancer-related pain not adequately responding to chronic opioid treatment, subjects were randomized into groups receiving a THC-dominant oromucosal spray, a combined THC/CBD spray, or a placebo spray and allowed to titrate the dose within basic guidelines over the course of a week with minimal dosing adjustments thereafter. By the end of 2 weeks, the THC group took an average of ~10 sprays/day, for a total of ~27 mg of THC daily, while the combined THC/CBD group took ~11.5 sprays/day, for a total of ~31 mg of THC and ~29 mg of CBD daily. The two groups experienced similar rates of adverse effects, despite the slightly higher dose of THC in the combined THC/CBD group, but the THC/CBD recipients were nearly twice as likely to experience a ≥30% reduction in pain scores compared to

the THC-only group (43% vs. 23%, respectively).[33] In this study, the combination of THC and CBD resulted in improved tolerance of a higher total cannabinoid dose with improved benefits.

Clinicians with access to products containing a variety of THC:CBD ratios are able to adjust the dosing based on patient goals, personal preference, and clinical response. For example, those with a history of being highly sensitive to adverse effects from THC may want to start with a THC:CBD ratio in the range 1:10–1:5, whereas others may do well on a nabiximols-like formula in the range of 1:1.

From a practical standpoint, it is important to note that for many patients a lower THC:CBD ratio results in the need for a higher total daily dose of cannabinoids, which typically results in a more expensive treatment. For example, a patient with pain may experience relief at THC (5 mg) that is comparable but less well tolerated than the relief experienced with THC (4 mg) + CBD (8 mg), but the latter treatment may cost more than twice as much, based on the total number of milligrams needed per dose.

Though it is widely reported that CBD mitigates euphoria and psychoactive adverse effects of THC while enhancing its benefits, some contrary data have been reported. In one study of 31 healthy cannabis users, treatment with 200, 400, or 800 mg of oral CBD 90 min prior to inhalation of THC-dominant cannabis did not change ratings of feeling "high," an experience typically desired by nonmedical users but avoided in medical users.[34] Older studies report that CBD changes the type of psychological reaction induced by THC in infrequent cannabis smokers, reducing their anxiety and thereby rendering THC more enjoyable.[35] Thus, CBD may heterogeneously mitigate the adverse effects of THC, especially in regard to psychoactivity, and this likely varies significantly among individuals and delivery methods.

Furthermore, some evidence exists that CBD may pharmacodynamically inhibit the analgesic effects of THC. In a small study on women with fibromyalgia that compared three chemovars of vaporized cannabis (type 1, THC-dominant; type 2, mixed THC and CBD; and type 3, CBD-dominant) with placebo, pharmacokinetic data suggested that CBD inhalation increased THC plasma concentrations but diminished THC-induced analgesic effects, indicative of synergistic pharmacokinetic but antagonistic pharmacodynamic interactions.[36] Since the analgesic effects of THC are mainly CB1-mediated and CBD somewhat antagonizes this signaling, it should not be surprising that CBD can decrease the power of therapeutic effects of THC in addition to its adverse effects.

Adverse and Beneficial Psychoactive Effects

The psychoactive effects of cannabis, primarily related to CB1 agonism, are often reported as adverse effects. Patients may feel somnolence, time distortion, impaired short-term memory, confusion, anxiety, and perceptual disturbances that can interfere with function and may be dangerous when combined with driving and other activities. When the goal of treatment is typically to improve patients' function while reducing their symptoms, THC-related intoxication and impairment are undesirable.

Often grouped with other psychoactive properties of cannabis, euphoria is also frequently considered an adverse effect, the sixth most commonly reported based on one meta-analysis.[37] Euphoria is defined as "a feeling of great happiness or well-being."[38] An older definition from The Century Dictionary, which I find especially applicable to cannabis, is "the state of feeling well, especially when occurring in a diseased person."

Is it undesirable for our diseased patients to feel well? In most situations, euphoria should be considered a valuable side benefit, and clinical strategies to maximize the euphoric properties of cannabis are appropriate in many cases. In fact, hundreds of my patients have reported this feeling of well-being as the primary therapeutic effect of cannabis, with reduction in symptoms noted as less profound and less important to their clinical course.

An Israeli qualitative study of 19 patients, ages 28–79 years, using cannabis to treat chronic pain illustrates this concept. All the interviewees described the experience of chronic pain in terms of "losing one's self" and "losing one's life," and many used the word "normal" to describe their lives after beginning to use cannabis. They talked about how cannabis allowed them to sleep, focus, and function and, through this, to attain a sense of normality in their lives once again. Interviewees generally reported that cannabis changed their pain to a different and more tolerable form and more often distanced the pain rather than reducing it. They frequently described a return to normality or "restored self," a term proposed by the authors: for example, "I can smoke and feel normal," "I behave much more normal than before. It is simply easier," and "I was a shadow of my former self, and now I am a normal human being again."[39]

I also frequently hear these phrases in my clinic—perhaps the most common is, "cannabis has given me my life back." Importantly, in the qualitative data mentioned above, regaining oneself was not described as a passive process facilitated solely by the use of medical cannabis; the interviewees consistently described a state in which they became more proactive in reclaiming their lives. I also observe this in my patients, many of whom become more inspired and engaged in meaningful and therapeutic activities after they start using cannabis. After observing this trend for a couple of years in

my most successful patients, I began promoting it to all my patients by first suggesting they explore the psychoactive effects of cannabis as an adjunct to meditation, prayer, reflection, and exercise.

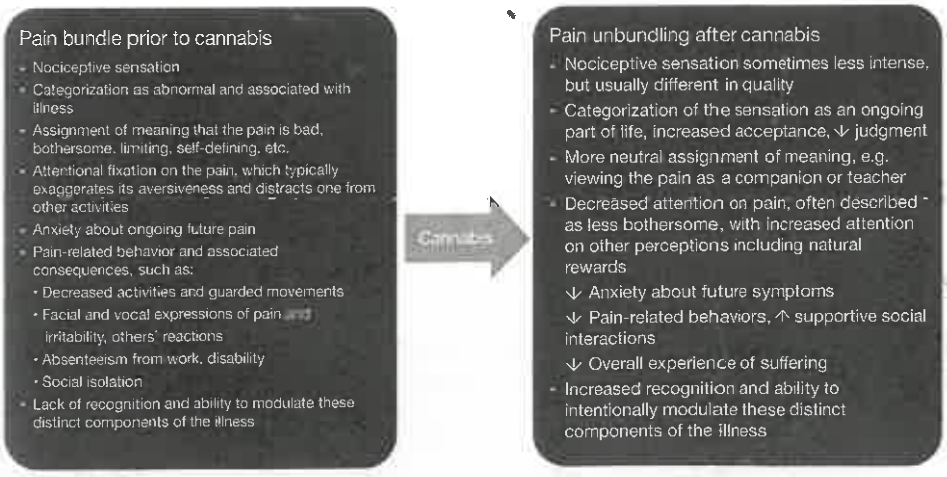
Beyond restored self, my patients report a variety of psychological benefits attributed to the psychoactive effects of cannabis, many of which are experienced or learned during the acute effects but often persist long after the drug effects resolve:

- greater acceptance of symptoms and illness
- increased self-awareness and insight into one's situation
- enhanced capacity for mindfulness and decreased emotional reactivity
- ability to view oneself from a different vantage point
- increased ability to find creative solutions to problems
- increased mental, emotional, and physical flexibility and capacity for change
- increased desire to interact socially and help others
- sense of connection to God, nature, or the universe

Furthermore, many patients with pain describe a process of unbundling, or increasing their ability to recognize and modulate the various aspects of their condition, illustrated in Figure 17.3:

FIGURE 17.3

Unbundling of Chronic Pain Perception and Behavior



Source: Dustin Sulak

Most of my cannabis-naïve patients wish to experience symptom relief with minimal to no psychoactive effects, which we can typically accomplish using the dosing strategies suggested in this chapter. At some point during their follow-up, however, I explain that while symptom reduction is important, *healing* often involves a repatterning of perceptions, thoughts, and behavior and that the psychoactivity of cannabis, perhaps its most powerful medicinal property, can promote such changes, especially when intentionally used for that purpose.

Many patients assume that cannabis-induced psychoactivity is intense or hallucinogenic, which it can be at high doses, so I explain that the desired experience can be achieved with a mild effect, similar to 1–2 glasses of wine but typically more pleasurable. This context is reassuring and patients are usually willing to follow my instructions. Since my patients often use an oral or oromucosal dose of THC before bed to promote restorative sleep, I invite patients to, once weekly, take their before-bed dose of THC a couple of hours earlier than usual, turn off or set aside screens and communication devices, prevent other distractions, and prepare a safe environment conducive to gentle movement, meditation, reflecting, and journaling.* If they do not notice a shift in consciousness, I encourage them to increase the dose by 10%–25% each week until they do.

Treat the Patient, Not the Diagnosis

Clinicians are often surprised to learn that they might give similar cannabis dosing plans to patients with a wide range of diagnoses. Patients with Crohn's disease, multiple sclerosis, posttraumatic stress disorder, and chronic musculoskeletal pain may respond to similar regimens; conversely, two patients with the same condition may succeed with significantly different regimens. This challenges the way we think about condition-specific treatments and forces us to individually tailor treatment plans for our patients.

While this may seem complex and challenging, it actually simplifies the practice of cannabinoid medicine. The cannabis clinician typically becomes somewhat of a generalist, attracting refractory patients from all the other specialty fields, but we need not become experts in every field. While it is important to learn the basic pathophysiology of, and current conventional treatment options for, our patients' conditions, this information only sometimes impacts my cannabis recommendations. There are certainly clinical

* My patient education website Healer.com includes a section titled Wellness Activities with video instructions for gentle movements, meditations, and breathing practices I find useful for patients exploring the psychoactive benefits of cannabis.

pearls that can help with specific conditions and diagnoses that warrant caution or specific considerations, but the overall success of the cannabis clinician is largely based on one's general knowledge of dosing, delivery methods, and physiological effects of the various active constituents.

The most important information that will guide your individualized cannabis recommendations is an understanding of the patient and their context in life. Synchronize with their goals and personal preferences, understand their highest values, and identify high-impact opportunities for improving function and quality of life. Not only will this inform your therapeutic strategy, but the process of gathering this information will invoke trust and build the foundation for a therapeutic relationship, a partnership in healing.

When I begin an initial visit, I usually lead with these three questions to elucidate the goals and values of my patient:

- If you could get anything out of this visit, what would it be?
- Imagine you are much healthier 2 months from now: how is life different? Please paint the picture for me.
- Please describe your greatest successes and biggest challenges in dealing with this condition.

The responses help me craft two or three concrete goals that I always include in the patient's plan, which I print and provide to the patient at the end of their visit. If restorative sleep is lacking, that is always one of the goals at the end of an initial visit. I like the goals to include concrete details, demonstrating that I understand what makes my patients' lives more meaningful, whether it is "be able to tolerate and enjoy three hours of rabbit hunting," "be able to drive to work without stopping to use the bathroom," or "be able to enjoy your time with your grandchildren without being distracted by pain."

Dosing Strategies for Cannabis-Naïve Patients

For patients with no recent or historical experience with cannabis, my goal is to introduce and titrate cannabis with the greatest likelihood of benefits with the lowest risk of adverse effects, including mild adverse effects. Recall the basic principles of cannabis dosing that inform my strategy for the cannabis-naïve:

- broad safe and effective dosing range with interindividual variability and sometimes intraindividual variability (different symptoms responding to different doses)

- nonlinear dose-response effects, necessitating methodical titration and self-assessment
- narrow therapeutic window of THC, which can broaden over time and when combined with CBD

For patients with chronic, daily symptoms, I typically start with the oromucosal route of delivery, usually drops of infused oil, for easy titration, intermediate onset and duration, and overall efficacy and tolerability. I provide a starting dose intended to be subtherapeutic, and I explain to the patient that by starting low and going slow we are most likely to achieve success. I provide a titration schedule and ask the patient to use the Inner Inventory, a scale I developed to help patients identify when they begin responding to dose adjustments (see Example Patient B box for details). At the minimal dose required to elicit a mild response, I instruct the patient to temporarily stop titration and continue at that dose for 3 days. Thereafter, if they are not satisfied with the therapeutic effects, they may resume titrating until they experience either satisfactory benefits, in which case they should stop titrating and continue on that dose, or adverse effects, in which case they should reduce their dose slightly.

I usually recommend three times daily dosing after meals, and unless there is a reason for a lower THC:CBD ratio, I start with 1:1, which is broadly effective, well-tolerated, and supported by a large body of (nabiximols) clinical trial data. The starting dose is usually 1–2 mg of THC with a corresponding dose of CBD if indicated. If the patient has disturbed sleep, this becomes our first therapeutic target. Because I have observed mixed results with CBD administration before bed, with some patients reporting enhanced sleep and others reporting disturbed sleep, I typically recommend THC-dominant, low-CBD formulas with sedating terpene profiles for this purpose.

I determine the frequency of titration based on my assessment of the urgency of the patient's need for relief, aversiveness to side effects, and self-efficacy. If the urgency is low, I may begin with before-bed dosing only and then proceed to daytime dosing after benefits are obtained, but for faster results, the day and night titration can occur simultaneously.

The accompanying boxes are examples of cannabis plan components that I print and provide to patients. I keep these as modifiable template items in my electronic health record so I can create custom recommendations for each patient. Please feel free to use them in your practice and modify as needed.

What Products Do I Need and How Do I Take Them?

Obtain a cannabis oil or tincture with a known potency and clear labeling so you can administer an accurate dosage using a dropper or oral syringe. For faster onset, hold the liquid in your mouth for 1–5 min before swallowing, and for better absorption, take after meals containing fat. If you purchase separate THC-dominant and CBD-dominant products, this will allow the ratio of these components to be adjusted if needed.

Driving, Adverse Effects, and Overdose

After starting cannabis and after each dosage increase, avoid driving or operating machinery until you know how a particular dose affects you. When a person is impaired from cannabis, they are usually aware of this impairment and able to make a good decision about whether they are safe to drive.

Unwanted side effects of cannabis are usually due to an excessive dose of THC. The most common mild adverse effects are dizziness, impaired coordination, dry mouth, and sleepiness. Overdose symptoms include confusion, nausea, vomiting, diarrhea, anxiety or panic, pain, and hallucination. Cannabis overdose is unpleasant but safe and resolves within 4–24 hr. The best treatment for accidental overdose is a calm, comfortable environment. Contact emergency medical services if you experience chest pain, palpitations, or trouble breathing or you suspect dehydration related to vomiting and/or diarrhea.

Example Patient A: Cannabis-Naïve, Low Urgency, Slower Approach

Step 1: Address the need for restorative sleep.

- Take before bed (1–2 hr in advance for trouble falling asleep, right before bed for trouble staying asleep).
- Use a THC-dominant product, preferably made from sedating cannabis varieties.
- Take a starting dose of 2 mg.
- Increase the dose by 1 mg every 2–3 nights until you experience satisfactory restorative sleep and wake feeling rested. If you experience adverse effects like morning grogginess or disorientation in the middle of the night, reduce the dose by 1 mg.
- Until our next meeting, your maximum dose should be 15 mg.

Step 2: After restorative sleep is achieved, address daytime symptoms.

- Take in the morning and afternoon.
- Take a starting dose of 2 mg of THC and 2 mg of CBD.
- Increase the dose by 1 mg of THC and 1 mg of CBD every 3 days until you achieve satisfactory benefits or bothersome side effects. If you experience side effects, reduce the dose slightly.
- Until our next meeting, your maximum dose should be 10 mg of THC and 10 mg of CBD twice daily.

Example Patient B: Cannabis-Naïve, Higher Urgency, Faster Approach

Step 1: Address the need for restorative sleep.

- Take before bed (1–2 hr in advance for trouble falling asleep, right before bed for trouble staying asleep).
- Use a THC-dominant product, preferably made from sedating cannabis varieties.
- Take a starting dose of 2 mg.
- Increase the dose by 1 mg every night until you experience satisfactory restorative sleep and wake feeling rested. If you experience adverse effects like morning grogginess or disorientation in the middle of the night, reduce the dose by 1 mg.
- Until our next meeting, your maximum dose should be 15 mg.

Step 2: Address daytime symptoms (can be concurrent with step 1).

- Take in the morning and afternoon.
- Take a starting dose of 2 mg of THC and 2 mg of CBD.
- Increase the dose by 1 mg of THC and 1 mg of CBD every day until you achieve a mild improvement in your Inner Inventory scores associated with the cannabis oil.
- Temporarily stop increasing and remain at this minimal noticeable dose for 3 days.
- Resume increasing the dose by 1 mg of THC and 1 mg of CBD every 3 days until you achieve satisfactory benefits or bothersome side effects. If you experience side effects, reduce the dose slightly.
- Until our next meeting, your maximum dose should be 10 mg of THC and 10 mg of CBD twice daily.

How to Check Your Inner Inventory:

Rate each item on a scale of 1 to 10 (1 = worst and 10 = best) before and 1–2 hr after taking each dose. Set an alarm reminder and record your scores for best results.

- **Breath:** How easy and smooth is your breath?
- **Body:** How comfortable and calm does your body feel? How easy is it to remain still and comfortable?
- **Mood:** How easy is it for you to feel a sense of contentment and appreciation? How easy is it for you to smile right now?
- **Symptoms:** How severe are your symptoms? (1 = minimal and 10 = severe)

I also appreciate the titration schedule of nabiximols oromucosal spray, which allows slow introduction starting with before-bed dosing and gives time for widening of the therapeutic window of THC using a slightly different method.[40] Prior to adopting this slower and lower dose titration schedule, previous nabiximols clinical trials yielded significantly higher rates of adverse effects.[41] In Table 17.1, each spray refers to THC (2.7 mg) + CBD (2.5 mg).

TABLE 17.1
Titration Schedule of Nabiximols Oromucosal Spray

	Number of sprays		
Day	**In the morning**	**In the evening**	**Total per day**
1	0	1	1
2	0	1	1
3	0	2	2
4	0	2	2
5	1	2	3
6	1	3	4
7	1	4	5
8	2	4	6
9	2	5	7

	Number of sprays		
Day	In the morning	In the evening	Total per day
10	3	5	8
11	3	6	9
12	4	6	10
13	4	7	11
14	5	7	12

Source. Sativex® Summary of Product Characteristics, https://www.medicines.org.uk/emc/product/602. Accessed 9 October, 2020

For cannabis-naïve patients with episodic or infrequent symptoms that are more amenable to the inhaled route of delivery, such as migraine, nausea or vomiting, and acute spasms, I recommend starting with 1–2 inhalations, waiting 10–15 min, and then reassessing (using the Inner Inventory) whether another inhalation is needed. I advise my patients to be cautious when using flower vaporizers the first time; many will otherwise take several inhalations in a row while trying to determine if the device is turned on and set correctly. The respiratory sensations are much less noticeable and the exhalation is much less visible compared to smoking, so it is easy for novices to assume they did not receive a proper inhalation.

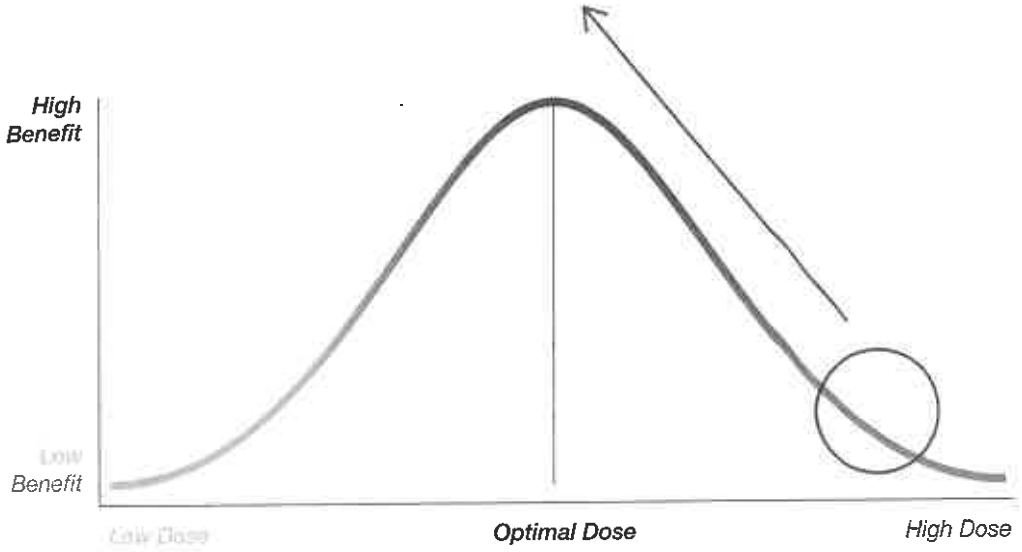
Dosing Strategies for Experienced Cannabis Users

I frequently encounter patients who have been using cannabis for psychoactive, social, creative, or spiritual purposes for years or decades prior to becoming ill. They consult me to learn how to modify their use pattern to achieve better symptom control with fewer side effects. I have found three strategies essential in helping such patients: reversing tolerance, noninhaled routes of delivery, and combining CBD with THC.

I first screen for signs of cannabis tolerance in daily users. It is common for the occasional cannabis smoker, who develops a symptom responsive to cannabis, to gradually increase the frequency and dosage until they are smoking all day every day. At first, they clearly note the increased intake is associated with symptomatic improvement, but over time the treatment loses efficacy. I explain the concept of biphasic dose–response, usually with a diagram (see Figure 17.4), and emphasize that by reversing cannabis tolerance they can achieve increased benefits and decreased side effects with significantly less expense.

FIGURE 17.4

Biphasic Dose–Response



Source. Dustin Sulak

It is also common for experienced users to inappropriately rely on inhaled cannabis to treat persistent symptoms. I help them identify that they experience only temporary relief, and typically wait until the symptoms recur to an intensity that is significantly bothersome or limiting before deciding to readminister cannabis. The mental and physiological distress of breakthrough symptoms occurring numerous times daily is far from ideal. I explain that oromucosal and/or oral delivery can provide baseline relief so that the troughs between sessions of inhalation are less intense and the need to inhale is noticed less often (Figure 17.5). I also explain that many people who rely on inhalation to treat constant symptoms build tolerance and diminish the therapeutic power of their medicine.

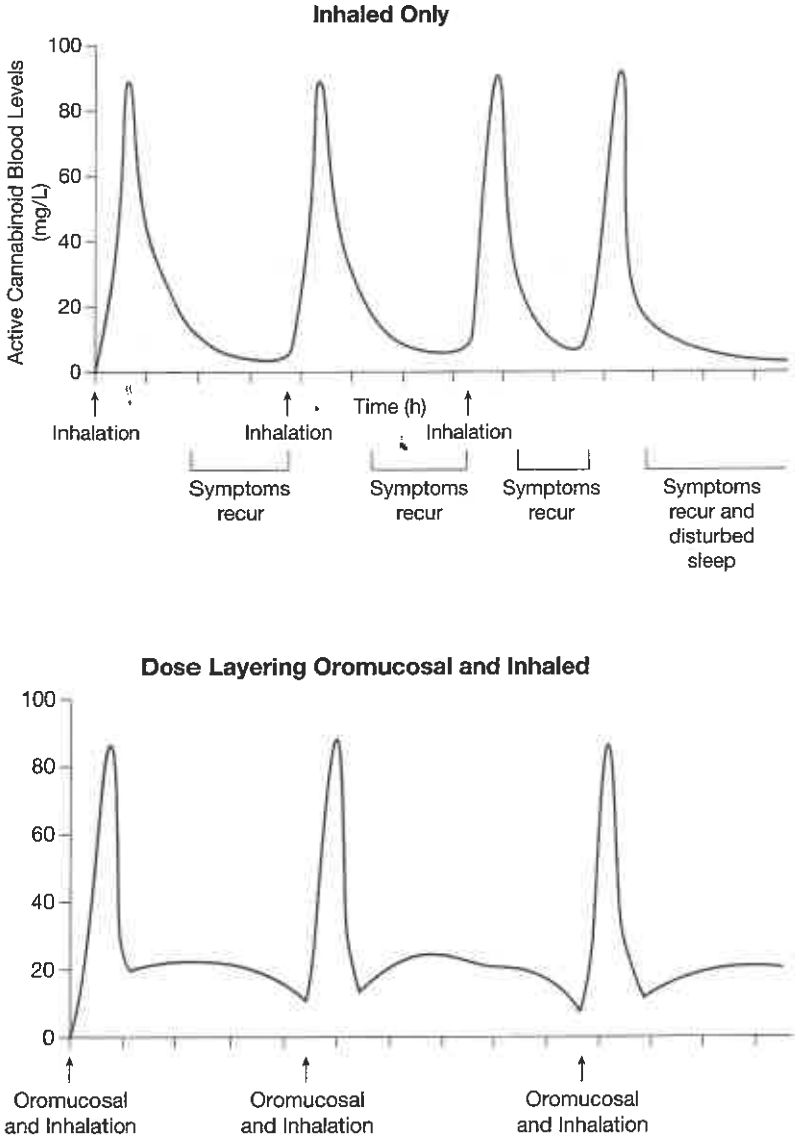
Finally, I frequently meet experienced users who reserve cannabis for after work or on the weekends because they chose to avoid driving or working while impaired. As they age and their symptoms intensify, they find themselves suffering through the day and experiencing excellent relief at night. Some may have tried low or moderate doses of hemp-based CBD products with unsatisfactory results. I explain the concept of the therapeutic window of THC, that they can take a certain (usually oromucosal) dose of THC during the day with some relief and no impairment, and that adding CBD to the THC can improve their results and diminish potential impairment. Many experienced cannabis

FIGURE 17.5A AND FIGURE 17.5B

Dose Layering:

(A) Inhaled Only and

(B) Oromucosal and Inhaled



Source. Dustin Sulak

users fail to appreciate CBD until they try using it as an adjunct to THC and recognize the combination is fully compatible with their daily activities.

Cannabis Tolerance and How to Reverse It

I see many patients using cannabis with minimal benefit because they have built tolerance not only to the psychoactive or adverse effects but also to the therapeutic effects. For example, consider a 62-year-old woman with severe anxiety and chronic pain who smokes six joints daily: her symptoms are so severe she can barely leave the house to make an appointment, but she knows the cannabis must be helping because when she does not smoke she feels even worse. In this situation, layering in baseline treatment with oromucosal delivery or adding CBD will unlikely yield additional benefit. The priority, in this situation, is to first reverse her tolerance and then proceed with other dosing strategies.

Over the years I have interviewed hundreds of cannabis patients who have figured out how to reverse their own tolerance. Remarkably, most find a 2-day period of abstinence is all they need to experience stronger effects from a lower dose. This was confirmed by an imaging study that showed cannabis-dependent men had 15% lower CB1 receptor availability in the brain compared to healthy controls (significant in all brain regions except the thalamus and cerebellum), but after just 2 days of abstinence the cannabis-dependent subjects had no difference in CB1 availability compared to healthy controls. The CB1 availability increased only slightly between day 2 and day 28 of abstinence, and the magnitude of withdrawal symptoms was strongly inversely correlated with CB1 availability.[42] These findings suggest that avoiding and reversing cannabis tolerance is important not only for therapeutic efficacy but also for preventing dependence and withdrawal.

I developed a 6-day "sensitization protocol" that effectively helps patients reverse cannabis tolerance while developing self-awareness and self-efficacy, available free on Healer.com. In an email survey sent to my practice in 2012, 48 patients responded after completing the protocol. Five patients did not note benefit, but the other 43 reduced their dose by an average of 56% while universally reporting improvements in therapeutic effects. To help patients succeed, I include a variety of supports (and distractions) such as worksheets, exercises, and diet strategies.* The essential components of the protocol, however, are straightforward:

- **Days 1 and 2:** Begin with 48 hr of cannabis abstinence.†
- **Day 3:** Break the "cannabis fast" with the lowest dose required to produce a mini-

* www.healer.com/programs/sensitization-protocol/

† Can be modified to 24–36 hr in patients who cannot tolerate 48 hr.

mal noticeable response, using the Inner Inventory scale to help determine when this occurs.

- **Days 3–5:** Continue to administer cannabis up to three times daily to achieve only a minimal noticeable response.
- **Day 6:** Gradually increase the dose to achieve equal or superior therapeutic effects compared to those experiences prior to starting the protocol.

Interestingly, some patients report ongoing sensitization to cannabis during days 3–5. Perhaps they are simply becoming more aware of the subtle effects of cannabis by exercising their faculties of self-perception, or perhaps these minimal doses of THC are gently upregulating the sensitivity of the endocannabinoid system. Some preclinical data support the latter hypothesis: THC increased the production of endocannabinoids in brain cells;[43] coadministration of THC and morphine caused an upregulation of CB1 receptor levels in mouse spinal cords;[44] and an acute dose of THC increased cannabinoid receptor affinity in rats.[45]

When recommending the sensitization protocol, I am sure to emphasize several potential benefits, beyond improved efficacy, to help patients overcome their hesitancy about taking a break from their favorite medicine. Suggesting that they might reduce the monthly cost of their medicine by 50% or more is usually the most compelling, along with the likelihood of reducing adverse effects, especially fatigue.

For patients demonstrating some signs of problematic cannabis use, the sensitization protocol is an excellent exercise for regaining control over their relationship with cannabis. It provides a challenge they can usually overcome, contributing to self-efficacy; it often gives them a short experience of cannabis withdrawal, which can motivate them to avoid building tolerance in the future; and it helps reframe the relationship with cannabis, prioritizing the therapeutic effects.

Over the years, numerous patients have also reported that avoiding prolonged use of a particular chemovar helps prevent tolerance; rotating between several chemovars every 1–12 weeks seems to be helpful for many patients who use cannabis flower. It is possible that oromucosal and oral cannabis products that have some standardization for content of major constituents, but also have inherent variability in minor constituents related to batch differences in starting materials, may produce better results with greater retention of efficacy over time.

Summary: How to Craft Individualized Plans for Your Patients

Dosing principles:

- Wide safe and effective dosing range (0.01–50 $mg \cdot kg^{-1} \cdot day^{-1}$) with most patients responding in the 0.1–2 $mg \cdot kg^{-1} \cdot day^{-1}$ range. Start low and go slow, but do not be afraid to go all the way.
- Biphasic and bidirectional dose–response effects are common. Methodical titration, self-awareness, and concrete goals are essential.
- The therapeutic window of THC is narrow in the cannabis-naïve. Use slow titration strategies and coadministration with CBD to improve tolerability and efficacy. Consider treating with a bedtime dose of THC for several days before starting daytime THC dosing.
- For those wishing to avoid psychoactive effects, use precision oromucosal dosing with a THC:CBD ratio ≤1:5, acidic cannabinoids, or topical products. Consider suggesting gentle psychoactive experiences at a follow-up visit.
- Expect most patients to eventually get best results with dose layering of various delivery methods to address baseline, breakthrough, and episodic symptoms.
- For patients who inhale cannabis frequently, suggest a flower vaporizer to minimize or eliminate potential harm. Avoid vape pens with oil cartridges until they are proven safe.
- Set concrete goals congruent with your patients' highest values and recognize that symptom amelioration may be less important than "restored self" and improvement in function.
- Prioritize correcting disturbed sleep, an essential element of healing that often potentiates other treatments. Because I often see mixed results in patients using CBD before bed, I suggest starting with low-dose oromucosal THC, which is much more often effective in my experience.
- Identify and reverse tolerance and problematic use by employing the sensitization protocol or intermittent 2-day periods of abstinence.

Elements of a cannabis plan:

- goal(s)
- route of delivery

- starting dose
- titration amount and frequency
- expectations and what to look for while titrating: signs of efficacy and adverse effects
- maximum dose (until the next follow-up visit)
- what to look for when selecting products compatible with the plan

Epilepsia, 51(5):768–772, 2010
doi: 10.1111/j.1528-1167.2009.02334.x

FULL-LENGTH ORIGINAL RESEARCH

Cerebrospinal fluid levels of the endocannabinoid anandamide are reduced in patients with untreated newly diagnosed temporal lobe epilepsy

*†Andrea Romigi, ‡Monica Bari, *†Fabio Placidi, *†M. Grazia Marciani, §Marilena Malaponti, *Federico Torelli, *†Francesca Izzi, *Chiara Prosperetti, *Silvana Zannino, *Francesca Corte, ¶Carlo Chiaramonte, and **§Mauro Maccarrone

*Dipartimento di Neuroscienze, Università degli Studi di Roma Tor Vergata, Roma, Italy; †Fondazione Santa Lucia, Via Ardeatina 306, Roma, Italy; ‡Dipartimento di Medicina Sperimentale e Scienze Biochimiche, Università degli Studi di Roma Tor Vergata, Roma, Italy; §Centro Europeo per la Ricerca sul Cervello (CERC)/Fondazione Santa Lucia, Roma, Italy; ¶University of Rome La Sapienza, Cattedra di Statistica Assicurativa Roma, Roma, Italy; and **Dipartimento di Scienze Biomediche, Università degli Studi di Teramo, Roma, Italy

Summary

Purpose: The endocannabinoid system is involved in excitatory/inhibitory balance mechanisms within the central nervous system (CNS). Growing evidence shows that its perturbation leads to development of epileptic seizures in experimental models, thus indicating that endocannabinoids play an intrinsic protective role in suppressing pathologic neuronal excitability. Experimental data also demonstrate that the endocannabinoid anandamide (AEA) can antagonize epileptic discharges in hippocampal tissue. The objective of our study was to measure endocannabinoids levels in the cerebrospinal fluid (CSF) of drug-naive patients affected by temporal lobe epilepsy (TLE).

Methods: We measured the levels of both AEA and the other endocannabinoid, 2-arachidonoylglycerol (2-AG), in the CSF of drug-naive patients with TLE.

Results: A significant reduction of AEA was found in the CSF of patients with compared with healthy controls (epileptic patients = 2.55 ± 1.78 pmol/ml; healthy controls = 11.65 ± 7.53 pmol/ml; n = 9 for both groups, $p < 0.01$). 2-AG levels, however, were not affected (epileptic patients = 209.5 ± 146.56; healthy controls = 159.6 ± 110.2) (n = 6 for both groups, $p = 0.48$).

Discussion: Our findings seem to be consistent with experimental evidence demonstrating a significant prevention of epileptic seizures induced by endocannabinoids in models of epilepsy. Furthermore, they support the hypothesis that AEA may be involved in its pathogenesis, suggesting a hypothetical primary impairment of the endocannabinoid system in untreated TLE. The actual role of this in vivo dysregulation still remains unclear.

KEY WORDS: Endocannabinoids, Anandamide, 2-Arachidonoylglycerol, Temporal lobe epilepsy.

Epilepsy is related to pathologic hyperexcitability and hypersynchronous activity in large neural networks. Seizure seems to be the result of an imbalance between the two basic and antagonist neuronal properties—excitation and inhibition—toward excitation. The role of excitatory versus inhibitory mechanisms, electrical gap junctions, neuronal network oscillations, and rewiring of neuronal circuits in the pathogenesis of epilepsy is still unclear. Endogenous cannabinoids (endocannabinoids) are lipid mediators, mainly *amides* and *esters* of long-chain polyunsaturated fatty acids. They act as endogenous agonists for type-1 and type-2 cannabinoid receptors (CB1R and CB2R, respectively), thus mimicking in central and peripheral tissues the pharmacologic effects of delta-9-tetrahydrocannabinol (Δ^9-THC), the main psychoactive ingredient of *Cannabis sativa* extracts such as hashish and marijuana (Maccarrone et al., 2007; Di Marzo, 2008). Arachidonyl ethanolamide (anandamide, AEA) and 2-arachidonoylglycerol (2-AG) are the most studied endocannabinoids.

The endocannabinoid system is constituted of endocannabinoids, their target receptors, and the associated enzymes involved in their synthesis, transport, and degradation (Di Marzo, 2008). CB1R is the most expressed cannabinoid receptor subtype in the central nervous system (CNS), located in or near synaptic terminals (Chevaleyre et al.,

Accepted August 6, 2009; Early View publication October 8, 2009.
Address correspondence to Dr Andrea Romigi, Servizio di Neurofisiopatologia, Università di Roma Tor Vergata, Policlinico Tor Vergata, Viale Oxford 81, 00133 Rome, Italy. E-mail: a_romigi@inwind.it
Professor Mauro Maccarrone, Dipartimento di Scienze Biomediche, Università degli Studi di Teramo, Piazza A. Moro 45, 64100 Teramo, Italy. E-mail: mmaccarrone@unite.it

2006). Its activation inhibits synaptic transmitter release by means of Ca^{2+}or K^+ channel modulation, and the inhibition of adenylyl cyclase (Maccarrone et al., 2007; Di Marzo, 2008). It is now widely accepted that the activation of a postsynaptic cell leads to the production of endocannabinoids, which spread in a retrograde direction across the postsynaptic membrane and the synaptic cleft. This allows the binding to and the activation of CB1Rs on presynaptic terminals. The final outcome is the inhibition of neurotransmitter release (Chevaleyre et al., 2006).

Endocannabinoids are involved in an ''on demand'' protection against seizures, as demonstrated in experimental murine models of epilepsy (Monory et al., 2006). The anticonvulsant effects of cannabinoids are mediated primarily by the CB1R activation (Wallace et al., 2001). Whether marijuana and other cannabis derivatives could have anticonvulsant properties in humans is still controversial (Consroe et al., 1975; Cunha et al., 1980; Gross et al., 2004; Mortati et al., 2007). Several cannabinoids have demonstrated anticonvulsant effects in animal models; however, proconvulsant effects have also been reported. In addition, CB1R activation in in vivo animal models by means of AEA, 2-AG, and selective synthetic agonists demonstrated significant prevention of epileptic seizure (Panikashvili et al., 2001; van der Stelt et al., 2001a,b; Wallace et al., 2001, 2002).

The anticonvulsant effects of the endocannabinoid system in experimental models may bring up some therapeutic issues. Although *Cannabis sativa* was used to treat epilepsy since antiquity (Mechoulam & Lichtman, 2003), its anticonvulsant effect was not yet demonstrated; however, although the selective enhancement of endocannabinoid levels may induce an anticonvulsant effect, the therapeutic exploitation of CB1R agonists is not viable because of their negative psychotropic effects.

Furthermore, a marked downregulation of CB1R expression and glutamatergic axon terminals equipped with this receptor in human hippocampus affected by severe refractory epilepsy has recently been reported (Ludányi et al., 2008). The downregulation was also associated with a decreased expression of a CB1R-interacting protein and the 2-AG synthesizing enzyme.

In order to determine whether the dysregulation of endocannabinoid signaling is involved in the pathogenesis of temporal lobe epilepsy (TLE) in humans, we evaluated AEA and 2-AG levels in the cerebrospinal fluid (CSF) of drug-naive patients with TLE.

Materials and Methods

Patients and controls

Peripheral blood and CSF were collected from 12 untreated inpatients (9 F, 3 M, mean age 46 ± 15.58 years, range 27–72 years) affected by partial epilepsy. Patients gave their written informed consent and were admitted to the Neurological Clinic of the University of Rome Tor Vergata to participate in the diagnostic study. On the basis of clinical, neuroradiologic [1,5 or 3 Tesla brain magnetic resonance imaging (MRI)] and electroencephalography (EEG) characteristics (interictal and ictal EEG, when available), all patients were diagnosed as affected by cryptogenic (probably symptomatic) TLE, according to International League Against Epilepsy (ILAE) criteria (Engel, 2001). Patients were included after the second or third seizure, and the delay between the first seizure and CSF collection was determined. Patients were also completely seizure-free for >24 h before CSF collection. The following exclusion criteria were applied: (1) major psychiatric or medical illnesses, (2) migraine history, (3) intake of drugs affecting the CNS, and (4) CNS disorders other than epilepsy.

Twelve control subjects (9 F, 3 M, mean age 42.71 ± 15.18 years; range 17–82 years), matched for age and sex, were enrolled; all of them were inpatients at the same clinic and underwent lumbar puncture for diagnostic purposes. In all these subjects, clinical and instrumental data excluded CNS or systemic diseases. In particular, seven patients were admitted for suspected multiple sclerosis (five with paresthesias and two with dizziness); two patients were admitted for suspected subarachnoid hemorrhage (headache), and three patients for loss of consciousness due to vasovagal (one patient) or cardiogenic syncope (two patients).

Controls were drug-free for at least 3 months and none took any medication at the time of CSF collection or had personal or family history of epilepsy. Routine determinations in patients and controls included total cell count and measurement of the concentration of total proteins and albumin, both in CSF and serum. Samples were stored at −80°C until analysis.

Participants were informed about the study purpose and procedures, and written informed consent was obtained from all subjects. The study was approved by the institutional ethics committee.

CSF determination of endocannabinoids

Human blind specimens were sent to the biochemistry laboratory. Lipids were extracted from CSF, and the organic phase was dried under nitrogen in order to evaluate AEA or 2-AG endogenous levels. The dry pellet was resuspended in 20 μl methanol; it was processed and analyzed by high performance liquid chromatography (HPLC) with fluorometric detection, as reported (Centonze et al., 2007). It should be noted that AEA and 2-AG were detected independently, using different aliquots of CSF. The amount and quality of CSF withdrawn from the both groups was suitable to detect AEA in nine samples, and 2-AG in six samples only.

Statistical analysis

Statistical analysis was performed by the nonparametric Mann-Whitney *U* test, elaborating experimental data by

means of the STATISTICA 7.0 program (StatSoft, Tulsa, OK, U.S.A.).

Results

AEA and 2-AG levels in the CSF were measured both in patients and controls. An approximately 5-fold AEA decrease was detected in the CSF of epileptic patients compared with healthy controls [mean ± standard deviation (SD) of epileptic patients = 2.55 ± 1.78 pmol/ml; mean ± SD of healthy controls = 11.65 ± 7.53 pmol/ml] ($n = 9$ for both groups, $p < 0.01$). Conversely, 2-AG was not affected (mean ± SD of epileptic patients = 209.5 ± 146.56; mean ± SD of healthy controls = 159.6 ± 110.2) ($n = 6$ for both groups, $p = 0.48$) (see Fig. 1). Demographic, clinical, EEG, and CSF data of each epileptic patient are summarized in Table 1.

Discussion

Despite the vast literature on the neuroprotective role of endocannabinoid pathways against neuronal hyperexcitability and epileptic seizures in experimental models, its failure has been poorly investigated in epileptic patients and ignored in drug-naive subjects. A growing body of evidence seems to demonstrate a significant alteration of the endocannabinoid AEA, mainly in animal model of mesial temporal epilepsy and, as a matter of fact, we found that AEA, but not 2-AG, is significantly reduced in the CSF of patients affected by TLE compared with healthy controls. This means that the two major endocannabinoids may be differentially engaged in TLE. Our narrow sample presumably encompassed both mesial and neocortical TLE. It is unfortunate that the small and heterogeneous sample did not allow us to detect any possible correlation between AEA levels and age and/or disease duration because of the low statistical power. Other parameters regarding disease severity, such as drug resistance and seizure frequency, could not be evaluated because patients were both drug-naive and newly diagnosed.

Our findings were obtained in untreated, newly diagnosed patients who were seizure-free for >24 h before CSF collection. In these patients, confounding factors such as antiepileptic drugs, drug-resistance, and seizures that may modulate ''endogenous'' antiepileptic mechanisms were excluded.

These data may be in agreement with the growing body of evidence that hypothesizes an experimental neuroprotective role of endocannabinoids (Marsicano et al., 2003) and their effective inhibition of refractory epilepsy in hippocampal neuronal culture models (Katona et al., 2006).

In addition, our findings may be consistent with the emerging concept that AEA and 2-AG have different regulatory mechanisms, and that AEA is preferentially involved in pathologic events (Marsicano et al., 2003; Monory et al., 2006; Centonze et al., 2007; Deshpande et al., 2007). Furthermore, Wallace et al. (2002) showed that AEA and its analog O-1812 act as anticonvulsants in the maximal electroshock seizure model, further implicating CB1R as a main site of seizure modulation. Similarly, Marsicano et al. (2003) confirmed the neuroprotective role of AEA, demonstrating that kainic acid treatment induced the increase of AEA in hippocampal neurons, without affecting 2-AG, thus providing ''on demand'' protection against acute excitotoxicity in CNS neurons.

On the basis of the available evidence, it can be inferred that the failure of AEA-mediated inhibition both via γ-aminobutyric acid (GABA)ergic and antiglutamatergic networks can contribute to the pathogenesis of untreated and newly diagnosed patients with TLE. In line with this, a previous study demonstrated that CB1R and AEA are involved in the control of neuronal excitability, thus reducing excitatory neurotransmission at a presynaptic site. This mechanism might be involved in the prevention of excessive excitability, leading to epileptiform activity; indeed the CB1R antagonist SR141716 blocked the inhibition evoked by exogenous cannabinoids (Ameri et al., 1999).

Because CB1R are expressed on both GABAergic interneurons (Katona et al., 1999; Chevaleyre et al., 2006) and glutamatergic hippocampal neurons (Marsicano & Lutz, 1999; Freund et al., 2003), a growing body of evidence

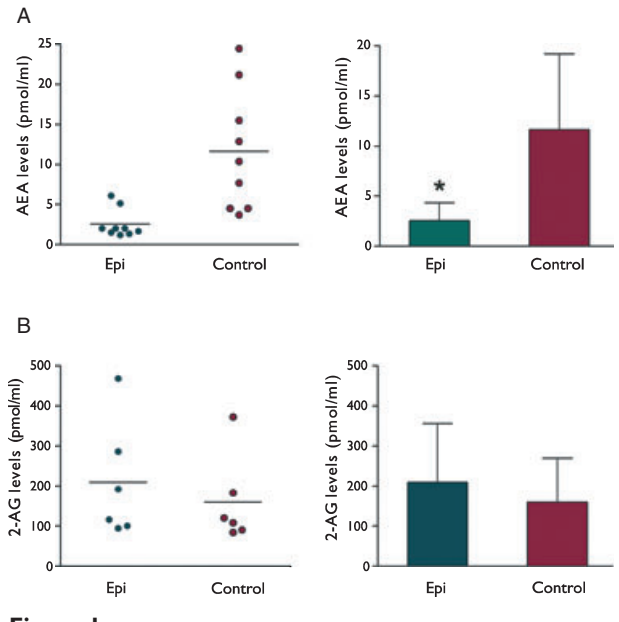


Figure 1.
AEA and 2-AG levels in cerebrospinal fluid (CSF) of epileptic and control patients. (**A**) The graphs show that AEA levels are significantly reduced in the CSF of epileptic versus control patients. (**B**) The graphs show that there is no significant difference in 2-AG levels in CSF of epileptic versus control patients.
Epilepsia © ILAE

Table 1. Demographic, clinical features, and CSF dosages

Pt	Sex	Age (years)	Delay (months)	Interictal EEG	Ictal EEG	Seizure type	AEA pmol/ml	2-AG pmol/ml
1	F	58	48	Right FT Left FT	Right FT	CPS Verbal and oral automatisms; Loss of contact	N.A.	94.18
2	F	34	48	Left T	Left T	CPS, SG; Psychomotor arrest, speech arrest, jerks, and paresthesias of right face and arm	N.A.	468.7
3	F	33	36	Right FT	Right posterior T	CPS, SG Auditory aura	N.A.	286.26
4	F	28	12	Bilateral FT with right prevalence	N.A.	CPS Epigastric aura, psychomotor arrest;	2	192.15
5	F	30	12	Right FT	N.A.	CPS, SG Psychomotor arrest;	1.35	N.A
6	F	72	48	Left T	Left T	CPS, SG Confusion followed by GTC	1.5	N.A.
7	F	60	2	Right FCT	N.A.	CPS, SG Psychomotor arrest	1.67	N.A.
8	F	58	2	Bilateral FCT	N.A.	SPS, SG Epigastric aura	5.13	N.A.
9	M	55	12	Left FT	Left T	CPS, SG Orofacial automatism, confusion	2	N.A.
10	F	27	12	Left FT	N.A.	CPS, SG Speech arrest	6.1	N.A.
11	M	58	9	Right FT Left FT	Right FT Left FT	SPS, SG Psychomotor arrest, confusion, ictal tachycardia	1.2	100
12	F	54	12	Left T	N.A.	CPS Epigastric aura, speech arrest	2	116

F, female; M, male; SPS simple partial seizure; CPS complex partial seizure; SG secondarily generalized; TLE, temporal lobe epilepsy; N.A., not available; FT, frontotemporal; FCT, fronto-centro-temporal; T, temporal.

supports the role played by endocannabinoids in key epileptogenic circuits in the hippocampus, resulting in a protective action of CB1R stimulation against kainic acid–induced seizures (Monory et al., 2006). An important issue is whether the AEA reduction occurs in glutamatergic or GABAergic cell populations. Recently, Ludányi et al. (2008) observed a decreased ratio of CB1R positive excitatory axon terminals in the epileptic human hippocampus, as similarly observed in the rodent hippocampus (Katona et al., 2006; Kawamura et al., 2006; Monory et al., 2006). In addition, these authors (Ludányi et al., 2008) provided direct anatomic evidence that CB1Rs are also located presynaptically on glutamatergic axon terminals in human hippocampus but that they were severely reduced in the hippocampal formation of epileptic patients.

Consequently, the disruption of protective endocannabinoid signaling in patients with refractory TLE may be sustained by a reduction of CB1R density in human hippocampus (Ludányi et al., 2008). These authors evaluated patients affected by drug-refractory symptomatic TLE, hypothesizing that the lack of effective treatment may be due to their severe vulnerability to perturbations of network excitability. Our sample is different because it included drug-naive, newly diagnosed patients potentially treatable affected by mesial or lateral TLE. Nevertheless, very recently Kozan et al. (2009) demonstrated a CB1R role in regulating epileptiform activity in penicillin-induced epilepsy in rats, an experimental model that resembles human focal interictal discharges (Purpura et al., 1972). As a consequence, the neuroprotective endocannabinoid signaling may be diminished regardless of severity, disease duration, drug-resistance, or seizure frequency. Furthermore, epilepsy itself may be related to an increase of glutamatergic and/or a decrease of GABAergic currents, not sufficiently antagonized by AEA. The role of endocannabinoid signaling in human epileptogenesis has to be clarified by more extensive clinical studies regarding TLE.

Despite the small sample size of this study, our findings are particularly noteworthy being ''in vivo'' data obtained from untreated epileptic patients, which is difficult to reproduce in experimental models. It is difficult to determine if the AEA decrease may be a cause or a consequence of the seizures. However, the recent history of epilepsy, the narrow number of seizures, and the seizure-free delay before

CSF collection suggest a hypothetical primary impairment of the endocannabinoid system in untreated cryptogenic TLE.

The actual role of this ''in vivo'' dysregulation remains unclear. Further studies are necessary to clarify if this pathway is involved in each type of epilepsy (i.e., primary generalized or extra temporal lobe epilepsy) or whether it is typical of TLE.

However, the ineffectiveness of conventional antiepileptic drugs in one-third of patients affected by epilepsy (French, 2007) highlights the need to develop novel therapeutic targets, where endocannabinoid system could play an intriguing role.

Acknowledgments

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Disclosure: The authors have no conflicts of interest to report.

References

Ameri A, Wilhelm A, Simmet T. (1999) Effects of the endogenous cannabinoid, anandamide, on neuronal activity in rat hippocampal slices. *Br J Pharmacol* 126:1831–1839.

Centonze D, Bari M, Rossi S, Prosperetti C, Furlan R, Fezza F, De Chiara V, Battistini L, Bernardi G, Bernardini S, Martino G, Maccarrone M. (2007) The endocannabinoid system is dysregulated in multiple sclerosis and in experimental autoimmune encephalomyelitis. *Brain* 130:2543–2553.

Chevaleyre V, Takahashi KA, Castillo PE. (2006) Endocannabinoid-mediated synaptic plasticity in the CNS. *Annu Rev Neurosci* 29:37–76.

Consroe PF, Wood GC, Buchsbaum H. (1975) Anticonvulsant nature of marihuana smoking. *JAMA* 234:306–307.

Cunha JM, Carlini EA, Pereira AE, Ramos OL, Pimentel C, Gagliardi R, Sanvito WL, Lander N, Mechoulam R. (1980) Chronic administration of cannabidiol to healthy volunteers and epileptic patients. *Pharmacology* 21:175–185.

Deshpande LS, Blair RE, Ziobro JM, Sombati S, Martin BR, DeLorenzo RJ. (2007) Endocannabinoids block status epilepticus in cultured hippocampal neurons. *Eur J Pharmacol* 558:52–59.

Di Marzo V. (2008) Targeting the endocannabinoid system: to enhance or reduce? *Nat Rev Drug Discov* 7:438–455.

Engel J Jr. (2001) ; International League Against Epilepsy (ILAE). A proposed diagnostic scheme for people with epileptic seizures and with epilepsy: report of the ILAE Task Force on Classification and Terminology. *Epilepsia* Jun 42:796–803.

French JA. (2007) Refractory epilepsy: clinical overview. *Epilepsia* 48: 3–7.

Freund TF, Katona I, Piomelli D. (2003) Role of endogenous cannabinoids in synaptic signaling. *Physiol Rev* 83:1017–1066.

Gross DW, Hamm J, Ashworth NL, Quigley D. (2004) Marijuana use and epilepsy: prevalence in patients of a tertiary care epilepsy center. *Neurology* 62:2095–2097.

Katona I, Sperlágh B, Sík A, Käfalvi A, Vizi ES, Mackie K, Freund TF. (1999) Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. *J Neurosci* 19:4544–4558.

Katona I, Urbán GM, Wallace M, Ledent C, Jung KM, Piomelli D, Mackie K, Freund TF. (2006) Molecular composition of the endocannabinoid system at glutamatergic synapses. *J Neurosci* 26:5628–5637.

Kawamura Y, Fukaya M, Maejima T, Yoshida T, Miura E, Watanabe M, Ohno-Shosaku T, Kano M. (2006) The CB1 cannabinoid receptor is the major cannabinoid receptor at excitatory presynaptic sites in the hippocampus and cerebellum. *J Neurosci* 26:2991–3001.

Kozan R, Ayyildiz M, Agar E. (2009) The effects of intracerebroventricular AM-251, a CB1-receptor antagonist, and ACEA, a CB1-receptor agonist, on penicillin-induced epileptiform activity in rats. *Epilepsia* 50:1760–1767.

Ludányi A, Eross L, Czirják S, Vajda J, Halász P, Watanabe M, Palkovits M, Maglóczky Z, Freund TF, Katona I. (2008) Downregulation of the CB1 cannabinoid receptor and related molecular elements of the endocannabinoid system in epileptic human hippocampus. *J Neurosci* 28:2976–2990.

Maccarrone M, Battista N, Centonze D. (2007) The endocannabinoid pathway in Huntington's disease: a comparison with other neurodegenerative diseases. *Prog Neurobiol* 81:349–379.

Marsicano G, Lutz B. (1999) Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. *Eur J Neurosci* 11:4213–4225.

Marsicano G, Goodenough S, Monory K, Hermann H, Eder M, Cannich A, Azad SC, Cascio MG, Gutiérrez SO, van der Stelt M, López-Rodriguez ML, Casanova E, Schütz G, Zieglgänsberger W, Di Marzo V, Behl C, Lutz B. (2003) CB1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science* 302:84–88.

Mechoulam R, Lichtman AH. (2003) Neuroscience. Stout guards of the central nervous system. *Science* 3; 302:65–67.

Monory K, Massa F, Egertová M, Eder M, Blaudzun H, Westenbroek R, Kelsch W, Jacob W, Marsch R, Ekker M, Long J, Rubenstein JL, Goebbels S, Nave KA, During M, Klugmann M, Wölfel B, Dodt HU, Zieglgänsberger W, Wotjak CT, Mackie K, Elphick MR, Marsicano G, Lutz B. (2006) The endocannabinoid system controls key epileptogenic circuits in the hippocampus. *Neuron* 51:455–466.

Mortati K, Dworetzky B, Devinsky O. (2007) Marijuana: an effective antiepileptic treatment in partial epilepsy? A case report and review of the literature *Rev Neurol Dis* 4:103–106.

Panikashvili D, Simeonidou C, Ben-Shabat S, Hanus L, Breuer A, Mechoulam R, Shohami E. (2001) An endogenous cannabinoid (2-AG) is neuroprotective after brain injury. *Nature* 4; 413:527–531.

Purpura DP, Penry JK, Tower DB, Woodbury DM, Walter RD. (1972) *Experimental models of epilepsy*. Raven Press, New York.

van der Stelt M, Veldhuis WB, van Haaften GW, Fezza F, Bisogno T, Bar PR, Veldink GA, Vliegenthart JF, Di Marzo V, Nicolay K. (2001a) Exogenous anandamide protects rat brain against acute neuronal injury *in vivo*. *J Neurosci* 15:21.

van der Stelt M, Veldhuis WB, Bär PR, Veldink GA, Vliegenthart JF, Nicolay K. (2001b) Neuroprotection by Delta9-tetrahydrocannabinol, the main active compound in marijuana, against ouabain-induced *in vivo* excitotoxicity. *J Neurosci* 21:6475–6479.

Wallace MJ, Wiley JL, Martin BR, DeLorenzo LJ. (2001) Assessment of the role of CB1 receptors in cannabinoid anticonvulsant effects. *Eur J Pharmacol* 428:51–57.

Wallace MJ, Martin BR, DeLorenzo RJ. (2002) Evidence for a physiological role of endocannabinoids in the modulation of seizure threshold and severity. *Eur J Pharmacol* 452:295–301.

Cannabis and Cannabinoid Research
Volume 1.1, 2016
DOI: 10.1089/can.2016.0009

Cannabis and Cannabinoid Research

Mary Ann Liebert, Inc. publishers

REVIEW Open Access

Clinical Endocannabinoid Deficiency Reconsidered: Current Research Supports the Theory in Migraine, Fibromyalgia, Irritable Bowel, and Other Treatment-Resistant Syndromes

Ethan B. Russo*

Abstract

Medicine continues to struggle in its approaches to numerous common subjective pain syndromes that lack objective signs and remain treatment resistant. Foremost among these are migraine, fibromyalgia, and irritable bowel syndrome, disorders that may overlap in their affected populations and whose sufferers have all endured the stigma of a psychosomatic label, as well as the failure of endless pharmacotherapeutic interventions with substandard benefit. The commonality in symptomatology in these conditions displaying hyperalgesia and central sensitization with possible common underlying pathophysiology suggests that a clinical endocannabinoid deficiency might characterize their origin. Its base hypothesis is that all humans have an underlying endocannabinoid tone that is a reflection of levels of the endocannabinoids, anandamide (arachidonylethanolamide), and 2-arachidonoylglycerol, their production, metabolism, and the relative abundance and state of cannabinoid receptors. Its theory is that in certain conditions, whether congenital or acquired, endocannabinoid tone becomes deficient and productive of pathophysiological syndromes. When first proposed in 2001 and subsequently, this theory was based on genetic overlap and comorbidity, patterns of symptomatology that could be mediated by the endocannabinoid system (ECS), and the fact that exogenous cannabinoid treatment frequently provided symptomatic benefit. However, objective proof and formal clinical trial data were lacking. Currently, however, statistically significant differences in cerebrospinal fluid anandamide levels have been documented in migraineurs, and advanced imaging studies have demonstrated ECS hypofunction in post-traumatic stress disorder. Additional studies have provided a firmer foundation for the theory, while clinical data have also produced evidence for decreased pain, improved sleep, and other benefits to cannabinoid treatment and adjunctive lifestyle approaches affecting the ECS.

Key words: anandamide; anorexia nervosa; cannabidiol; cannabinoids; depression; endocannabinoids; fibromyalgia; Huntington disease; irritable bowel syndrome; migraine; motion sickness; multiple sclerosis; Parkinson disease; post-traumatic stress disorder; prebiotics; THC

Introduction: Background History and Theory of Clinical Endocannabinoid Deficiency

The theory of clinical endocannabinoid deficiency (CED) was presented in 2001 in two publications,[1,2] but more thoroughly explored in 2004[3] in an article that has subsequently been cited frequently in the literature.[4] The theory of CED was based on the concept that many brain disorders are associated with neurotransmitter deficiencies, affecting acetylcholine in Alzheimer's disease, dopamine in parkinsonian syndromes, serotonin and norepinephrine in depression, and that a comparable deficiency in endocannabinoid

PHYTECS, Vashon Island, Washington.

*Address correspondence to: Ethan B. Russo, MD, PHYTECS, 1875 Century Park East, Suite 2250, Los Angeles, CA 90067, E-mail: ethanrusso@comcast.net

levels might be manifest similarly in certain disorders that display predictable clinical features as sequelae of this deficiency.

All humans possess an underlying endocannabinoid tone that reflects of levels of anandamide (AEA) and 2-arachidonoylglycerol (2-AG), the centrally acting endocannabinoids, their synthesis, catabolism, and the relative density of cannabinoid receptors in the brain. If endocannabinoid function were decreased, it follows that a lowered pain threshold would be operative, along with derangements of digestion, mood, and sleep among the almost universal physiological systems subserved by the endocannabinoid system (ECS).[5] The CED theory also posits that such deficiencies could arise due to genetic or congenital reasons or be acquired due to intercurrent injury or disease that consequently produces characteristic pathophysiological syndromes with particular symptomatology.

The greatest evidence for CED is present for migraine, fibromyalgia, and irritable bowel syndrome (IBS).[3] A strong case can be advanced for unifying pathophysiological trends in the three conditions:

- All manifest hyperalgesic states must be clinically diagnosed based on subjective criteria as all lack characteristic tissue pathology or easily accessible objective laboratory findings
- All are diagnoses of exclusion that often generate extensive negative diagnostic work-ups
- They display elevated incidence of anxiety and depression (in a chicken vs. egg dilemma) and have been labeled psychosomatic in origin or worse, wastebasket diagnoses, at one time or another by skeptical clinicians
- Comorbidity is quite clear in the three diagnoses. Primary headaches co-occurred in 97% of 201 fibromyalgia patients,[6] 35.6% of 101 chronic daily headache (transformed migraine) subjects also fit clinical criteria of fibromyalgia,[7] and 31.6% of IBS subjects were also diagnosable with fibromyalgia, while 32% of fibromyalgia patients also fit for IBS[8]
- While some patients suffer from only one of these syndromes, lifetime risk to develop another or all three is quite common (Fig. 1).

An extensive list of other disorders previously cited that may fall under the CED rubric included[3] neonatal failure to thrive,[9] cystic fibrosis,[10] causalgia,[11] brachial plexopathy,[12] phantom limb pain, infantile colic, glaucoma,[13] dysmenorrhea,[14] *hyperemesis gravidarum*,[15] unexplained fetal wastage (repetitive miscarriages),

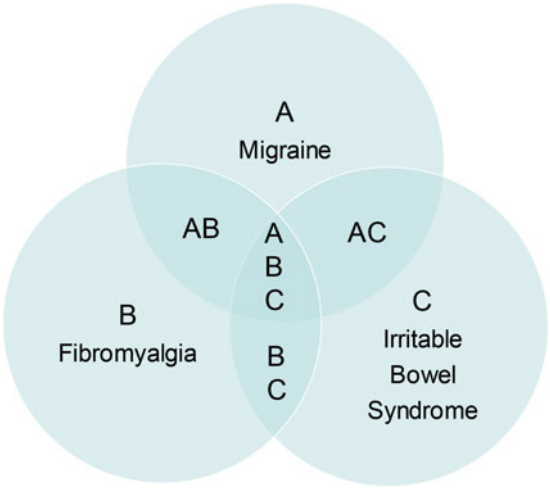


FIG. 1. Diagram depicting comorbidity of migraine, fibromyalgia, and irritable bowel syndrome.

post-traumatic stress disorder (PTSD),[16,17] bipolar disease,[18] and possibly many others. All display as yet unfathomed pathophysiological features and remain treatment resistant. Might their underlying nature have been missed? Recently, in a seminal article on the ECS and its optimization,[4] the authors added support for these and various other conditions.

Materials and Methods

Standard searches were undertaken of the PubMed/National Library of Medicine database for the listed keywords and references from pertinent literature for pertinence to clinical cannabinoid deficiency.

IBS, CED, and the Microbiome–Gut–Brain Axis

IBS, also known as spastic colon, is a functional disorder characterized by gastrointestinal (GI) pain, spasm, discomfort, and altered bowel movements, either predominantly diarrhea, predominantly constipation, or alternating between those states. Attacks are highly correlated with anxiety, but debate continues as to which incites the other. Individual episodes may be triggered by some specific foods or dietary indiscretions such as overeating on holidays. While frequently assessed as a life-long condition,[19] it is clear that significant gastrointestinal insults such as food poisoning or antibiotic administration may generate attacks that persist, often indefinitely. IBS is the most frequent diagnosis in gastroenterology practices in the United States,

with prevalence in the Western world of 10–15%.[19] As an idiopathic disorder, no physical signs are pathognomonic, and even diagnostic procedures such as laboratory tests, including those for gluten enteropathy, colonoscopy, or barium studies, most often fail to identify other causes,[3] but more formal Rome criteria have been established.[19] Those authors characterized the status of IBS as (p. 409) a disorder of unknown origin being treated by agents with an unknown mechanism of action. It has been posited that IBS represents a visceral hypersensitivity, with features of GI allodynia and hyperalgesia.[20] A seminal review of the ECS and its relationship with the GI tract appeared that year.[21] To summarize, GI propulsion, secretion, and inflammation in the gut are all modulated by the ECS, providing a rationale for cannabinoids as treatment candidates for IBS.[22] As examples, GI propulsion is under tonic control of the ECS,[21] and cannabis was one of the first effective clinical interventions in the 19th century for the intense secretory diarrhea associated with cholera,[23] a finding which was more recently validated with modern methodology.[24]

The use, by its sufferers, of cannabis-based agents to treat IBS has eventuated in large part due to the unfortunate fact that conventional treatment with anticholinergics, opioids, and antidepressants has been quite suboptimal, while three dedicated agents have been withdrawn from certain markets after prior regulatory approval. Two 5-HT_3 antagonists, alosetron and cilansetron, were associated with ischemic colitis, while tegaserod, a 5-HT_4 agonist, produced cardiovascular adverse events.

Additional support for the ECS as a key modulator of GI function was provided in an examination of circular muscle fibers from colonoscopic biopsies of surgical specimens from 31 normal patients.[25] AEA colocalized with cholinergic receptors in normal colon and inhibited the cholinergic contractile force of circular and longitudinal muscles through a non-CB_1 mechanism or possibly an alternative cannabinoid mechanism not mediated by CB_1 or CB_2. It was posited that inflammatory and disease states in the gut rendered the ECS more functionally important.

A 3.5-fold elevation in TRPV1-immunoreactive nerve fibers was observed in biopsies from IBS sufferers compared with controls ($p < 0.0001$).[26] The authors observed (p. 923) that the increased TRPV1 nerve fibers may contribute to visceral hypersensitivity and pain in IBS and provide a novel therapeutic target. Thus, a rationale exists for therapeutic interventions that would boost AEA levels or desensitize TRPV1, such as cannabidiol (CBD), to treat the condition.[27] Although fatty acid amide hydrolase (FAAH) inhibition of CBD has been questioned by some, its ability to raise serum AEA levels was clearly indicated when administered in high doses to schizophrenic patients.[28]

Genetic variation affecting endocannabinoid metabolism was observed in diarrhea-predominant IBS patients.[29] THC (dronabinol) treatment slowed colonic transit time in subjects harboring the *CNRI* rs806378 *CT/TT* genotype. Subsequently, a statistically significant association of this gene with colonic transit in IBS with diarrhea (IBS-D) was demonstrated ($p = 0.014$).[30] They observed (p. G559) that CB_1 receptor-related mechanisms modify colonic transit and sensation and may influence the development of symptoms in Caucasian patients with IBS, particularly IBS-D.

Unfortunately, while many patient surveys have touted benefits of cannabinoid treatment of IBS symptoms[31] and abundant anecdotal support is evident on the Internet, little actual clinical work has been accomplished. In a randomized controlled trial (RCT) of 52 normal patients taking single doses of 7.5 mg of THC versus placebo, the drug increased colonic compliance ($p = 0.045$) and inhibited postprandial colonic tone ($p = 0.048$) and fasting and postprandial phasic pressure ($p = 0.008$), with a trend toward relaxation of fasting colon tone ($p = 0.096$).[32] Another study focused on visceral sensitivity to rectal distention as measured by a barostat in normal ($N = 12$) versus IBS ($N = 10$) patients after administration of THC.[33] No significant differences were noted, but adverse events were reported in 100% of participants at the 10 mg dosage. A third small (23 IBS patients) trial of synthetic THC for a brief interval (2 days) showed no change in transit time.[29] More formal studies with whole cannabis extracts would be illuminating.

Additional interventions may be practical on the nutritional front utilizing new knowledge of the utility of probiotics and prebiotics. A direct effect of *Lactobacillus acidophilus* NCFM strain through oral administration to induce *CNR2* mRNA expression above that of resting human HT-29 epithelial cells ($p < 0.01$) was demonstrated along with an enhancement of morphine antinociceptive effect in rats ($p < 0.001$), which was inhibited by administration of the CB_2 antagonist, AM-630 ($p < 0.001$).[34] A review of human studies of probiotic supplements to treat IBS revealed that 34/42 trials demonstrated beneficial effects for one or more end-points or target symptoms (pain, discomfort, bloating, distention, laboratory parameters).[19] The interplay

of the microbiome–gut–brain axis in IBS is underscored by the recent finding that THC altered the microfloral balance in obese diet-induced obese mice, affecting the Firmicutes:Bacteroidetes ratio ($p = 0.021$) and preventing its increase or weight gain despite a high-fat diet.[35] Thus, optimal gut health without pain and with maintenance of appropriate body weight seems to require a complex interplay between diet, enteric flora, and endocannabinoid balance.

Experimental models have obvious limitations, and contrary findings are always possible. A recent study[36] demonstrated in a mouse model of accelerated GI transit that palmitoylethanolamide, an entourage endocannabinoid, indirectly activated CB_1 receptors only under conditions in which AEA or the receptors were upregulated, not deficient. Furthermore, it is unfortunate that laboratory measures of serum or tissue endocannabinoid levels have not been systematically examined in IBS.

Migraine and CED

Migraine is an extremely prevalent headache syndrome affecting 14% of Americans, with a 3:1 female:male ratio and $20 billion annual cost in that country.[37] This author has previously reported on migraine's treatment by cannabis,[1,3,38] and two major reviews have recently appeared.[39,40] Migraine is far more complex than merely cranial pain. It has a genetic predilection and female predominance and presents as a predominantly hemicranial beating headache associated with unusual associated manifestations: nausea, photophobia, and phonophobia, with hormonal and environmental triggers.

The possible relationship of migraine with the ECS is highlighted by numerous findings. Anandamide produced serotonin receptor responses consisting of 89% potentiation of 5-HT_{1A} and 36% inhibition of 5-HT_{2A},[41] findings that have been associated with profiles of effective pharmacological migraine interventions that would seem to support respective activity in acute and chronic migraine (CM), respectively. The migraine epiphenomena of photophobia and phonophobia suggest an overactive sensory hyperalgesia, just the kind of homeostatic imbalance that the ECS tends to correct in central nervous system (CNS) function.[5] The periaqueductal gray matter is a putative migraine generator in which AEA is tonically active, producing analgesia when administered or hyperalgesia when CB_1 is pharmacologically blocked.[42]

A great deal of additional support for the integral role of the ECS in migraine pathophysiology has been provided by a series of investigations linking endocannabinoids to the trigeminovascular system, which many consider to lie at the root of its pathophysiology. The first experiment[43] resulted in several pertinent findings: AEA diminished blood vessel dilation in the dura mater induced by calcitonin gene-related peptide (CGRP) 30%, capsaicin 45%, and nitric oxide (NO) 40%. Additionally, AEA acted presynaptically to prevent release of NO by CGRP in dural artery smooth muscle. AEA also was released in tonic manner and displayed modulatory activity in the trigeminovascular system.

A subsequent article focused on vascular phenomena associated with migraine.[44] AEA caused dose-dependent dural vessel dilation that was diminished by capsazepine, a TRPV1 antagonist, and by $CGRP_{8-37}$, a CGRP antagonist. (While the vascular effects of this and the prior study may appear contradictory, it should be noted that migraine produces vasoconstriction or vasodilation in different phases and that these are epiphenomena of the disorder, rather than its etiology.) The concentration of AEA that produced these findings was far higher than that required to activate CB_1. This suggests the possibility that repetitive administration with a TRPV1 agonist such as CBD[27] could conceivably desensitize the receptor and thus alleviate these pathophysiological mechanisms, much as capsaicin has successfully reduced peripheral neuropathic pain with regular cutaneous administration. Capsaicin has even been utilized intranasally as an acute migraine treatment,[45] and it is thus reasonable to consider CBD as a less noxious alternative desensitizing intervention.

A third publication examined trigeminovascular neuronal responses[46] with findings that WIN 55,212-2, a potent CB_1 agonist, inhibited trigeminocervical complex A and C-fiber afferent activity, which was abrogated by SR141716A, a CB_1 inverse agonist. However, this finding was only obtained with AEA after prior TRPV1 blockade by capsazepine. These findings support possible clinical application of CB_1-agonists in migraine and cluster headache, although the authors warned of psychoactive sequelae of agents such as THC.

In an animal model of migraine,[47] AEA reduced nitroglycerin-induced neuronal activation in the nucleus trigeminalis caudalis and area postrema, the latter being an emetic chemoreceptor. There was likewise an induction of expression of the immediate early gene transcription factor Fos in the hypothalamic paraventricular and supraoptic nuclei, in the parabrachial nucleus, and in the brainstem periaqueductal gray matter of the brainstem. These findings reinforce an important role of the ECS in generation of migraine episodes.

Various studies in Italy have focused on the etiological relationship of platelets with migraine in affected patients. In one[48] of the studies, increased function in AEA membrane transporter and AEA hydrolase (now known as fatty acid amidohydrolase [FAAH], the enzyme that catabolizes AEA) in platelets of women with migraine without aura was observed in comparison with patients with episodic tension headache or controls with no headaches. Interestingly, there were no differences in CB_1 receptor density in the groups, but AEA hydrolysis was elevated in platelets of migraine sufferers. Consequent decreased serum AEA levels could theoretically lower the pain threshold in such patients.

In another study,[49] female and male migraineurs both displayed lower FAAH and AEA membrane transporter platelet activity, hypothesized as a possible adaptive response to CM or a reaction to overuse of pain killers known as analgesic rebound. An additional study[50] showed that 2-AG and AEA levels were both profoundly reduced in the platelets of patients with episodic migraine without aura ($N=20$) and CM ($N=20$) versus controls ($N=20$) ($p<0.0001$).

Perhaps the strongest evidence of the existence of CED in migraine or any disorder comes from a study[51] that assayed cerebrospinal fluid (CSF) AEA levels in 15 chronic migraineurs versus 20 controls with a phenomenal statistically significant difference ($p<0.0001$) (Fig. 2). The authors opined concerning what they termed a system failure in migraine (p. 1387):

> Reduced AEA levels in the CSF of CM [chronic migraine] patients support the hypothesis of the failure of this endogenous CB [cannabinoid] system in CM, which seems to be related to increased CGRP and NO production in this pathological condition. This finding might be due to a failure of the inhibitory role of the endocannabinoid AEA on the trigeminovascular system activation—.

THC (1–20 μM) and other CB_1 agonists dose-dependently diminished cortical spreading depression amplitude, duration, and propagation velocity ($p<0.001$) in a rat brain model, supporting its ability to inhibit the trigeminovascular in migraine with aura.[52]

A clinical study examined 27 medication-overuse headache patients, a common precipitant of migraine exacerbation.[53] Before treatment, patients displayed decreased temporal summation thresholds, increased pain sensation, and reduced platelet FAAH (the enzyme that breaks down AEA) expression versus controls. After medication withdrawal treatment and elimination of analgesic rebound effects, FAAH activity, and temporal summation thresholds significantly normalized (both $p=0.001$), supporting an etiological ECS dysfunction in these patients.

In subsequent experiments in mice,[54] intraperitoneal injection of nitroglycerine induced mechanical hyperalgesia that was almost totally eliminated by FAAH deletion or administration of FAAH inhibitors ($p<0.0001$).

Additional supportive data on the migraine-ECS relationship are derived from genetic investigation. The CB_1 gene, *CNR1* mapped to chromosome 6q14-15, was linked to migraine through haplotypic tagging with

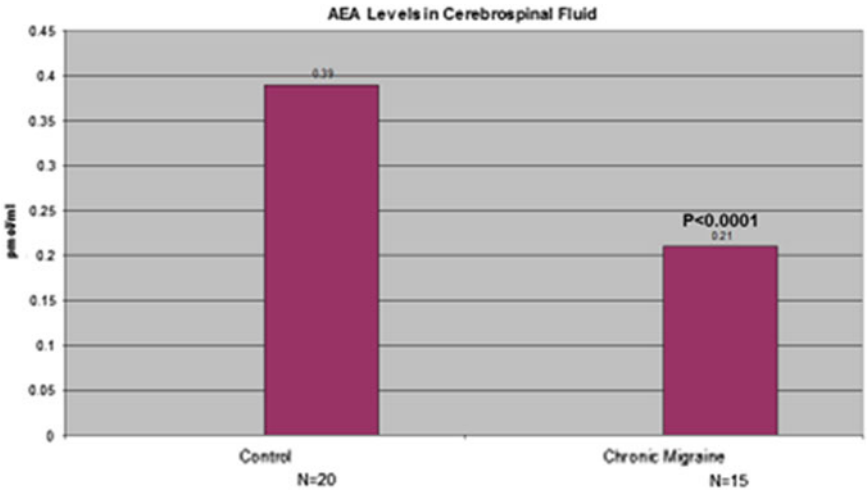


FIG. 2. Anandamide levels in cerebrospinal fluid of chronic migraine patients versus controls, adapted from data obtained from Sarchielli et al.[51]

high significance ($p=0.008$) and indicative of a genetic effect altering trigeminovascular activation.[55] The strongest linkage was to HT6 haplotype ($p=0.002$), which correlated highly with migraine symptoms of photophobia > nausea > disability. Migraineurs also showed greater degrees of neuroticism ($p<0.001$), depression ($p<0.001$), and reported drug/alcohol abuse ($p<0.005$). Of late, many pharmaceutical companies have pursued development of antibodies aimed at CGRP as a therapeutic target in migraine prophylaxis,[37] but it remains to be seen whether this represents a more fundamental target than strategies focusing on the ECS.

Until recently, only case reports and surveys of use of THC and cannabis and its effects on migraine have been published,[31,56] but a more formal observational trial has been reported[57] from a cannabis-oriented clinic in the state of Colorado. Among 120 adults with migraine for whom cannabis prophylaxis was recommended, and of which 67.8% had previously used cannabis, the frequency of headache diminished from 10.4 to 4.6 attacks per month ($p<0.0001$) (Fig. 3). Overall, 85.1% had decreased migraine frequency, with 39.7% reporting positive effects: prevention of or reduced headache frequency (19.8%) or aborted headache (11.6%) in this selected and uncontrolled population employing a mixture of administration techniques with unanalyzed but presumably high-THC cannabis.

It is worth remembering that cannabis was a mainstay of treatment of migraine in Europe and North America for a century between 1843 and 1943,[1] similarly supporting claims of a high degree of efficacy of cannabis treatment in both acute and prophylactic treatments of migraine. Further study utilizing modern techniques and standardized preparations with low THC and higher titers of CBD in proper RCTs is long overdue.

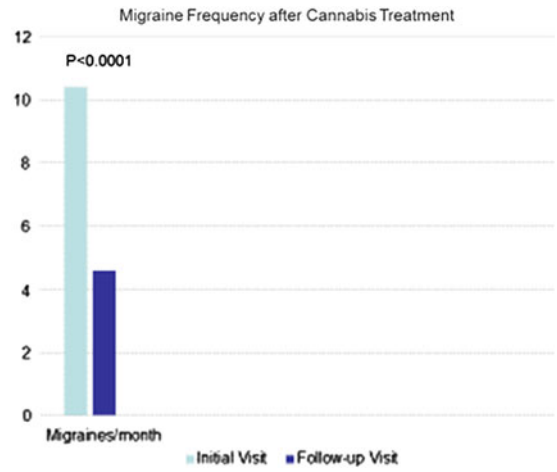


FIG. 3. Bar graph of change in migraine frequency after cannabis treatment, adapted from data from Rhyne et al.[57]

Focus on Fibromyalgia

Fibromyalgia was probably first described by Sir William Gowers[58] as fibrositis, a condition characterized as soft tissue pain that could wander in the body, and which was aggravated by overuse. In the 1980s, fibromyalgia became the preferred term due to a failure to identify inflammation or other objective changes in tissue biopsies from affected patients. Formal diagnostic parameters (Rome Criteria) were established thereafter. While a recent report indicated the presence of small fiber neuropathy in a subset of patients with fibromyalgia symptoms[59] creating possible diagnostic confusion, this finding by no means explains all such cases. Fibromyalgia is noteworthy for its characteristic painful nodules dubbed as trigger points that are particularly prevalent in the shoulder and neck that are frequently of sufficient severity to limit physical activity. The disorder has a clear association with depression and anxiety, but debate surrounds the timing and relationship of these comorbidities. Like migraine, it is more prevalent in women and invariably disrupts sleep. The disorder remains controversial in some quarters, but it is nonetheless the most common diagnosis in American rheumatology practices.[60] Many authorities now posit a central sensitization consistent with neuropathic pain at the root of the syndrome.[61] In Italy, it was noted that fibromyalgia, like migraine, was associated with secondary hyperalgesia, that is, a lowered threshold to pain in areas adjacent to the primarily affected parts,[62] for which the authors suggested pharmacological NMDA blockade for what they interpreted as a deficit in serotonergic analgesia. That same year, hyperalgesia was observed in association with central endocannabinoid hypofunction in the spinal cord and that endocannabinoids reduced associated hyperalgesia,[63] making the ECS a prime target and CED a rational explanation. The authors proposed that cannabinoid treatments would be indicated for various maladies driven by a primary afferent barrage, which would include visceral hyperalgesia (as hypothesized in IBS), allodynia associated with neuropathic pain states, and reflex sympathetic dystrophy or complex regional pain syndrome.

Cannabis or cannabinoids have been frequently utilized by fibromyalgia patients to treat its myriad

symptoms. In an uncontrolled trial in nine patients, THC was administered in doses of 2.5–15 mg a day for 3 months.[64] Surprisingly, the ethics committee would not permit placebo use in the study. Unfortunately, all but four patients left the study early secondary to THC side effects, but those completing had marked reductions in subjective pain visual analog scales (VAS) ($p < 0.01$) (Fig. 4). No benefits on touch-evoked allodynia, nor pinprick hyperalgesia, were documented.

Another group examined nabilone, a semisynthetic THC analog and CB_1 agonist of 10-fold higher potency.[65] Forty fibromyalgia patients received nabilone 1 mg BID for 4 weeks. Visual analog scales of pain, a Fibromyalgia Impact Questionnaire, and anxiety scores were all statistically significantly benefited compared with placebo ($p < 0.02$). The effects on sleep were also assessed with nabilone[66] in 31 patients with doses of 0.5–1 mg at bedtime compared with patients taking amitriptyline 10–20 mg. Nabilone was superior on an Insomnia Severity Index, but no benefits on pain, measure of mood, or quality of life were observed.

Herbal cannabis was utilized in an open-label manner in 28 fibromyalgia patients in comparison with an equal number of matched control patients[67] in another report. Two hours after cannabis use, VAS scores showed a statistically significant ($p < 0.001$) reduction of pain and stiffness, enhancement of relaxation, and an increase in somnolence and feeling of well-being. The mental health component summary score of the Short Form (36) Health Survey (SF-36) was significantly higher ($p < 0.05$) in cannabis users than in nonusers.

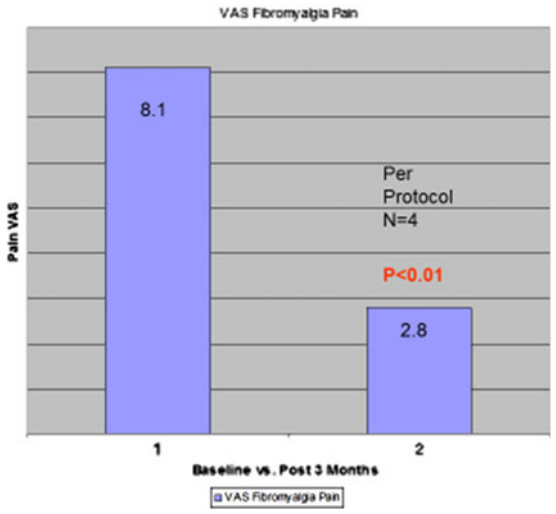


FIG. 4. Bar graph depicting decreases in pain in the per-protocol subset of fibromyalgia patients taking THC, adapted from data from Schley et al.[64] THC, tetrahydrocannabinol (dronabinol).

Other cannabis-based medicine clinical trials have been noteworthy in their benefits on symptomatic reduction allowing sleep (reviewed in Refs.[68,69]), and the same would likely be obtained in fibromyalgia, which displays many features in common with other causes of peripheral neuropathic pain. A notable example would be adjunctive use of Sativex (USAN: nabiximols) in a 5-week RCT in 125 patients with intractable peripheral neuropathic pain with allodynia in which it proved superior to placebo ($p = 0.00$) in Box Scale-11 (BS-11) score change and reduced dynamic allodynia test scores versus placebo ($p = 0.0420$).[70]

While this degree of benefit is yet to be shown in formal RCTs in fibromyalgia, the court of public opinion supports its utility. A recent survey on efficacy of three regulatory body-approved pharmaceutical fibromyalgia treatments versus cannabis recently garnered in excess of 1300 respondents and is available online from the National Pain Report.[71]

Of the approved drugs for fibromyalgia, duloxetine and milnacipran are mixed serotonin and adrenergic uptake inhibitors, while pregabalin is an anticonvulsant drug repurposed to treat neuropathic pain. Results of the survey (Fig. 5) strongly favor cannabis over the poorly effective prescription medicines. These results certainly support an urgent need for more definitive RCTs of a well-formulated and standardized cannabis-based medicine in fibromyalgia inasmuch as existing current medicines with regulatory approval seem to fall quite short of the mark.

Additional Conditions Suggesting CED

The ECS has been demonstrated to play a key role in the pathophysiology of motion sickness[72] assessed by subjecting volunteers to parabolic flight maneuvers producing microgravity. Seven of 21 adults so tested developed acute motion sickness with significant reductions in AEA ($p = 0.04$) and 2-AG ($p = 0.01$) in blood. Nausea scores correlated negatively with AEA ($p = 0.02$), and even CB_1 receptor mRNA gene expression in leukocytes diminished significantly ($p = 0.03$) 4 h after exposure in the most adversely affected.

Animal models have clearly established the role of the ECS in multiple sclerosis (MS).[73] Direct assays of AEA

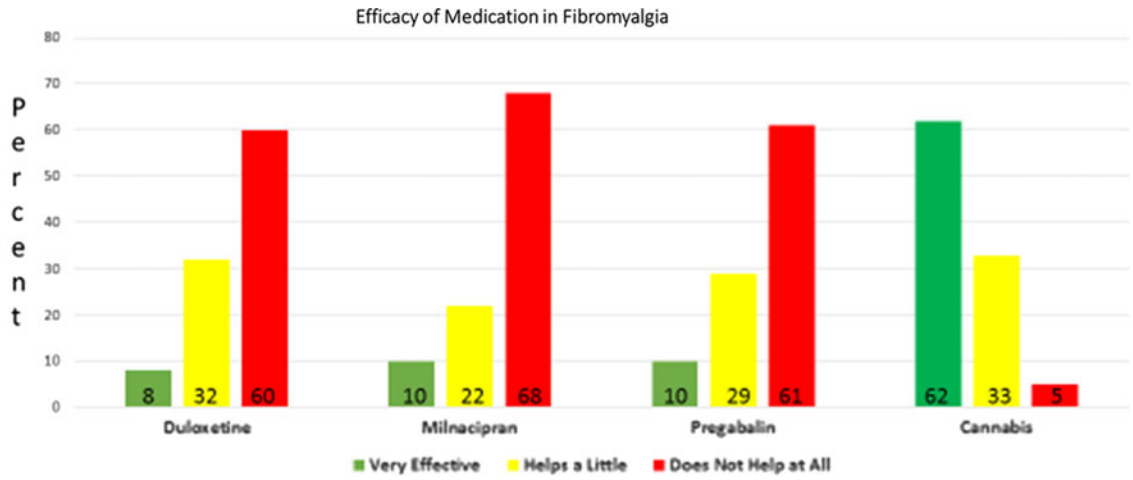


FIG. 5. Efficacy of approved pharmaceuticals compared with cannabis in fibromyalgia according to patient survey results, adapted from data from National Pain Report.[71]

and 2-AG in the CSF of MS patients versus controls confirm significant deficits in affected patients, particularly in secondary progressive cases, confirming an impaired endocannabinoid system,[74] and affirming the value of such measurements as a functional disease marker.

In a recent interesting finding assessing central pain mechanisms in neuropathy due to diabetes, streptozotocin was administered in a rat model that demonstrated reduced rostroventromedial medullary AEA levels, and in which the TRPV1 desensitizer, capsaicin, decreased nociceptive behavioral signs,[75] as well as demonstrating the central effects of a supposedly peripheral disorder. If corroborated in human studies, this finding might add diabetic neuropathy to the growing list of putative endocannabinoid deficiency disorders.

In 2008, a mouse model of Huntington's disease (HD) demonstrated a widespread impairment of endocannabinoid function.[76] Subsequently, in the postmortem brains of human patients with HD,[77] a striking loss of immunoreactivity of CB_1 in putamen and globus pallidus was demonstrated throughout the time course of the disorder. The degree of change was much higher than that for enkephalin or substance P, making CB_1 a superior marker, even at earlier clinical stages. This loss of CB_1 was felt to be a potential compensatory response as it could reduce GABA release in the striatum. A subsequent positron emission tomography (PET) study in living HD sufferers,[78] employing 18F-MK9470, a CB_1 ligand, demonstrated significant decreases in receptor availability versus controls ($p < 0.0001$). These reductions ranged from 15% in cerebellum up to 25% in frontal cortex, confirming underactivity of the ECS in HD that would disrupt neurotransmission and correlated inversely with disease severity.

Direct laboratory measurements were also performed in untreated Parkinson's disease (PD) patients, examining CSF,[79] and demonstrated a doubling of AEA levels over age-matched controls ($p < 0.001$), irrespective of disease stage. The authors posited this as a compensatory mechanism in the striatum of PD patients in an effort to alleviate dopamine depletion. Subsequently, another study[80] was the first to demonstrate the role of the ECS in synaptic long-term depression in motor circuits in PD. The motor deficits present in rodents with dopamine lesions were reversed by combining a D2 agonist with an endocannabinoid reuptake inhibitor. This finding suggests that progressive dopamine loss in PD in striatal circuits may decrease endocannabinoid tone and that the elevations in anandamide in PD patients may be an attempt to compensate for this loss.

Prior animal research has elucidated the relationship between the ECS, extinction of aversive memories,[16] and stress-induced analgesia.[17] This has been supplemented by additional evidence that stress-induced anxiety is directly related to central anandamide deficiency in mice.[81]

One genetic study in humans has linked genetic variants of CNR1, the CB_1 receptor gene, to fear extinction mechanisms.[82] Homozygote and heterozygote G-allele carriers of the gene rs2180619 showed prominent extinction of fear in a virtual reality experiment,

while A/A homozygotes displayed an absence of fear-potentiated startle reactions, confirming the role of the ECS in human fear extinction.

Recent research in humans has clarified the role of the ECS in post-traumatic stress. Forty-six survivors of the World Trade Center attacks were studied.[83] Serum 2-AG was significantly reduced in PTSD victims versus those without PTSD symptoms, especially those with direct exposure, suggesting a promotion of retention of aversive memories. A negative relationship was also noted between AEA levels and intrusive symptoms. The authors indicated that research to date suggests a good correlation of lower serum AEA levels to increased CB_1 receptor binding sites in CNS, as was demonstrated in a PET study of untreated PTSD patients.[84] The CB_1-selective radioligand [^{11}C]OMAR on PET revealed higher volume of distribution (V_T) with lower AEA tone in PTSD ($p=0.001$) by 19.5% over healthy controls and 14.5% over traumatized patients without PTSD. Cortisol levels were lower in PTSD and trauma patients versus controls and OMAR V_T, AEA, and cortisol together correctly identified 85% of PTSD cases. Women had greater CB_1 receptor availability under basal conditions, suggesting greater susceptibility to development of PTSD, in accord with epidemiological observations. Agents increasing AEA availability were suggested as possible therapy and such availability might reflect compensatory upregulation as a reaction to reduced endocannabinoid levels. Three excellent recent reviews reinforce these findings.[85–87]

The criticality of ECS function in other psychiatric syndromes has been evidenced in studies of major depression, which is now thought of less as a failure of monoamine neurotransmission and more as a disorder of CNS plasticity with an inflammatory component, or even as a degenerative disease[88] directly linked to endocannabinoid deficiency. Additionally, AEA levels were eightfold higher in CSF of untreated acute schizophrenics than in controls ($p=0.000$), and AEA was negatively correlated with psychotic symptoms ($p=0.001$), representing a compensatory mechanism to the disorder.[89] Recent clinical trial work supports the utility of cannabidiol in its treatment.[28]

PET was also employed in a study of adult female anorexia nervosa and bulimia patients,[90] demonstrating that global CB_1 receptor availability was increased in anorexia over controls in cortical and subcortical areas ($p=0.0003$), in the insula in both anorexia and bulimia patients ($p=0.01$ and $p=0.004$, respectively), and in the inferior frontal and temporal areas in anorexia ($p=0.02$). The authors related these chronic upregulations of CB_1 activity to presumed ECS hypoactivity (p. 780). Interestingly, peripheral serum AEA is elevated in anorexia. Long ago, a single RCT was undertaken in anorexia nervosa in 11 female patients comparing THC to diazepam in a double-blind crossover study.[91] No increased weight gain was noted in the THC group, but dosing was seemingly excessive (up to 30 mg daily), as evidenced by paranoid ideation and loss of control in three patients (27%). More recent experience would suggest that lower THC dosing with a cannabis-based preparation, as opposed to pure THC, might yield different results with prospects for not only fewer adverse events, but increased efficacy as well.[92–94] Certainly, additional trials are warranted in this common and difficult clinical context.

Given the current seemingly increased incidence and recognition of autistic spectrum disorders, it is useful to note their possible relationship with the ECS. Genes associated with these disorders also regulate ECS function: neuroligin-3 R451C-knockin and neuroligin-3 knockout mutations in mice impaired tonic endocannabinoid signaling,[95] with the authors suggesting therapeutic approaches in the human affliction to address this finding. Similarly, presynaptic β-neurexins controlled synaptic signals in excitatory synapses through regulation of postsynaptic 2-AG production[96] and were said to be essential for control of tonic endocannabinoid signaling.

Conclusions, Caveats, and Suggestions for Additional Research on and Treatment of CED

The current review has examined the concept of CED and presented more than a decade of supportive objective evidence. However, certain caveats are necessary. One is that contradictory findings are not only possible but also common. This is due, in part, to the often reciprocal relationships between the two major endocannabinoids, AEA and 2-AG, as expansively demonstrated in a current review[87]: Anandamide is most often the tonic signaling agent of the ECS and regulator of synaptic transmission, while 2-arachidonoylglycerol acts as a phasic signal activator in neuronal depolarization and mediator of synaptic plasticity. Thus, discordant levels of the two endocannabinoids may frequently be encountered. Additionally, while CED may be harmful, excesses clearly are, as well, with obvious examples of obesity, metabolic syndrome, and hepatic fibrosis.[97]

Aside from the evidence of depressed AEA levels in the CSF of migraine sufferers[51] and the other examples

presented here, there has been little direct objective evidence of the CED theory in patients until quite recently. Additional investigations in a similar vein to assess endocannabinoid levels in the serum or spinal fluid of migraine, IBS, and fibromyalgia versus controls would be illuminating. Anatomic and physiological scanning techniques (e.g., fMRI, PET) are not yet capable of producing real-time direct assessments of endocannabinoid levels in living patients, but hopefully research will soon allow this type of screening assessment in health and disease. Similarly, genomic testing has produced great strides in elucidating the mutations responsible for many congenital conditions, but has not yet fully plumbed the depths of regulation of gene function that may well underlie the putative CED conditions discussed herein.

RCTs of CED conditions are certainly well justified on the basis of current data and should replace the current largely uncontrolled black market experiments that desperate patients with these afflictions are contemporaneously forced to undertake in their quest for relief of their symptoms.

Various strategies to treat CED conditions are possible. A direct approach with CB_1 agonists must recognize the fact that the ECS operates as a homeostatic regulator that sometimes requires a gentle pharmacological nudge, rather than a forceful shove, by synthetic full agonists. Thus, small doses of a weak partial agonist (e.g., THC) should be considered, which would not induce tolerance and may jump-start the ECS. Even THC alone is poorly tolerated or appreciated by patients,[98] and standardized whole cannabis extracts that contain additional synergistic and buffering components, such as CBD and cannabis terpenoids, are certainly preferable.[93] Alternatively, FAAH inhibitors will also raise AEA levels, but only CBD among them has achieved current legal commercial market availability. Pharmaceutical approaches affecting endocannabinoid transport or its genetic regulation would also hold promise. Beyond drug interventions, a growing body of knowledge supports the realistic goal that lifestyle approaches should be integral to the treatment of CED; specifically, low-impact aerobic regimens have demonstrated beneficial effects on endocannabinoid function,[99] and as discussed above, dietary manipulations with probiotics and prebiotics may ameliorate not only IBS symptoms but also the entire spectrum of CED conditions. Ultimately, multimodality approaches are most likely to be fruitful in treatment of these common yet difficult clinical challenges.

Acknowledgment

The assistance of Interlibrary Loan of Mansfield Library, University of Montana, is gratefully acknowledged.

Author Disclosure Statement

No competing financial interests exist.

References

1. Russo EB. Hemp for headache: an in-depth historical and scientific review of cannabis in migraine treatment. J Cannabis Ther. 2001;1:21–92.
2. Russo EB. Handbook of psychotropic herbs: a scientific analysis of herbal remedies for psychiatric conditions. Haworth Press: Binghamton, NY, 2001.
3. Russo EB. Clinical endocannabinoid deficiency (CECD): can this concept explain therapeutic benefits of cannabis in migraine, fibromyalgia, irritable bowel syndrome and other treatment-resistant conditions? Neuroendocrinol Lett. 2004;25:31–39.
4. McPartland JM, Guy GW, Di Marzo V. Care and feeding of the endocannabinoid system: a systematic review of potential clinical interventions that upregulate the endocannabinoid system. PLoS One. 2014;9:e89566.
5. Pacher P, Kunos G. Modulating the endocannabinoid system in human health and disease—successes and failures. FEBS J. 2013;280:1918–1943.
6. Nicolodi M, Sicuteri F. Fibromyalgia and migraine, two faces of the same mechanism. In: Recent Advances in Tryptophan Research (Filippini GA, ed). Plenum Press: New York, 1996, pp. 373–379.
7. Peres MF, Young WB, Kaup AO, et al. Fibromyalgia is common in patients with transformed migraine. Neurology. 2001;57:1326–1328.
8. Sperber AD, Atzmon Y, Neumann L, et al. Fibromyalgia in the irritable bowel syndrome: studies of prevalence and clinical implications. Am J Gastroenterol. 1999;94:3541–3546.
9. Fride E, Bregman T, Kirkham TC. Endocannabinoids and food intake: newborn suckling and appetite regulation in adulthood. Exp Biol Med (Maywood). 2005;230:225–234.
10. Fride E. Cannabinoids and cystic fibrosis: a novel approach. J Cannabis Ther. 2002;2:59–71.
11. Notcutt W, Price M, Miller R, et al. Initial experiences with medicinal extracts of cannabis for chronic pain: results from 34 "N of 1" studies. Anaesthesia. 2004;59:440–452.
12. Berman JS, Symonds C, Birch R. Efficacy of two cannabis based medicinal extracts for relief of central neuropathic pain from brachial plexus avulsion: results of a randomised controlled trial. Pain. 2004;112:299–306.
13. Jarvinen T, Pate D, Laine K. Cannabinoids in the treatment of glaucoma. Pharmacol Ther. 2002;95:203–220.
14. Russo E. Cannabis treatments in obstetrics and gynecology: a historical review. J Cannabis Ther. 2002;2:5–35.
15. Westfall R, Janssen P, Lucas P, et al. Survey of medicinal cannabis use among childbearing women: patterns of its use in pregnancy and retroactive self-assessment of tis efficacy against 'morning sickness'. Complement Ther Clin Pract. 2006;12:27–33.
16. Marsicano G, Wotjak CT, Azad SC, et al. The endogenous cannabinoid system controls extinction of aversive memories. Nature. 2002;418:530–534.
17. Hohmann AG, Suplita RL, Bolton NM, et al. An endocannabinoid mechanism for stress-induced analgesia. Nature. 2005;435:1108–1112.
18. Ashton CH, Moore PB, Gallagher P, et al. Cannabinoids in bipolar affective disorder: a review and discussion of their therapeutic potential. J Psychopharmacol. 2005;19:293–300.
19. Clarke G, Cryan JF, Dinan TG, et al. Review article: probiotics for the treatment of irritable bowel syndrome—focus on lactic acid bacteria. Aliment Pharmacol Ther. 2012;35:403–413.
20. Holzer P. Gastrointestinal afferents as targets of novel drugs for the treatment of functional bowel disorders and visceral pain. Eur J Pharmacol. 2001;429:177–193.
21. Pertwee RG. Cannabinoids and the gastrointestinal tract. Gut. 2001;48:859–867.
22. Di Carlo G, Izzo AA. Cannabinoids for gastrointestinal diseases: potential therapeutic applications. Expert Opin Investig Drugs. 2003;12:39–49.
23. O'Shaughnessy WB. On the preparations of the Indian hemp, or gunjah (*Cannabis indica*); their effects on the animal system in health, and their

utility in the treatment of tetanus and other convulsive diseases. Trans Med Phys Soc Bengal. 1838–1840;71–102:421–461.
24. Izzo AA, Capasso F, Costagliola A, et al. An endogenous cannabinoid tone attenuates cholera toxin-induced fluid accumulation in mice. Gastroenterology. 2003;125:765–774.
25. Smid SD, Bjorklund CK, Svensson KM, et al. The endocannabinoids anandamide and 2-arachidonoylglycerol inhibit cholinergic contractility in the human colon. Eur J Pharmacol. 2007;575:168–176.
26. Akbar A, Yiangou Y, Facer P, et al. Increased capsaicin receptor TRPV1-expressing sensory fibres in irritable bowel syndrome and their correlation with abdominal pain. Gut. 2008;57:923–929.
27. Bisogno T, Hanus L, De Petrocellis L, et al. Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. Br J Pharmacol. 2001;134:845–852.
28. Leweke FM, Piomelli D, Pahlisch F, et al. Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. Transl Psychiatry. 2012;2:e94.
29. Wong BS, Camilleri M, Eckert D, et al. Randomized pharmacodynamic and pharmacogenetic trial of dronabinol effects on colon transit in irritable bowel syndrome-diarrhea. Neurogastroenterol Motil. 2012;24:358-e169.
30. Camilleri M, Kolar GJ, Vazquez-Roque MI, et al. Cannabinoid receptor 1 gene and irritable bowel syndrome: phenotype and quantitative traits. Am J Physiol Gastrointest Liver Physiol. 2013;304:G553–G560.
31. Ware MA, Adams H, Guy GW. The medicinal use of cannabis in the UK: results of a nationwide survey. Int J Clin Pract. 2005;59:291–295.
32. Esfandyari T, Camilleri M, Busciglio I, et al. Effects of a cannabinoid receptor agonist on colonic motor and sensory functions in humans: a randomized, placebo-controlled study. Am J Physiol Gastrointest Liver Physiol. 2007;293:G137–G145.
33. Klooker TK, Leliefeld KE, Van Den Wijngaard RM, et al. The cannabinoid receptor agonist delta-9-tetrahydrocannabinol does not affect visceral sensitivity to rectal distension in healthy volunteers and IBS patients. Neurogastroenterol Motil. 2011;23:30–35, e2.
34. Rousseaux C, Thuru X, Gelot A, et al. *Lactobacillus acidophilus* modulates intestinal pain and induces opioid and cannabinoid receptors. Nat Med. 2007;13:35–37.
35. Cluny NL, Keenan CM, Reimer RA, et al. Prevention of diet-induced obesity effects on body weight and gut microbiota in mice treated chronically with delta9-tetrahydrocannabinol. PLoS One. 2015;10:e0144270.
36. Capasso R, Orlando P, Pagano E, et al. Palmitoylethanolamide normalizes intestinal motility in a model of post-inflammatory accelerated transit: involvement of CB(1) receptors and TRPV1 channels. Br J Pharmacol. 2014;171:4026–4037.
37. Underwood E. A shot at migraine. Science. 2016;351:116–119.
38. Russo E. Cannabis for migraine treatment: the once and future prescription? An historical and scientific review. Pain. 1998;76:3–8.
39. Baron EP. Comprehensive review of medicinal marijuana, cannabinoids, and therapeutic implications in medicine and headache: what a long strange trip it's been. Headache. 2015;55:885–916.
40. Greco R, Gasperi V, Maccarrone M, et al. The endocannabinoid system and migraine. Exp Neurol. 2010;224:85–91.
41. Boger DL, Patterson JE, Jin Q. Structural requirements for 5-HT2A and 5-HT1A serotonin receptor potentiation by the biologically active lipid oleamide. Proc Natl Acad Sci U S A. 1998;95:4102–4107.
42. Walker JM, Hohmann AG, Martin WJ, et al. The neurobiology of cannabinoid analgesia. Life Sci. 1999;65:665–673.
43. Akerman S, Kaube H, Goadsby PJ. Anandamide is able to inhibit trigeminal neurons using an in vivo model of trigeminovascular-mediated nociception. J Pharmacol Exp Ther. 2003;309:56–63.
44. Akerman S, Kaube H, Goadsby PJ. Anandamide acts as a vasodilator of dural blood vessels in vivo by activating TRPV1 receptors. Br J Pharmacol. 2004;142:1354–1360.
45. Fusco BM, Barzoi G, Agro F. Repeated intranasal capsaicin applications to treat chronic migraine. Br J Anaesth. 2003;90:812.
46. Akerman S, Holland PR, Goadsby PJ. Cannabinoid (CB1) receptor activation inhibits trigeminovascular neurons. J Pharmacol Exp Ther. 2007;320:64–71.
47. Greco R, Mangione AS, Sandrini G, et al. Effects of anandamide in migraine: data from an animal model. J Headache Pain. 2011.
48. Cupini LM, Bari M, Battista N, et al. Abnormal degradation of endocannabinoids in migrainous women. Cephalalgia. 2003;23:684.
49. Cupini LM, Costa C, Sarchielli P, et al. Degradation of endocannabinoids in chronic migraine and medication overuse headache. Neurobiol Dis. 2008;30:186–189.
50. Rossi C, Pini LA, Cupini ML, et al. Endocannabinoids in platelets of chronic migraine patients and medication-overuse headache patients: relation with serotonin levels. Eur J Clin Pharmacol. 2008;64:1–8.
51. Sarchielli P, Pini LA, Coppola F, et al. Endocannabinoids in chronic migraine: CSF findings suggest a system failure. Neuropsychopharmacology. 2007;32:1384–1390.
52. Kazemi H, Rahgozar M, Speckmann EJ, et al. Effect of cannabinoid receptor activation on spreading depression. Iran J Basic Med Sci. 2012;15:926–936.
53. Perrotta A, Arce-Leal N, Tassorelli C, et al. Acute reduction of anandamide-hydrolase (FAAH) activity is coupled with a reduction of nociceptive pathways facilitation in medication-overuse headache subjects after withdrawal treatment. Headache. 2012;52:1350–1361.
54. Nozaki C, Markert A, Zimmer A. Inhibition of FAAH reduces nitroglycerin-induced migraine-like pain and trigeminal neuronal hyperactivity in mice. Eur Neuropsychopharmacol. 2015;25:1388–1396.
55. Juhasz G, Lazary J, Chase D, et al. Variations in the cannabinoid receptor 1 gene predispose to migraine. Neurosci Lett. 2009;461:116–120.
56. el-Mallakh RS. Marijuana and migraine. Headache. 1987;27:442–443.
57. Rhyne DN, Anderson SL, Gedde M, et al. Effects of medical marijuana on migraine headache frequency in an adult population. Pharmacotherapy. 2016;36:505–510.
58. Gowers WR. A lecture on lumbago: its lessons and analogues. Br Med J. 1904;1:117–121.
59. Caro XJ, Winter EF. The role and importance of small fiber neuropathy in fibromyalgia pain. Curr Pain Headache Rep. 2015;19:55.
60. Bennett RM. Rational management of fibromyalgia. Rheum Dis Clin North Am. 2002;28:xiii–xv.
61. Bennett RM. The rational management of fibromyalgia patients. Rheum Dis Clin North Am. 2002;28:181–199, v.
62. Nicolodi M, Volpe AR, Sicuteri F. Fibromyalgia and headache. Failure of serotonergic analgesia and N-methyl-D-aspartate-mediated neuronal plasticity: their common clues. Cephalalgia. 1998;18(Suppl 21): 41–44.
63. Richardson JD, Aanonsen L, Hargreaves KM. Hypoactivity of the spinal cannabinoid system results in NMDA-dependent hyperalgesia. J Neurosci. 1998;18:451–457.
64. Schley M, Legler A, Skopp G, et al. Delta-9-THC based monotherapy in fibromyalgia patients on experimentally induced pain, axon reflex flare, and pain relief. Curr Med Res Opin. 2006;22:1269–1276.
65. Skrabek RQ, Galimova L, Ethans K, et al. Nabilone for the treatment of pain in fibromyalgia. J Pain. 2008;9:164–173.
66. Ware MA, Fitzcharles MA, Joseph L, et al. The effects of nabilone on sleep in fibromyalgia: results of a randomized controlled trial. Anesth Analg. 2010;110:604–610.
67. Fiz J, Duran M, Capella D, et al. Cannabis use in patients with fibromyalgia: effect on symptoms relief and health-related quality of life. PLoS One. 2011;6:e18440.
68. Russo EB, Guy GW, Robson PJ. Cannabis, pain, and sleep: lessons from therapeutic clinical trials of Sativex, a cannabis-based medicine. Chem Biodivers. 2007;4:1729–1743.
69. Russo EB, Hohmann AG. Role of cannabinoids in pain management. In: Comprehensive Treatment of Chronic Pain by Medical, Interventional and Behavioral Approaches (Deer T, Gordin V, eds.). Springer: New York, 2013, pp. 181–197.
70. Nurmikko TJ, Serpell MG, Hoggart B, et al. Sativex successfully treats neuropathic pain characterised by allodynia: a randomised, double-blind, placebo-controlled clinical trial. Pain. 2007;133:210–220.
71. National Pain Report. Marijuana rated most effective for treating fibromyalgia. National Pain Report, 2014. Available at: http://nationalpainreport.com/marijuana-rated-most-effective-for-treating-fibromyalgia-8823638.html
72. Chouker A, Kaufmann I, Kreth S, et al. Motion sickness, stress and the endocannabinoid system. PLoS One. 2010;5:e10752.
73. Baker D, Pryce G, Croxford JL, et al. Endocannabinoids control spasticity in a multiple sclerosis model. FASEB J. 2001;15:300–302.
74. Di Filippo M, Pini LA, Pelliccioli GP, et al. Abnormalities in the cerebrospinal fluid levels of endocannabinoids in multiple sclerosis. J Neurol Neurosurg Psychiatry. 2008;79:1224–1229.

75. Silva M, Martins D, Charrua A, et al. Endovanilloid control of pain modulation by the rostroventromedial medulla in an animal model of diabetic neuropathy. Neuropharmacology. 2016;107:49–57.
76. Bisogno T, Martire A, Petrosino S, et al. Symptom-related changes of endocannabinoid and palmitoylethanolamide levels in brain areas of R6/2 mice, a transgenic model of Huntington's disease. Neurochem Int. 2008;52:307–313.
77. Allen KL, Waldvogel HJ, Glass M, et al. Cannabinoid (CB(1)), GABA(A) and GABA(B) receptor subunit changes in the globus pallidus in Huntington's disease. J Chem Neuroanat. 2009;37:266–281.
78. Van Laere K, Casteels C, Dhollander I, et al. Widespread decrease of type 1 cannabinoid receptor availability in Huntington disease in vivo. J Nucl Med. 2010;51:1413–1417.
79. Pisani A, Fezza F, Galati S, et al. High endogenous cannabinoid levels in the cerebrospinal fluid of untreated Parkinson's disease patients. Ann Neurol. 2005;57:777–779.
80. Kreitzer AC, Malenka RC. Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. Nature. 2007;445:643–647.
81. Bluett RJ, Gamble-George JC, Hermanson DJ, et al. Central anandamide deficiency predicts stress-induced anxiety: behavioral reversal through endocannabinoid augmentation. Transl Psychiatry. 2014;4:e408.
82. Heitland I, Klumpers F, Oosting RS, et al. Failure to extinguish fear and genetic variability in the human cannabinoid receptor 1. Transl Psychiatry. 2012;2:e162.
83. Hill MN, Bierer LM, Makotkine I, et al. Reductions in circulating endocannabinoid levels in individuals with post-traumatic stress disorder following exposure to the World Trade Center attacks. Psychoneuroendocrinology. 2013;38:2952–2961.
84. Neumeister A, Normandin MD, Pietrzak RH, et al. Elevated brain cannabinoid CB1 receptor availability in post-traumatic stress disorder: a positron emission tomography study. Mol Psychiatry. 2013;18: 1034–1040.
85. Neumeister A, Seidel J, Ragen BJ, et al. Translational evidence for a role of endocannabinoids in the etiology and treatment of posttraumatic stress disorder. Psychoneuroendocrinology. 2015;51:577–584.
86. Gabbay FE, Choi KH, Wynn GH, et al. The role of endoncannbinoid function in posttraumatic stress disorder: modulation the risk phenotype and rendering effects of trauma. In: Cannabinoids in Neurologic and Mental Disease (Fattore L, ed.). Academic Press: London, 2015, pp. 247–288.
87. Morena M, Patel S, Bains JS, et al. Neurobiological interactions between stress and the endocannabinoid system. Neuropsychopharmacology. 2016;41:80–102.
88. Hill MN, Gorzalka BB. Is there a role for the endocannabinoid system in the etiology and treatment of melancholic depression? Behav Pharmacol. 2005;16:333–352.
89. Giuffrida A, Leweke FM, Gerth CW, et al. Cerebrospinal anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. Neuropsychopharmacology. 2004;29:2108–2114.
90. Gerard N, Pieters G, Goffin K, et al. Brain type 1 cannabinoid receptor availability in patients with anorexia and bulimia nervosa. Biol Psychiatry. 2011;70:777–784.
91. Gross H, Ebert MH, Faden VB, et al. A double-blind trial of delta 9-tetrahydrocannabinol in primary anorexia nervosa. J Clin Psychopharmacol. 1983;3:165–171.
92. Portenoy RK, Ganae-Motan ED, Allende S, et al. Nabiximols for opioid-treated cancer patients with poorly-controlled chronic pain: a randomized, placebo-controlled, graded-dose trial. J Pain. 2012;13:438–449.
93. Russo EB. Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. Br J Pharmacol. 2011;163:1344–1364.
94. Russo EB, Mead AP, Sulak D. Current status and future of cannabis research. Clin Researcher. 2015:58–63.
95. Foldy C, Malenka RC, Sudhof TC. Autism-associated neuroligin-3 mutations commonly disrupt tonic endocannabinoid signaling. Neuron. 2013;78:498–509.
96. Anderson GR, Aoto J, Tabuchi K, et al. Beta-neurexins control neural circuits by regulating synaptic endocannabinoid signaling. Cell. 2015;162:593–606.
97. Pacher P, Kunos G. Cardiovascular, metabolic, liver kidney and inflammatory disorders. In: Handbook of Cannabis (Pertwee R, ed.). Oxford University Press: Oxford, United Kingdom, 2014, pp. 564–581.
98. Calhoun SR, Galloway GP, Smith DE. Abuse potential of dronabinol (Marinol). J Psychoactive Drugs. 1998;30:187–196.
99. Raichlen DA, Foster AD, Gerdeman GL, et al. Wired to run: exercise-induced endocannabinoid signaling in humans and cursorial mammals with implications for the 'runner's high'. J Exp Biol. 2012;215:1331–1336.

Cite this article as: Russo EB (2016) Clinical endocannabinoid deficiency reconsidered: current research supports the theory in migraine, fibromyalgia, irritable bowel and other treatment-resistant syndromes, *Cannabis and Cannabinoid Research* 1:1, 154–165, DOI: 10.1089/can.2016.0009.

Abbreviations Used

2-AG = 2-arachidonoylglycerol
5-HT = 5-hydroxytryptamine (serotonin)
AEA = arachidonylethanolamide (anandamide)
BS-11 = Box Scale-11
CB = cannabinoid
CB_1/CB_2 = cannabinoid receptor 1 or 2
CBD = cannabidiol
CED = clinical endocannabinoid deficiency
CGRP = calcitonin gene-related peptide
CM = chronic migraine
CNS = central nervous system
CSF = cerebrospinal fluid
ECS = endocannabinoid system
FAAH = fatty acid amide hydrolase
fMRI = functional magnetic resonance imaging
GABA = gamma-aminobutyric acid
GI = gastrointestinal
HD = Huntington's disease
IBS = irritable bowel syndrome
IBS-D = irritable bowel syndrome with diarrhea
MS = multiple sclerosis
NMDA = N-methyl-D-aspartate
NO = nitric oxide
PD = Parkinson's disease
PET = positron emission tomography
PTSD = post-traumatic stress disorder
RCT = randomized controlled trial
SF-36 = Short Form (36) Health Survey
THC = tetrahydrocannabinol (dronabinol)
TRPV = transient receptor potential vanilloid receptor
VAS = visual analog scale

J Autism Dev Disord (2015) 45:2582–2593
DOI 10.1007/s10803-015-2430-9

ORIGINAL PAPER

Coping and Well-Being in Parents of Children with Autism Spectrum Disorders (ASD)

Wei Wei Lai[1] · Tze Jui Goh[2] · Tian P. S. Oei[1] · Min Sung[2]

Published online: 24 March 2015

Abstract This study examined psychological well-being and coping in parents of children with ASD and parents of typically developing children. 73 parents of children with ASD and 63 parents of typically developing children completed a survey. Parents of children with ASD reported significantly more parenting stress symptoms (i.e., negative parental self-views, lower satisfaction with parent–child bond, and experiences of difficult child behaviors), more depression symptoms, and more frequent use of Active Avoidance coping, than parents of typically developing children. Parents of children with ASD did not differ significantly in psychological well-being and coping when compared as according to child's diagnosis. Study results reinforced the importance of addressing well-being and coping needs of parents of children with ASD.

Keywords Well-being · Depression · Anxiety · Coping · Parenting stress · Autism spectrum disorders · Asia

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder defined by deficits in communication and social interaction, and the engagement in restricted and repetitive patterns of behaviors (American Psychiatric Association 2013). Individuals with ASD face daily challenges in multiple domains of their lives, including poor adaptive functioning, anxiety, hyperactivity, and obsessive–compulsive behaviors (Bauman 2010; Huang et al. 2014; Peters-Scheffer et al. 2012; Simonoff et al. 2008; Wang et al. 2011). Recently, studies have cited a rising trend in ASD prevalence worldwide, with increasing incidence of children with ASD in both Caucasian and Asian populations (Centers for Diseases Control and Prevention 2012; Elsabbagh et al. 2012). Disease burden research in Singapore also cited ASD-related health problems to be most debilitating when compared to other child and adolescent physical and mental health disorders (Ministry of Health 2004). The chronic nature of ASD and associated behavioral and emotional challenges contribute to persistent caregiving and parenting stress among parents of children with ASD (Benson and Karlof 2009).

Previously, parents of children with ASD have been reported to have poorer psychological outcomes. They have been reported to experience higher parenting stress (e.g., Hayes and Watson 2012; Griffith et al. 2010; Wang et al. 2011), and more depression and anxiety symptoms (e.g., Baker et al. 2011; Benson and Karlof 2009; Estes et al. 2009; Gallagher et al. 2008), compared to parents of typically developing children or children with other developmental disabilities such as intellectual disability and Down Syndrome. Child characteristics such as the severity of condition (e.g., Abbeduto et al. 2004; Konstantareas and Papageorgiou 2006), adaptive functioning level of the child (e.g., Hall and Graff 2011), ASD-related behaviors (e.g., Huang et al. 2014), behavior problems (e.g., Gray 2006; Hall 2012; Lecavalier et al. 2006), child's age (e.g., Smith et al. 2008) and gender (e.g., Mandell and Salzer 2007), have been suggested to impact the psychological well-being and coping approaches of parents of children with ASD. However, in particular, ASD-related behaviors and

✉ Wei Wei Lai
weiwei.lai@my.jcu.edu.au

[1] Department of Psychology, James Cook University Singapore, 149 Sims Drive, Singapore 387380, Singapore

[2] Department of Child and Adolescent Psychiatry, Institute of Mental Health, Singapore, Singapore

behavioral functioning of the child were reported to impact parenting stress inconsistently (Huang et al. 2014; Mori et al. 2009; Pearson et al. 2006; Phetrasuwan and Miles 2009; Wang et al. 2011). For instance, some studies noted a positive relationship between parenting stress and severity level of child's ASD-related behaviors (e.g., Konstantareas and Papageorgiou 2006; Mori et al. 2009; Phetrasuwan and Miles 2009; Wang et al. 2011). However, other researchers such as Huang et al. (2014) and Pearson et al. (2006) reported that parents of children showing mild to moderate levels of ASD-related behaviors felt the most stress in parenting their child. Although child's behaviors, functioning level, age and gender were observed to affect parenting and caregiving stress, our understanding of the effects of these factors has not been conclusive. Further duplication and examination of current research is needed for a well-rounded understanding of parental and caregivers' well-being (Griffith et al. 2010; Abbeduto et al. 2004).

Parents of children with ASD use a range of coping strategies and resources when faced with parenting/caregiving stress (Hall and Graff 2011; Hastings et al. 2005; Lai and Oei 2014; Luong et al. 2009). In a review paper, Lai and Oei (2014) highlighted that parents of children with ASD used both adaptive (e.g., cognitive reframing; seeking social support) and maladaptive (e.g., avoidance and disengagement) coping strategies, with an inclination towards adaptive coping methods such as seeking social support and positive reinterpretation. Among parents of children with ASD, the use of adaptive coping strategies has also been linked to positive mental health outcomes (Benson 2010; Penley et al. 2002; Taylor and Stanton 2007). While it may then be expected that parents of children with ASD adapt well to parenting stress, past studies have also consistently reported elevated stress symptoms in these parents (Hayes and Watson 2012). It is therefore unclear if parents of children with ASD are coping with parenting/caregiving stress adequately and effectively (Hayes and Watson 2012). Moreover, some studies suggested more frequent use of maladaptive coping strategies among parents of children with ASD than parents of children with non-ASD developmental disabilities or those of typical development (e.g., Montes and Halterman 2007; Piazza et al. 2014; Sivberg 2002; Wang et al. 2011). In general, the nature of parenting stress and coping in parents of children with ASD, especially when compared to parents of children not diagnosed with this disorder, remains inconclusive (Lai and Oei 2014; Sivberg 2002; Wang et al. 2011).

Currently, there is a paucity of research in parental well-being and coping among Asian parents of children with ASD residing in Asian countries (Lai and Oei 2014; Moh and Magiati 2012; Yeo and Lu 2012). Previously, Asia-based studies have either (1) reported differences in parenting/caregiving experiences by comparing parents of children with ASD residing in different countries (e.g., Yeo and Lu 2012) or (2) examined parents' overall experience of the diagnostic process for their child (e.g., Moh and Magiati 2012). While having their merits, comparisons across different countries overlook the differences in parenting culture, environment and expectations that is inherent in the population, which can alter the impact of raising a child with ASD (Lai and Oei 2014).

Studies based on parents of different ethnic backgrounds have suggested that Caucasian parents of children with ASD engage in emotion-focused coping methods such as passive appraisal and avoidance more frequently than Asian parents (Lin et al. 2008; Luong et al. 2009; Oyserman et al. 2002), while Asian parents of children with ASD engage more frequently in problem-focused coping strategies than Caucasian parents (Luong et al. 2009; Twoy et al. 2007). In the general stress and coping literature, emotion-focused coping is suggested to be psychologically maladaptive and problem-focused coping is linked to adaptive psychological adjustment (Penley et al. 2002; Taylor and Stanton 2007). Implicatively, Asian parents of children with ASD may then cope better with stress than Caucasian parents (Benson 2010; Penley et al. 2002; Taylor and Stanton 2007). Despite this, it is also possible that Asian-related ideologies such as "saving face" can influence Asian parents to internalize any stressful feelings felt, and not reach out for support, to avoid the social stigma of having a child with a developmental disability (Kawachi and Berkman 2001; Mak and Ho 2007; Uchino 2006).

As discussed, inconsistent findings on stress and coping research in parents of children with ASD and existing cultural differences between Asian- and western-based studies render any direct application of western literature on Asian populations limited. Therefore, this paper aimed to examine the psychological well-being and coping strategies of parents of children with ASD in Singapore, an Asian but multi-ethnic population. It is hypothesized that (1) parents of children with ASD would report more parenting stress, depression and anxiety symptoms, than parents of typically developing children. Since ASD-related behaviors can impact parenting stress to varying degrees (ref. Huang et al. 2014; Wang et al. 2011), the current study also sought to understand parenting stress, and parent-reported depression and anxiety symptoms based on the diagnosis of the child (i.e., comparing between parents of children with Autism, Asperger's Syndrome, PDD-NOS and of typical development), as an elaboration of Hypothesis (1). Practically, this categorization of parental experiences could also be valuable in assisting clinicians to tune in quickly to the needs of specific parent populations based on child's reported diagnosis (Huang et al. 2014).

Finally, while previous research highlighted that parents of children with ASD coped well with stress (e.g., Lai and Oei 2014), they were also reported to experience higher parenting stress and to use more maladaptive than adaptive coping strategies when compared to parents of children not diagnosed with an ASD (Hayes and Watson 2012; Montes and Halterman 2007; Piazza et al. 2014; Wang et al. 2011). Therefore, this study sought to contribute to the limited literature on ASD-related parental stress and coping, by examining whether parents of children with ASD used more adaptive or maladaptive coping strategies than parents of typically developing children.

Method

Participants

One hundred and thirty-six parents participated in the study. 54 % of recruited parents reported having a child with ASD and 46 % of parents had a child who did not have a diagnosis of ASD or other chronic medical conditions. Parents reported a mean age of 43.68 years (SD = 6.36) and were (1) mostly mothers (80.9 %), (2) not working or retired (35.3 %), and (3) with graduate/postgraduate education (36.8 %). The sample consisted mainly of parent–child pairs who were Chinese (81.6 %). Most of the children being rated were boys (56.6 %). Table 1 summarizes additional information on sample demographics.

Measures

Demographics Screening Form

Participants completed a form on personal demographics (i.e., age, gender, ethnicity), socio-economic statuses (i.e., profession, education level), child's characteristics (i.e., age, gender, diagnosis), and family variables (i.e., additional child with ASD in the family, additional help engaged for caregiving) that may impact parent outcomes and coping.

Parenting Stress Index: Short Form (PSI/SF; Abidin 1992)

The PSI/SF is a 36-item self-report form measuring parenting-related stressful behaviors and feelings based on three subscales of parental distress (PD), parent–child dysfunctional interaction (P-CDI), and difficult child (DC). A PSI/SF Total score is computed from the three subscale scores of items rated on a five-point rating scale (i.e., from 1 = "Strongly Disagree" to 5 = "Strongly Agree"). The PSI/SF is a widely used tool in clinical research with adequate psychometric properties (Abidin 1992). All scales from the PSI/SF displayed strong internal consistencies, with Cronbach's alpha coefficients of .93 (PSI/SF Total), .84 (PD), .86 (P-CDI), and .82 (DC) for the present study (see diagonals in Table 2).

Depression Anxiety Stress Scales (DASS-21; Lovibond and Lovibond 1995)

The DASS-21 is a self-report screening tool, which measures frequency of behaviors or intensity of feelings based on three subscales of anxiety (DASS-A), depression (DASS-D) and stress (DASS-S). It is used to measure the status of psychological well-being of parents in the current study. A DASS total score is computed from the three subscale scores of items rated on a four-point scale (i.e., from 0 = "Did not apply to me" to 3 = "Applied to me very much or most of the time"). The DASS-21 demonstrated sound psychometric properties, is used widely in clinical and non-clinical samples, and has also been validated for use in Asia (Lovibond 2011; Oei et al. 2013). In this study, strong internal consistencies of the DASS Total Scale, DASS-D, DASS-A, and DASS-S subscales were achieved with Cronbach's coefficient alphas of .94 (DASS Total), .88 (DASS-D), .83 (DASS-A) and .92 (DASS-S; see diagonals in Table 2).

Brief COPE (Carver et al. 1989)

The Brief COPE is a self-reporting, 28-item version of the COPE instrument that measures the usage frequencies of broad-based maladaptive and adaptive coping strategies. Items are rated on a four-point rating scale (i.e., from 1 = "I haven't been doing this at all" to 4 = "I've been doing this a lot"; Carver 2007). The psychometric properties of the Brief COPE have been previously examined, and the instrument is used in many studies on stress and coping, with clinical or non-clinical samples (Carver 1997).

In this study, the Brief COPE was analyzed using four sub-domains (i.e., Active Avoidance coping, problem-focused coping, positive coping, and religious/denial coping) as derived in Hastings et al. (2005) based on a sample of parents of children diagnosed with ASD. This approach was adopted to maximize construct validity of the Brief COPE on the current study sample. Internal consistency evaluations of the Brief COPE total and subscale scores were adequate, with Cronbach's coefficient alphas of .92 (Brief COPE total), .76 (Active Avoidance coping), .89 (problem-focused coping), .83 (positive coping), and .69 (religious/denial coping; see diagonals in Table 2).

Table 1 Means, standard deviations, frequencies and ANOVA/Chi-square comparisons of participant characteristics by child's diagnosis

	AUT (N = 43)	AS (N = 15)	PDD-NOS (N = 15)	TD (N = 63)	Total (N = 136)	ANOVA (DSM-5)		ANOVA (DSM-IV)	
						F(1, 129)	ηp^2	F(3, 127)	ηp^2
Age, M (SD)									
Parent's age (years)	46.10 (5.50)	46.00 (4.36)	48.30 (5.73)	41.00 (6.23)	43.68 (6.36)	25.19	.17	10.02*	.19
Child's age (years)	14.10 (3.60)	12.90 (4.00)	13.25 (2.72)	10.80 (3.19)	12.35 (3.67)	25.97	.16	8.93*	.17
Chi-square goodness-of-fit						Chi-square statistic		Chi-square statistic	
Gender									
Male (parent)	8 (5.9 %)	1 (.7 %)	6 (4.4 %)	11 (8.1 %)	26 (19.1 %)	.17		46.1	
Female (parent)	35 (25.7 %)	14 (10.3 %)	9 (6.7 %)	52 (38.2 %)	110 (80.9 %)	.56		8.67	
Male (child)	35 (25.7 %)	10 (7.4 %)	11 (8.1 %)	21 (15.4 %)	77 (56.6 %)	20.06*		23.22*	
Female (child)	8 (5.9 %)	5 (3.7 %)	4 (2.9 %)	42 (30.9 %)	59 (43.4 %)	9.93*		63.90*	
Ethnicity									
Chinese	37 (27.2 %)	14 (10.3 %)	14 (10.3 %)	46 (33.8 %)	111 (81.6 %)	4.48		24.07	
Malay	3 (2.2 %)	0	1 (.7 %)	10 (7.4 %)	14 (10.3 %)	2.57		9.57	
Indian	2 (1.5 %)	0	0	4 (2.9 %)	6 (4.4 %)	2.00		2.00	
Others (including Eurasian)	1 (.7 %)	1 (.7 %)	0	3 (2.2 %)	5 (3.7 %)	–		1.00	
Occupation									
Managerial/professional	10 (7.4 %)	6 (4.4 %)	7 (5.1 %)	12 (8.8 %)	35 (25.7 %)	4.24		2.00	
Sales/executive	5 (3.7 %)	0	0	13 (9.6 %)	18 (13.3 %)	3.56		3.56	
Clerical/technical	3 (2.2 %)	2 (1.5 %)	2 (1.5 %)	7 (5.1 %)	14 (10.3 %)	.07		3.93	
Self-employed	6 (4.4 %)	1 (.7 %)	0	5 (3.7 %)	12 (8.8 %)	.33		3.5	
Not working/retired	15 (11.0 %)	7 (5.1 %)	3 (2.2 %)	23 (16.9 %)	48 (35.3 %)	.38		17.62	
Others	3 (2.2 %)	0	3 (2.2 %)	3 (2.2 %)	9 (6.6 %)	1.00		–	
Education									
University/postgraduate	16 (11.8 %)	7 (5.1 %)	5 (3.7 %)	22 (16.2 %)	50 (36.8 %)	.33		.33	
Polytechnic/pre-university	8 (5.9 %)	3 (2.2 %)	2 (1.5 %)	17 (12.5 %)	30 (22.0 %)	.61		12.17	
Secondary/vocational	13 (9.6 %)	4 (2.9 %)	8 (5.9 %)	20 (14.7 %)	45 (33.1 %)	.13		15.33	
Primary or below	1 (.7 %)	0	0	2 (1.5 %)	3 (2.2 %)	1.65		10.02	
Others	5 (3.7 %)	1 (.7 %)	0	2 (1.5 %)	8 (5.9 %)	2.00		3.25	
Additional child with ASD									
Yes	2 (1.5 %)	1 (.7 %)	2 (1.5 %)	0	5 (3.7 %)	–		.40	
No	41 (30.1 %)	14 (10.3 %)	13 (9.6 %)	63 (46.3 %)	131 (96.3 %)	.79		44.1	
Help with caregiving									
Yes	10 (7.4 %)	2 (1.5 %)	4 (2.9 %)	13 (9.6 %)	29 (21.3 %)	1.67		27.20	
No	33 (24.3 %)	13 (9.6 %)	11 (8.1 %)	50 (36.8 %)	107 (78.7 %)	.13		14.00	

AUT Autism, *AS* Asperger's Syndrome; *PDD-NOS* pervasive developmental disorder—not otherwise specified, *TD* typically developing

* $p < .05$

Procedures

Parents of children with ASD registered with the Neuro-Behaviourial Clinic (NBC) at the Child Guidance Clinic (CGC), Institute of Mental Health (IMH) in Singapore between year 2006 and 2013 were recruited via study invitation letters. Based on medical records from the NBC Autism Services unit, registered patients with NBC Autism Services have either been diagnosed with Autism, Asperger's Syndrome or PDD-NOS, via psychiatrists' clinical assessment or a formal assessment session using the Autism diagnostic interview—revised (ADI-R) and Autism diagnostic observation schedule (ADOS; Lord et al. 1994, 2000). Parents of children with ASD completed consent and questionnaires anonymously via self-addressed envelopes. Consequently, child's diagnosis as reported by parents could not be crosschecked with medical records, as parent responses were anonymous. Parents of typically developing children (control group) were recruited via study advertisements posted at the Student Health Centre,

Table 2 Correlations and Cronbach's alpha (bold and diagonal) between PSI/SF, DASS-21, Brief COPE, and parent socio-demographic variables

	Child's condition[a]	PSI total	Parent distress	Parent child interaction	Difficult child	DASS total	Depression	Anxiety	Stress	Brief COPE total	Active Avoidance	Problem-focused	Positive	Religious/denial
PSI/SF total	−.36**	**.93**	.89**	.93**	.89**	.51**	.58**	.34**	.50**	.19*	.40**	.16	.07	.16
Parent distress	−.40**		**.84**	.74**	.65**	.53**	.61**	.33**	.50**	.13	.37**	.09	−.01	.12
Parent–child interaction	−.32**			**.86**	.77**	.45**	.50**	.33**	.42**	.16	.37**	.12	.06	.16
Difficult child	−.24**				**.82**	.41**	.46**	.25**	.42**	.23**	.35**	.22*	.16	.16
DASS total	−.06					**.94**	.92**	.90**	.95**	.43**	.65**	.32**	.24**	.37**
Depression	−.07						**.88**	.73**	.82**	.35**	.56**	.27**	.16	.32**
Anxiety	−.01							**.83**	.79**	.43**	.60**	.30**	.26**	.36**
Stress	−.09								**.92**	.41**	.63**	.32**	.25**	.34**
Brief COPE total	−.02									**.92**	.75**	.87**	.83**	.70**
Active Avoidance	−.14										**.76**	.55**	.51**	.54**
Problem-focused	−.09											**.89**	.79**	.57**
Positive	−.08												**.83**	.45**
Religious/denial	−.08													**.69**

Cronbach's coefficient alphas for total and subscales of PSI/SF, DASS, and Brief COPE in bold typesetting

[a] Child's condition nominally coded, i.e., 1, Autism; 2, Asperger's Syndrome; 3, PDD-NOS; 4, typically developing

* $p < .05$; ** $p < .01$

Health Promotion Board, in Singapore or via snowballing recruitment method. All parents provided informed consent. Study participation was (1) voluntary, (2) took an average of 20 minutes for each participant, (3) assured of total response confidentiality from public, and (4) offered a small appreciation fee of $10 Singapore dollars upon questionnaire completion. Ethics approval was obtained from the National Healthcare Group Domain-Specific Review Board (DSRB) Singapore and the James Cook University Human Research Ethics Committee (HREC).

Data Analyses

In consideration of Hypothesis 1, parent responses were first compared based on whether their child has been diagnosed with an ASD (i.e., between parents of children diagnosed with an ASD and parents of typically developing children). To further assess if parenting stress and parental psychological well-being varied with child's diagnosis, parent responses were also separated and compared in four groups according to child's diagnosis status as reported by parents: (1) Autism, (2) Asperger's Syndrome, (3) PDD-NOS, and (4) children who were not diagnosed with ASD or other chronic medical/mental health conditions.

MANOVA and Chi-square analyses were conducted to highlight differences in the demographical characteristics between all parent groups (i.e., Autism vs. Asperger's Syndrome vs. PDD-NOS vs. typical development). A MANOVA using two-tailed t test statistic was used to compare mean frequencies of self-reported parenting stress, psychological well-being (operationalized as depression and anxiety symptoms), and the use of coping strategies between parent groups. Total and subscale mean scores of each study measure were analyzed separately to identify group differences in the overall and sub-categorical measures of parenting stress, parental psychological well-being and coping. Analysis of variance (ANOVA) statistical methods were used for post hoc comparison testing of group means for specific dependent variables. Threshold for statistical significance was set at $p < .05$.

Results

Differences in Demographical Characteristics

Only differences in parent's and child's age, and child's gender, were statistically significant between parent groups at $p < .05$ (see Table 1). Post-hoc ANOVA comparisons indicated that only parents and their children in the Autism group were significantly older than parents and their typically developing children. Chi-square goodness-of-fit tests also indicated (1) significantly more male than female children being rated by their parents in the Autism group, and (2) significantly more female than male children of typical development being rated by their parents. Considering the observed differences, age and gender effects on parental outcomes and coping were controlled for by including parent's and child's age and child's gender as fixed-effect covariates in subsequent study analyses. However, this control for age and gender effects did not alter the interpretation of study results significantly. Implications of these differences are further discussed in the "Discussion" section.

Preliminary Data Analyses

Positive skewness on the PSI/SF and DASS Total and subscale mean scores were expected as the sample contained parents of typically developing children who may not experience as much parenting stress as parents of children with ASD in caregiving (ref. Hayes and Watson 2012). Transformation of data using square-root transformation technique did not alter results interpretation significantly. No outliers were found by examining box-and-whiskers plot diagrams. For all MANOVA analyses, Levene's test for homogeneity of variance also did not attain statistical significance at $p < .05$, suggesting that homogenous variance was observed for all comparison groups. Thus, untransformed data was used and reported for data analyses. Self-reported parenting stress, psychological well-being and coping scale scores did not differ significantly (a) between mothers and fathers in general, or (b) between gender of child being rated, $p < .05$.

Comparisons Between ASD and Typical Development

A one-way MANOVA was conducted to examine group differences in parenting stress, psychological well-being outcomes (i.e., self-reported depression and anxiety symptoms) and parental coping between parents of children with ASD and parents of typically developing children. Multivariate tests of group means reached statistical significance, Wilks' Lambda = .79, $F(3, 132) = 11.96$, $\eta p^2 = .21$. An estimate of 21 % of the error variance in the parenting stress, psychological well-being and coping total scale mean scores can be explained by differences in parents' group membership. MANOVA of group means in the sub-categories of parenting stress and psychological well-being also reached statistical significance, Wilks' Lambda = .70, $F(10, 125) = 5.35$, $\eta p^2 = .30$. An estimate of 30 % of the error variance in the parenting stress, psychological well-being and coping subscale mean scores can be explained by differences in parents' group membership. Based on these significant MANOVA results, group

differences in parent outcomes and coping scale scores were examined as below.

Parenting Stress

Post-hoc ANOVA of group means revealed statistically significant differences in (1) overall parenting stress levels as measured by PSI/SF total scale scores and (2) all three sub-domains of parenting stress as measured by the PSI/SF PD, P-CDI and DC subscale scores between parents of children with ASD and parents of typically developing children (see Table 3). Findings suggest that parents of children with ASD experienced higher parenting stress in general, and more symptoms in each sub-domain of parenting stress (i.e., more negative views of themselves as parents, poorer parent–child relationships and more child-related parenting stress) than parents of typically developing children (see Fig. 1).

Psychological Well-Being

Post-hoc ANOVA of group means showed statistically significant differences between parents of children with ASD and parents of typically developing children in DASS-D subscale scores only (see Table 3). It is suggested that parents of children with ASD experienced more depression symptoms than parents of typically developing children (see Fig. 1).

Parental Coping

Post-hoc ANOVA of group means showed statistically significant differences between parents of children with ASD and parents of typically developing children in the Brief COPE Active Avoidance subscale scores only (see Table 3). Findings suggest that parents of children with ASD engaged in Active Avoidance coping more frequently than parents of typically developing children.

Comparisons Between ASD Sub-Groups and Typical Development

To understand how parental outcomes differed with child's diagnosis as an extension of Hypothesis 1, a one-way MANOVA was employed to examine differences in parenting stress and psychological well-being outcomes between the four groups of (1) parents of children with Autism, (2) parents of child with Asperger's Syndrome, (3) parents of children with PDD-NOS, and (4) parents of typically developing children. Parental coping scores were not analyzed as coping differences between parents of children with ASD and parents of typically developing children were previously addressed.

Multivariate tests of group means reached statistical significance, Wilks' Lambda = .76, $F(9, 317) = 4.22$, $\eta p^2 = .88$. An estimate of 88 % of the error variance in the parenting stress, psychological well-being and coping total

Table 3 Group means, standard deviations, and ANOVA comparisons for total and subscale scores of PSI/SF, DASS-21, Brief COPE, based on DSM-V and DSM-IV criteria

ANOVA effects	AUT		AS		PDD-NOS		TD		ASD		ANOVA (DSM-5)		ANOVA (DSM-IV)	
	M	SD	M	SD	M	SD	M	SD	M	SD	F(3, 134)	ηp^2	F(3, 132)	ηp^2
PSI/SF total	97.70	21.97	101.40	19.68	105.47	27.58	79.27	18.67	90.29	23.37	32.11**	.19	11.63**	.21
PSI/SF parent distress	33.72	7.65	35.00	7.919	36.87	9.43	26.00	7.18	29.51	9.04	42.34**	.24	14.89**	.25
PSI/SF parent–child dysfunctional interaction	7.65	9.77	7.919	7.86	9.425	9.83	7.18	6.94	30.03	8.95	25.54**	.16	9.25**	.17
PSI/SF difficult child	31.79	8.00	33.00	7.63	33.47	10.29	27.60	6.51	32.12	8.43	11.95**	.08	4.69**	.10
DASS-21 total	10.77	9.27	12.73	9.92	14.73	13.48	9.41	9.98	10.86	10.20	2.39	.02	1.31	.03
DASS-depression	2.91	3.19	4.67	4.40	5.27	5.50	2.43	3.09	3.18	3.72	4.88*	.04	3.52*	.07
DASS-anxiety	2.77	2.90	2.20	1.78	3.20	4.20	2.62	3.63	2.71	3.29	.09	.00	.24	.01
DASS-stress	5.09	4.08	5.87	4.42	6.27	4.48	4.37	3.83	4.98	4.05	2.73	.10	1.24	.03
Brief COPE total	–	–	–	–	–	–	49.03	15.18	50.94	13.62	2.33	.17	–	–
Active Avoidance	–	–	–	–	–	–	13.37	4.005	14.09	3.76	4.44*	.04	–	–
Problem-focused	–	–	–	–	–	–	14.59	5.48	15.40	5.23	2.85	.04	–	–
Positive coping	–	–	–	–	–	–	12.24	4.70	12.43	4.21	.25	.62	–	–
Religious/denial	–	–	–	–	–	–	8.84	3.08	9.02	3.17	.38	.54	–	–

* $p < .05$

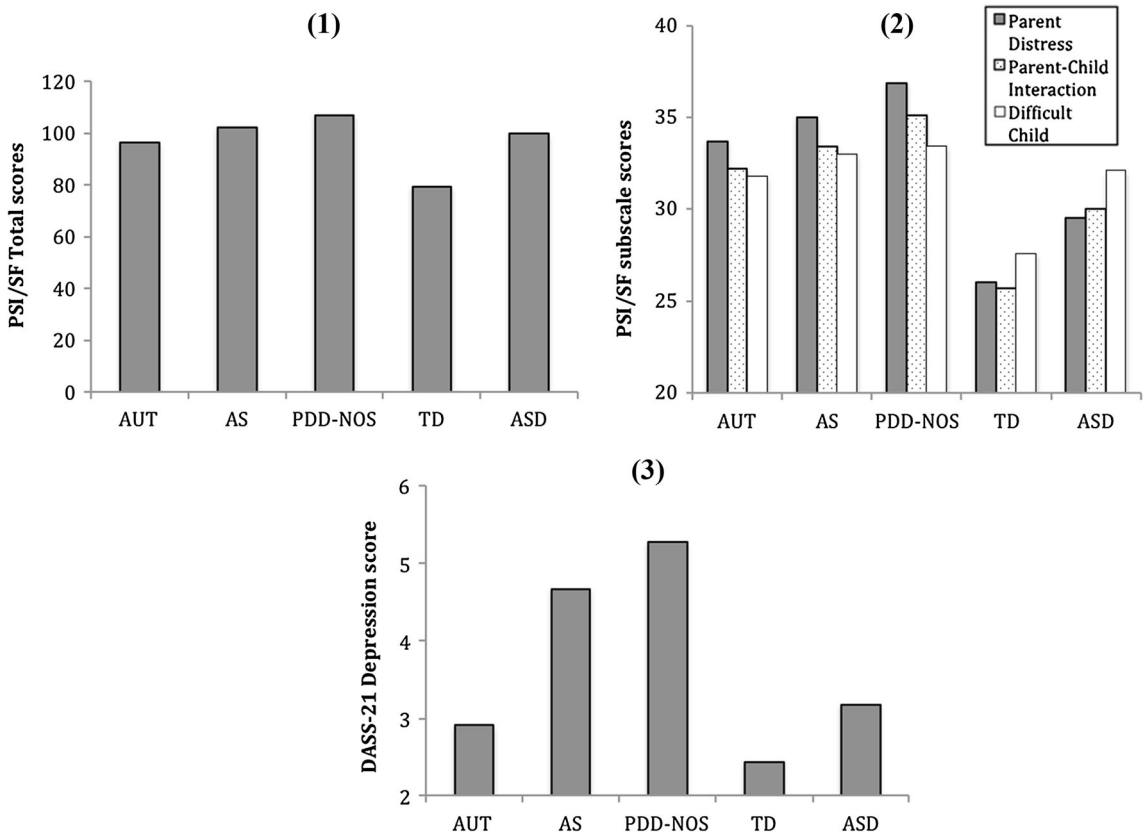


Fig. 1 PSI/SF total scores, PSI/SF subscale scores and DASS-21 depression subscale scores of **1** parents of children with Autism spectrum disorders (ASD) as a group, **2** parents of children with Autism (AUT) or Asperger's syndrome (AS) or pervasive developmental disorder—not otherwise specified (PDD-NOS) and **3** parents of typically developing (TD) children

scale mean scores can be explained by differences in child's diagnosis. Multivariate tests of group means in the subcategories of parenting stress, psychological well-being, and coping strategies also reached statistical significance, Wilks' Lambda = .58, $F(30, 362) = 2.47$, $\eta p^2 = .17$. An estimate of 17 % of the error variance in the parenting stress, psychological well-being and coping subscale mean scores can be explained by differences in child's diagnosis. Significant MANOVA effects as above suggest differences in parent outcomes. Further analyses were conducted to examine group differences in the sub-components of parenting stress and parental psychological well-being outcomes.

Parenting Stress

Post-hoc ANOVA revealed statistically significant differences between the four parent groups in (1) overall parenting stress as measured by PSI/SF total mean scores (see Table 3), and (2) all three sub-domains of parenting stress as measured by the PSI/SF PD, P-CDI and DC subscale mean scores (see Table 3). Comparison testing using post hoc ANOVA showed that PSI/SF total mean scores, and PSI/SF PD and P-CDI subscale mean scores, differed significantly between parents of typically developing children and each of the three parent groups with children with (1) Autism, (2) Asperger's Syndrome, or (3) PDD-NOS only. For PSI/SF DC subscale scores, significant differences were observed between parents of children with Autism or PDD-NOS and parents of typically developing children only.

In agreement with prior analyses based on parents of children with ASD as a group, findings suggest that parents of children diagnosed with any sub-category of ASD experience higher overall parenting stress, more negative views of themselves as parents, and less satisfaction in parent–child bond, than parents of typically developing children (see Fig. 1). Incongruent with analyses based on parents of children with ASD as a group, only parents of children diagnosed with either Autism or PDD-NOS reported more child-related parenting stress than parents of typically developing children (see Fig. 1). There were no significant differences in the overall and sub-domain measures of parenting stress between parents of children diagnosed with each sub-category of ASD.

Psychological Well-Being

Post-hoc ANOVA analyses observed statistically significant differences between parent groups in the DASS-D subscale mean scores only (see Table 3). Comparison testing using post hoc ANOVA showed that DASS-D subscale scores only differed significantly between parents of typically developing children and parents of children with PDD-NOS (see Table 3). Results imply that parents of children with PDD-NOS experienced more depression-related symptoms than parents of typically developing children (see Fig. 1). There were no significant differences in the overall and sub-domain measures of psychological well-being between parents of children diagnosed with each sub-category of ASD.

Discussion

Results showed that parents of children with a diagnosis of ASD reported more parenting stress and depression symptoms, and engaged in more maladaptive coping (i.e., Active Avoidance coping), than parents of typically developing children. Generally, findings from this study provided additional support that providing care for a child with ASD has a negative psychological effect on caregivers (e.g., Benson and Karlof 2009; Hayes and Watson 2012; Stuart and McGrew 2009).

Parental Psychological Well-Being Outcomes

In this study, parents of children with ASD experienced more parenting stress symptoms than parents of typically developing children. Previous research highlighted that stress proliferation and compounded caregiving demands aggravated the impact of negative caregiving experiences and feelings in parents of children with ASD over time (Benson 2010; Benson and Karlof 2009). Implicatively, and in agreement with previous literature, parenting stress is expected to heighten when parents care for any child with a chronic condition such as ASD in this study (Hayes and Watson 2012).

It is of note that the same pattern of findings in parenting stress and psychological well-being outcomes were observed whether responses from parents of children with ASD were compared as a group or based on child's reported diagnosis (i.e., aligned with DSM-4 criteria). Therefore, findings from this study suggest no discernable differences in parenting stress when parenting children diagnosed with any sub-category of ASD. Although parents of children with ASD did not differ among themselves in parenting stress and psychological well-being outcomes, the effects of some factors on parental well-being outcomes may warrant further investigation. For instance, previous research highlighted that parents' acceptance of child's social and communicative challenges played a role in the impact of parenting stress on psychological well-being outcomes (Lee 2009; Ling et al. 2010; Mori et al. 2009; Rao and Beidel 2009; Szatmari et al. 1995). In addition, factors such as child's daily and ASD-related behaviors (e.g., Lecavalier et al. 2006; Huang et al. 2014), the level of adaptive functioning of the child (e.g., Abbeduto et al. 2004; Hall and Graff 2011; Konstantareas and Papageorgiou 2006), and child's learning abilities and schooling arrangements (e.g., Lee et al. 2008; Nevo and Bin Khader 1995) can moderate the intensity of parenting stress felt by parents. In view of the above-mentioned findings, further examination of the factors that influence parenting stress experiences and parental psychological well-being outcomes is needed. Qualitative methods supporting more in-depth documentation of caregiving experiences can be considered to achieve this endeavor (Creswell 2012; Lai and Oei 2014).

In addition to stress, parents of children with ASD in our study reported more depressive symptoms, and no significant differences in anxiety symptoms, when compared to parents of typically developing children. Previous research has highlighted that parent-reported anxiety (but not depression) symptoms fluctuated across circumstances and time, while depression symptoms developed on a more stable trajectory (Baker et al. 2011; Benson and Karlof 2009; Gray 2003, 2006; Griffith et al. 2010; Luong et al. 2009). Considering this, it is then possible that parent-reported anxiety symptoms are insufficiently salient at this point in time to observe significant differences between parent comparison groups. Longitudinal studies evaluating parental psychological well-being at various time points of their child's development will provide further information for a well-rounded understanding of the mental health status, as well as parenting stress experiences, of parents of a child diagnosed with ASD (Gray 2003, 2006).

Parental Coping Strategies

In this study, parents of children with ASD were observed to engage in more maladaptive/emotion-focused coping (i.e., Active Avoidance coping) than parents of typically developing children. This is consistent with previous observations that parents of children with developmental disabilities (including ASD) used maladaptive/emotion-focused coping in managing caregiving stress (e.g., Piazza et al. 2014; Sivberg 2002; Wang et al. 2011). Of note is parents' increased use of Active Avoidance coping, which suggests that parents tended to criticize themselves for the problems they faced, vent negative emotions, distract themselves from thinking about problems or give up trying

to solve their problems (Carver et al. 1989; Hastings et al. 2005).

Parents' use of Active Avoidance coping strategies (as highlighted above) may be understood in light of the cultural background of parents in this study. In an Asian society such as that in Singapore, whereby high-achieving students are valued, parents of children with ASD could find themselves constantly worrying and looking for ways to help their child or having to reconcile with their disappointment when their child does not progress as quickly their peers (Fung and Cai 1998; Luong et al. 2009; Moh and Magiati 2012; Nevo and Bin Khader 1995). Eventually, when the stamina for problem-solving diminishes while caregiving problems continue to surface, parents may engage in maladaptive/emotion-focused coping such as avoidance to cope with caregiving challenges and stressful feelings (Gray 2006). Moreover, parents' use of avoidance coping can be reinforced by the Asian-related ideology of "saving face", whereby parents avoid seeking help from others out of fear of the social stigma and embarrassment associated with having a child with special needs (Kim et al. 2001; Luong et al. 2009; McCabe 2008; Nevo and Bin Khader 1995).

Findings from this study highlight the importance of acknowledging culture-specific coping behaviors. General stress and coping mechanisms provide a primary buffer against the immediate impact of parenting stress; however, general coping strategies are limited in optimizing available coping resources for culturally nuanced stressful experiences (Sawang et al. 2006). Healthcare professionals should dedicate closer attention and support to the individual needs of parents looking after children with special needs, especially when the children are functioning at a level that requires substantial support, and present with challenging behaviors and co-morbid medical problems (Bauman 2010; Lecavalier et al. 2006; Rao and Beidel 2009).

Study Limitations

There are some considerations to the application of study findings. Firstly, study findings may not be generalizable to parents of children with ASD who did not seek professional medical help at the NBC Autism Services at IMH Singapore. Future replications could include a nation-wide survey of the psychological health of parents of children with ASD in Singapore. Secondly, the study design was cross-sectional. As psychological well-being and coping are time- and context-dependent conditions, future studies could adopt longitudinal designs to observe changing trends or highlight possible factors contributing towards parental well-being or coping strategies (Benson and Karlof 2009; Taylor and Stanton 2007). Fourthly, the study could have benefited from comparisons with additional parent groups such as parents of children with intellectual or learning disabilities (e.g., Abbeduto et al. 2004), or other chronic medical or mental health conditions (e.g., Wang et al. 2011). This could help tease out parenting stress effects unique to parents of children with ASD for closer examination (Hayes and Watson 2012). Finally, due to the nature of convenience sampling for parents of children with ASD, sample characteristics for comparison groups (i.e., Autism, Asperger's Syndrome, PDD-NOS and typical development) could not be precisely matched to minimize secondary influences from demographical variables. In this study, significant differences were observed in parent's and child's age, and in child's gender, when comparing between parents in the ASD and typical development groups, although these differences did not affect interpretation of study results significantly. Previous research suggested potential moderating effects of child's age and gender on parental psychological outcomes (e.g., Mandell and Salzer 2007; Smith et al. 2008). Therefore, for a more detailed understanding of the effects of demographical factors on parental stress outcomes and coping, future studies may use well-matched comparison groups or track parent responses across several time points in the child's developmental trajectory (Gray 2006; Griffith et al. 2010).

Conclusion

Parents of children with ASD experienced significantly more parenting stress and depression symptoms, and engaged in more maladaptive coping, than parents of typically developing children. While parenting stress effects were consistently observed among different groups of parents in this study, parental coping on the other hand could be sensitive to cultural influences or caregiving demands from the environment. Healthcare professionals are thus reminded to stay mindful of parents' mental health statuses and individual caregiving needs when providing services to families of children with ASD.

Acknowledgments This study is supported by a grant from the Woodbridge Hospital Charity Fund in affiliation with the Institute of Mental Health, Singapore. Special thanks to the Neuro-Behavioral Clinic (Autism Services), Child Guidance Clinic, Institute of Mental Health, for the assistance in the conduct of this study.

References

Abbeduto, L., Seltzer, M. M., Shattuck, P., Krauss, M. W., Orsmond, G., & Murphy, M. M. (2004). Psychological well-being and coping in mothers of youths with Autism, Down Syndrome, or Fragile X Syndrome. *American Association on Intellectual and Developmental Disabilities, 109*(3), 237–254.

Abidin, R. R. (1992). The determinants of parenting behavior. *Journal of Clinical Child Psychology, 21*(4), 407–412.

American Psychiatric Association (APA). (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). Arlington, VA: American Psychiatric Publishing.

Baker, J. K., Seltzer, M. M., & Greenberg, J. S. (2011). Longitudinal effects of adaptability on behavior problems and maternal depression in families of adolescents with Autism. *Journal of Family Psychology, 25*(4), 601.

Bauman, M. L. (2010). Medical comorbidities in Autism: Challenges to diagnosis and treatment. *Neurotherapeutics, 7*(3), 320–327.

Benson, P. R. (2010). Coping, distress, and well-being in mothers of children with Autism. *Research in Autism Spectrum Disorders, 4*(2), 217–228.

Benson, P. R., & Karlof, K. L. (2009). Anger, stress proliferation, and depressed mood among parents of children with ASD: A longitudinal replication. *Journal of Autism and Developmental Disorders, 39*(2), 350–362.

Carver, C. S. (1997). You want to measure coping but your protocol's too long: consider the brief COPE. *International Journal of Behavioral Medicine, 4*(1), 92–100.

Carver, C. S. (2007). Brief COPE. Self-report measures available. Retrieved September 13, 2012 from http://www.psy.miami.edu/faculty/ccarver/CCscales.html.

Carver, C. S., Scheier, M. F., & Weintraub, J. K. (1989). Assessing coping strategies: A theoretically based approach. *Journal of Personality and Social Psychology, 56*(2), 267–283.

Centers for Diseases Control and Prevention (CDC). (2012). Prevalence of Autism spectrum disorders—Autism and developmental disabilities monitoring network, 14 sites, United States, 2008. *Surveillance Summaries, 61*(SS03), 1–19.

Creswell, J. W. (2012). *Educational research: planning, conducting, and evaluating quantitative and qualitative research* (4th ed.). Boston, MA: Pearson.

Elsabbagh, M., Divan, G., Koh, Y.-J., Kim, Y. S., Kauchali, S., Marcín, C., et al. (2012). Global prevalence of Autism and other pervasive developmental disorders. *Autism Research, 5*, 160–179. doi:10.1002/aur.239.

Estes, A., Munson, J., Dawson, G., Koehler, E., Zhou, X., & Abbott, R. (2009). Parenting stress and psychological functioning among mothers of preschool children with Autism and developmental delay. *Autism, 13*(4), 375–387.

Fung, D., & Cai, Y. M. (1998). *Help your child to cope: Understanding childhood stress*. Singapore: Times Books International.

Gallagher, S., Phillips, A. C., Oliver, C., & Carroll, D. (2008). Predictors of psychological morbidity in parents of children with intellectual disabilities. *Journal of Pediatric Psychology, 33*(10), 1129–1136.

Gray, D. E. (2003). Gender and coping: the parents of children with high functioning Autism. *Social Science and Medicine, 56*(3), 631–642.

Gray, D. E. (2006). Coping over time: The parents of children with Autism. *Journal of Intellectual Disability Research, 50*, 970–976. doi:10.1111/j.1365-2788.2006.00933.x.

Griffith, G. M., Hastings, R. P., Nash, S., & Hill, C. (2010). Using matched groups to explore child behavior problems and maternal well-being in children with Down Syndrome and Autism. *Journal of Autism and Developmental Disorders, 40*(5), 610–619.

Hall, H. R. (2012). Families of children with Autism: Behaviors of children, community support and coping. *Issues in Comprehensive Pediatric Nursing, 35*(2), 111–132.

Hall, H. R., & Graff, J. C. (2011). The relationships among adaptive behaviors of children with Autism, family support, parenting stress, and coping. *Issues in Comprehensive Pediatric Nursing, 34*(1), 4–25. doi:10.3109/01460862.2011.555270.

Hastings, R. P., Kovshoff, H., Brown, T., Ward, N. J., Espinosa, F. D., & Remington, B. (2005). Coping strategies in mothers and fathers of preschool and school-age children with Autism. *Autism, 9*(4), 377–391. doi:10.1177/1362361305056078.

Hayes, S. A., & Watson, S. L. (2012). The impact of parenting stress: A meta-analysis of studies comparing the experience of parenting stress in parents of children with and without Autism spectrum disorder. *Journal of Autism and Developmental Disorders, 43*(3), 629–642.

Huang, Chien-Yu., Yen, Hsui-Chen, Tseng, Mei-Hui, Tung, Li-Chen, Chen, Ying-Dar, & Chen, Kuan-Lin. (2014). Impacts of autistic behaviors, emotional and behavioral problems on parenting stress in caregivers of children with Autism. *Journal of Autism and Developmental Disorders, 44*(6), 1383–1390.

Kawachi, I., & Berkman, L. F. (2001). Social ties and mental health. *Journal of Urban Health: Bulletin of the New York Academy of Medicine, 78*(3), 458–467.

Kim, B. S. K., Atkinson, D. R., & Umemoto, D. (2001). Asian cultural values and the counseling process: Current knowledge and directions for future research. *The Counseling Psychologist, 29*, 570–603.

Konstantareas, M. M., & Papageorgiou, V. (2006). Effects of temperament, symptom severity and level of functioning on maternal stress in Greek children and youth with ASD. *Autism, 10*(6), 593–607.

Lai, W. W., & Oei, T. P. S. (2014). Coping in parents of children with Autism spectrum disorders: A review. *Review Journal of Autism and Developmental Disorders,*. doi:10.1007/s40489-014-0021-x.

Lecavalier, L., Leone, S., & Wiltz, J. (2006). The impact of behaviour problems on caregiver stress in young people with Autism spectrum disorders. *Journal of Intellectual Disability Research, 50*(3), 172–183.

Lee, G. K. (2009). Parents of children with high functioning Autism: How well do they cope and adjust? *Journal of Developmental and Physical Disabilities, 21*(2), 93–114.

Lee, L. C., Harrington, R. A., Louie, B. B., & Newschaffer, C. J. (2008). Children with Autism: Quality of life and parental concerns. *Journal of Autism and Developmental Disorders, 38*(6), 1147–1160.

Lin, C., Tsai, Y., & Chang, H. (2008). Coping mechanisms of parents of children recently diagnosed with Autism in Taiwan: A qualitative study. *Journal of Clinical Nursing, 17*(20), 2733–2740. doi:10.1111/j.1365-2702.2008.02456.x.

Ling, C. Y. M., Mak, W. W. S., & Cheng, J. N. S. (2010). Attribution model of stigma towards children with Autism in Hong Kong. *Journal of Applied Research in Intellectual Disabilities, 23*(3), 237–249.

Lord, C., Risi, S., Lambrecht, L., Cook, E. H, Jr, Leventhal, B. L., DiLavore, P. C., et al. (2000). The Autism diagnostic observation schedule—Generic: A standard measure of social and communication deficits associated with the spectrum of Autism. *Journal of Autism and Developmental Disorders, 30*(3), 205–223.

Lord, C., Rutter, M., & Le Couteur, A. (1994). Autism diagnostic interview-revised: A revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *Journal of Autism and Developmental Disorders, 24*(5), 659–685.

Lovibond, P. (2011). Depression Anxiety Stress Scales (DASS). DASS publications. Retrieved September 10, 2012 from http://www2.psy.unsw.edu.au/groups/dass//pub.htm.

Lovibond, P. F., & Lovibond, S. H. (1995). The structure of negative emotional states: Comparison of the Depression Anxiety Stress Scales (DASS) with the beck depression and anxiety inventories. *Behaviour Research and Therapy, 33*(3), 335–343.

Luong, J., Yoder, M. K., & Canham, D. (2009). Southeast asian parents raising a child with Autism: A qualitative investigation

of coping styles. *The Journal of School Nursing, 25*(3), 222–229. doi:10.1177/1059840509334365.

Mak, W. W., & Ho, G. S. (2007). Caregiving perceptions of Chinese mothers of children with intellectual disability in Hong Kong. *Journal of Applied Research in Intellectual Disabilities, 20*(2), 145–156.

Mandell, D. S., & Salzer, M. S. (2007). Who joins support groups among parents of children with Autism? *Autism, 11*(2), 111–122.

McCabe, H. (2008). The importance of parent-to-parent support among families of children with Autism in the People's Republic of China. *International Journal of Disability, Development and Education, 55*(4), 303–314.

Ministry of Health, Singapore (MOH). (2004). *Singapore burden of disease study 2004*. Retrieved August 02, 2013 from http://www.moh.gov.sg/content/dam/moh_web/Publications/Reports/2009/1%20Preface%20n%20Executive%20Summary.pdf.

Moh, T. A., & Magiati, I. (2012). Factors associated with parental stress and satisfaction during the process of diagnosis of children with Autism spectrum disorders. *Research in Autism Spectrum Disorders, 6*(1), 293–303.

Montes, G., & Halterman, J. S. (2007). Psychological functioning and coping among mothers of children with Autism: A population-based study. *Pediatrics, 119*(5), e1040–e1046.

Mori, Kyoko, Ujiie, Takeshi, Smith, Anna, & Howlin, Patricia. (2009). Parental stress associated with caring for children with Asperger's Syndrome or Autism. *Pediatrics International, 51*(3), 364–370.

Nevo, B., & Bin Khader, A. M. (1995). Cross-cultural, gender, and age differences in Singaporean mothers's conceptions of children's intelligence. *The Journal of Social Psychology, 135*(4), 509–517.

Oei, T. P., Sawang, S., Goh, Y. W., & Mukhtar, F. (2013). Using the Depression Anxiety Stress Scale 21 (DASS-21) across cultures. *International Journal of Psychology, 48*(6), 1–12.

Oyserman, D., Coon, H. M., & Kemmelmeier, M. (2002). Rethinking individualism and collectivism: Evaluation of theoretical assumptions and meta-analyses. *Psychological Bulletin, 128*(1), 3–72.

Pearson, D. A., Loveland, K. A., Lachar, D., Lane, D. M., Reddoch, S. L., Mansour, R., & Cleveland, L. A. (2006). A comparison of behavioral and emotional functioning in children and adolescents with autistic disorder and PDD-NOS. *Child Neuropsychology, 12*(4–5), 321–333.

Penley, J. A., Tomaka, J., & Wiebe, J. S. (2002). The association of coping to physical and psychological health outcomes: A meta-analytic review. *Journal of Behavioral Medicine, 25*(6), 551–603.

Peters-Scheffer, N., Didden, R., & Korzilius, H. (2012). Maternal stress predicted by characteristics of children with Autism spectrum disorder and intellectual disability. *Research in Autism Spectrum Disorders, 6*(2), 696–706. doi:10.1016/j.rasd.2011.10.003.

Phetrasuwan, S., & Shandor Miles, M. (2009). Parenting stress in mothers of children with Autism spectrum disorders. *Journal for Specialists in Pediatric Nursing, 14*(3), 157–165.

Piazza, Vivian E., Floyd, Frank J., Mailick, Marsha R., & Greenberg, Jan S. (2014). Coping and psychological health of aging parents of adult children with developmental disabilities. *American Journal on Intellectual and Developmental Disabilities, 119*(2), 186–198.

Rao, P. A., & Beidel, D. C. (2009). The impact of children with high-functioning Autism on parental stress, sibling adjustment, and family functioning. *Behavior Modification, 33*(4), 437–451.

Sawang, S., Oei, T. P. S., & Goh, Y. W. (2006). Are country and culture values interchangeable? A case example using occupational stress and coping. *International Journal of Cross Cultural Management, 6*, 2205–2219. doi:10.1177/1470595806066330.

Simonoff, E., Pickles, A., Charman, T., Chandler, S., Loucas, T., & Baird, G. (2008). Psychiatric disorders in children with Autism spectrum disorders: Prevalence, comorbidity, and associated factors in a population-derived sample. *Journal of American Academy of Child and Adolescent Psychiatry, 47*(8), 921–929.

Sivberg, B. (2002). Family system and coping behaviors. *Autism, 6*(4), 397–409. doi:10.1177/1362361302006004006.

Smith, L. E., Seltzer, M. M., Tager-Flusberg, H., Greenberg, J. S., & Carter, A. S. (2008). A comparative analysis of well-being and coping among mothers of toddlers and mothers of adolescents with ASD. *Journal of Autism and Developmental Disorders, 38*(5), 876–889.

Stuart, M., & McGrew, J. H. (2009). Caregiver burden after receiving a diagnosis of an Autism spectrum disorder. *Research in Autism Spectrum Disorders, 3*(1), 86–97.

Szatmari, P., Archer, L., Fisman, S., Streiner, D. L., & Wilson, F. (1995). Asperger's Syndrome and Autism: Differences in behavior, cognition, and adaptive functioning. *Journal of the American Academy of Child and Adolescent Psychiatry, 34*(12), 1662–1671.

Taylor, S., & Stanton, A. L. (2007). Coping Resources, Coping Processes, and Mental Health. *Annual Review of Clinical Psychology, 3*, 377–401.

Twoy, R., Connolly, P. M., & Novak, J. M. (2007). Coping strategies used by parents of children with Autism. *Journal of the American Academy of Nurse Practitioners, 19*, 251–260.

Uchino, B. N. (2006). Social support and health: A review of physiological processes potentially underlying links to disease outcomes. *Journal of Behavioral Medicine, 29*(4), 377–387. doi:10.1007/s10865-006-9056-5.

Wang, P., Michaels, C., & Day, M. (2011). Stresses and coping strategies of chinese families with children with Autism and other developmental disabilities. *Journal of Autism and Developmental Disorders, 41*(6), 783–795. doi:10.1007/s10803-010-1099-3.

Yeo, K. J., & Lu, X. (2012). Parenting stress and psychological distress among mothers of children with Autism in Johor Bahru and Hangzhou. *Journal of Educational Psychology and Counseling, 6*, 129–153.

World Journal of Psychiatry

Submit a Manuscript: https://www.f6publishing.com

World J Psychiatry 2023 May 19; 13(5): 174-181

DOI: 10.5498/wjp.v13.i5.174

ISSN 2220-3206 (online)

MINIREVIEWS

Deep brain stimulation for autism spectrum disorder

Stefano Marini, Lucia D'Agostino, Carla Ciamarra, Alessandro Gentile

Specialty type: Psychiatry

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Kaur M, United States; Moshref RH, Saudi Arabia

Received: December 23, 2022
Peer-review started: December 23, 2022
First decision: March 1, 2023
Revised: March 9, 2023
Accepted: March 29, 2023
Article in press: March 29, 2023
Published online: May 19, 2023

Stefano Marini, Lucia D'Agostino, Carla Ciamarra, Alessandro Gentile, Department of Mental Health, National Health Service, Termoli 86039, Italy

Corresponding author: Stefano Marini, MD, Doctor, Department of Mental Health, National Health Service, Via del Molinello n. 1, Termoli 86039, Italy. sfnmarini@gmail.com

Abstract

Deep brain stimulation (DBS) is a medical treatment that aims to obtain therapeutic effects by applying chronic electrical impulses in specific brain structures and neurological circuits. Over the years, DBS has been studied for the treatment of many psychiatric disorders. Scientific research on the use of DBS in people with autism has focused this interest mainly on treatment-resistant obsessive-compulsive disorder, drug-resistant epilepsy, self-injurious behaviors (SIB), and aggressive behaviors toward the self. Autism spectrum disorder (ASD) includes a group of developmental disabilities characterized by patterns of delay and deviance in the development of social, communicative, and cognitive skills and the presence of repetitive and stereotyped behaviors as well as restricted interests. People with autism often have numerous medical and psychiatric comorbidities that worsen the quality of life of patients and their caregivers. Obsessive-compulsive symptoms can be found in up to 81.3% of people with autism. They are often severe, refractory to treatment, and particularly difficult to treat. SIB has a high prevalence in severely retarded individuals and is often associated with autism. Drug treatment of both autism and SIB presents a therapeutic challenge. To describe the current state of the art regarding the efficacy of DBS in people with ASD, a literature search was conducted for relevant studies using the PubMed database. Thirteen studies have been considered in this paper. Up to date, DBS has been used for the stimulation of the nucleus accumbens, globus pallidus internus, anterior limb of the internal capsule, ventral anterior limb of the internal capsule, basolateral amygdala, ventral capsule and ventral striatum, medial forebrain bundle, and posterior hypothalamus. In the total sample of 16 patients, 4 were adolescents, and 12 were adults. All patients had symptoms resistant to multiple drug therapy. Many patients taken into consideration by the studies showed clinical improvements as evidenced by the scores of the psychopathological scales used. In some cases, clinical improvements have varied over time, which may require further investigation. Among the new therapeutic perspectives, DBS could be a valid option. However, further, and more in-depth research is needed in this field.

Key Words: Deep brain stimulation; Autism spectrum disorder; Comorbidities; Drug

resistant; New therapeutic perspectives

Core Tip: Deep brain stimulation (DBS) is a medical treatment that aims at obtaining therapeutic effects by applying chronic electrical impulses in specific brain structures and neurological circuits. Autism spectrum disorder comprises a group of developmental disabilities that are often associated with numerous medical and psychiatric comorbidities that worsen the quality of life of patients and their caregivers. Comorbidities often require multiple drug treatments with an increasing rate of treatment resistance. Thirteen studies have been considered in this paper. Up to date, DBS has been used for the stimulation of the nucleus accumbens, globus pallidus internus, anterior limb of the internal capsule, ventral anterior limb of the internal capsule, basolateral amygdala, ventral capsule and ventral striatum, medial forebrain bundle, and posterior hypothalamus. In the total sample of 16 patients, 4 were adolescents (all males), and 12 were adults (5 males and 7 females). All patients had symptoms resistant to multiple drug therapy. Only one patient was considered not a responder to DBS. Among the new therapeutic perspectives, as evidenced by the studies presented in this article, DBS could be a valid option. However, further, and more in-depth research is needed in this field.

Citation: Marini S, D'Agostino L, Ciamarra C, Gentile A. Deep brain stimulation for autism spectrum disorder. *World J Psychiatry* 2023; 13(5): 174-181
URL: https://www.wjgnet.com/2220-3206/full/v13/i5/174.htm
DOI: https://dx.doi.org/10.5498/wjp.v13.i5.174

INTRODUCTION

Deep brain stimulation (DBS) is a medical treatment that aims at obtaining therapeutic effects of certain neurological and psychiatric disorders by applying chronic electrical impulses in specific brain structures and neurological circuits[1]. The modern beginning of DBS can be traced back to the work of Benabid, Pollak, and colleagues at the Joseph Fourier University in Grenoble in the 1980s[2], based on several decades of clinical work and biophysical discoveries[3]. The clinical success of DBS has opened the door to other neurostimulation therapies such as transcranial magnetic stimulation and has motivated an intense analysis of the neural circuits affected by neurological disorders such as Parkinson's disease[4]. The first use of DBS for a psychiatric indication was published by Nuttin *et al*[5] in 1999. Over the years, DBS has been studied for the treatment of obsessive-compulsive disorder (OCD)[6], tardive dyskinesia (TD)[7], treatment-resistant depression[8-10], Tourette's syndrome[11], treatment-refractory anorexia nervosa[12].

Autism spectrum disorder (ASD) includes a group of developmental disabilities characterized by patterns of delay and deviance in the development of social, communicative, cognitive skills and the presence of repetitive and stereotyped behaviors as well as restricted interests[13]. In addition to core symptoms, people with ASD often have numerous medical and psychiatric comorbidities that worsen the quality of life of patients and their caregivers[14]. Obsessive-compulsive symptoms can be found in up to 81.3% of people with ASD. They are often severe, refractory to treatment, may be clinically confused with core symptoms of ASD, and are particularly difficult to treat[15,16].

Self-injurious behavior (SIB) has been defined as "behavior which produces physical injury to the individual's own body"[17]. SIB has a high prevalence in severely retarded individuals and is often associated with autism. Indeed, up to 42% of people with autism may exhibit repetitive SIBs[18]. Additionally, over 75% of children with SIB will have these behaviors persist into adulthood sometimes resulting in serious harm and even death[19-21].

In approximately two-thirds of cases, SIB is maintained by social variables[22], while in approximately one-quarter of cases, SIB occurs independently of social consequences [automatic reinforcement subtype, automatically maintained SIB (ASIB)][23]. ASIB is considered the most challenging subtype to treat, because the events that cause and maintain it are not known. Currently, ASIB is classified into three subtypes[24,25]. Subtype 1 ASIB is characterized by higher rates of SIB in conditions with minimal external stimulation. Subtype 2 ASIB is characterized by high or variable rates of SIB across high and low stimulation conditions. Subtype 3 ASIB is characterized by the presence of self-restraint[26].

Drug treatment of both autism and SIB presents a therapeutic challenge. Some drugs such as risperidone, aripiprazole, and fluoxetine have shown positive efficacy evidence for treating irritability in people with ASD but not for specifically reducing self-harm[27-30]. Currently, the most successful therapeutic strategies for SIBs are based on applied behavioral analysis techniques[31,32] combined

with pharmacological treatments with neuroleptics, mood stabilizers, sedatives, but some patients remain refractory[33].

The present work aims to describe the current state of the art regarding the efficacy of DBS in people with ASD.

A literature search was conducted for relevant studies using PubMed database. In drafting this paper, the authors decided to consider the published articles, classifying them according to the brain regions stimulated by DBS and not according to the pathologies treated.

There are clinical studies on animal models in the literature, but in this article, we will only consider human clinical studies, as we are more interested in the usefulness and efficacy of DBS in clinical practice.

Scientific research on the use of DBS in people with autism has focused this interest mainly on treatment-resistant OCD, drug-resistant epilepsy (DRE), SIBs, and aggressive behaviors toward self. Four studies in the literature have used DBS to treat OCD and other comorbidities in people with ASD [34-37] (see Table 1). Five studies investigated the efficacy of DBS in the treatment of SIB in people with autism[38-42]. A protocol for the application of DBS in children and young adults has recently been published, but the results are not yet available[43]. Furthermore, Heiden *et al*[44] published a retrospective study of the use of DBS in ten patients, including two patients with autism, but the results were not extrapolated for the different pathologies. This makes it impossible to consider the efficacy of DBS in the autistic patients included in the study. Torres *et al*[45] also published a study on the use of DBS for aggression in 7 patients, 5 of whom had autism. The results were not divided for a single patient not allowing to identify of the efficacy of DBS for autistic patients. Recently Benedetti-Isaac *et al* [46] published a follow-up study on the use of DBS in 5 pediatric autistic patients with aggressive behaviors resistant to drug therapy, but the results were not divided by single patient.

In the total sample of 16 patients, 4 were adolescents (all males), and 12 were adults (5 males and 7 females). All patients had symptoms resistant to multiple drug therapy. Generally, treatment resistance consists of three core components: Correct psychiatric diagnosis, adequate treatment, and symptoms not responding adequately despite treatment[47].

USE OF DBS IN PEOPLE WITH AUTISM

In patients with autism, the literature published so far has used DBS for the stimulation of the nucleus accumbens (NAc), Globus Pallidus internus (GPi), anterior limb of internal capsule (ALIC), ventral ALIC (vALIC), basolateral amygdala, ventral capsule and ventral striatum, medial forebrain bundle (MFB), posterior hypothalamus (PHyp).

Three studies[33,34,39] have applied DBS to the NAc of people with autism and numerous comorbidities. Past literature has shown that the NAc may be a key structure for the control of OCD symptoms[48,49], in modulating aggression[50], and in improving the response to social stimuli in ASD [51].

Segar *et al*[34] showed the efficacy of DBS in a 24-year-old female patient with Kleefstra Syndrome with comorbidities of ASD, OCD, and Tourette-like symptoms. The clinical improvements mainly concerned the patient's compulsive behaviors, coprolalia, language, and social interaction, with marked improvement in the global assessment of functioning scores.

In 2019, Doshi *et al*[35] reported a 42-year-old woman with autism who underwent bilateral NAc DBS for control of severe OCD and aggression (violent outbursts against others and hitting and injuring others and herself) refractory to pharmacological treatments. In the days following the surgery, the patient had shown a marked difference in her behavior and eye contact, and appropriate laughter. Clinical improvements were consistent with improvements in administered psychopathology scale scores [Yale-Brown obsessive-compulsive scale (Y-BOCS), Hamilton depression scale, Hamilton anxiety scale, and social communication questionnaire].

Park *et al*[40] observed remarkable clinical improvements in a 14-year-old boy with ASD and SIB treated with bilateral NAc DBS. The clinical improvements (assessed with the Y-BOCS, clinical global impression scale, attention deficit hyperactive disorder rating scale, and social responsiveness scale), were accompanied by functional and structural changes in the brain after DBS, demonstrated using fluorodeoxyglucose positron emission tomography/computed tomography imaging. Furthermore, at the 2-year post-operative evaluation, the boy showed improved language comprehension and expression skills, and improved eye contact.

Two studies[38,41] have applied GPi DBS to people with autism to improve movement impairments. Stocco *et al*[39] applied GPi DBS to two people with ASD, severe stereotypies, and SIB (one patient simultaneously received DBS in GPi and the Anterior limb of the internal capsule). Only the patient who received GPi DBS had maintained clinical improvements over time, even reducing drug therapy. As suggested by the authors, GPi DBS may provide relief for severe pharmacologically unresponsive stereotypies in some patients. Indeed, the characteristics of the ideal patient to be subjected to DBS should be better explored.

Table 1 Summary of deep brain stimulation studies for autism spectrum disorder

Ref.	Patients' age/sex	Diagnosis and comorbidities	Indications for DBS	DBS targets	Pre-BDS scores	Post-BDS scores	Main outcomes
Segar *et al*[34]	24, F	KS, OCD, ASD, epilepsy	Biting hands, picking skin	NAc	GAF 20	GAF 50-60	Clinical improvements mainly for compulsive behaviors, coprolalia, language and social interaction
Doshi *et al*[35]	42, F	OCD, ASD, epilepsy	OCD, aggression	NAc	Y-BOCS 19, HAMD 20, HAS 30, SCQ 26	Y-BOCS 5, HAMD 15, HAS 18, SCQ 16	Marked improvements in OCD symptoms, aggressive behavior, eye contact and appropriate laughter
Park *et al*[40]	13, M	ASD, Develop-mental Delay	Self-mutilation, face-hitting	NAc	CGI-S 6; ABC 106; CY-BOCS 22; K-ARS 54; SRS 101	CGI-S 4; ABC 40; CY- BOCS 7; K-ARS 36; SRS 98	Decreased in SIB and improvement in verbal communication
Stocco *et al* [39]	19, F	ASD, ID, monosomy 2q and trisomy 20p	Self-picking, Severe stereotypes	GPi	JHMRS 46	JHMRS 4	Marked improvement in the SIB and dystonia
	17, M	ASD, ID, anxiety	Punching of arms and legs, biting, Severe stereotypes	GPi and ALIC	JHMRS 67	JHMRS 19	Substantial initial improvement in SIB, but the benefit disappeared after 6 mo and was not regained
Kakko *et al* [42]	19, M	ASD, ID, epilepsy, TD	Aggression, self-mutilation, lacerations	GPi	NR	NR	TD symptoms were markedly improved. The anxiety, behavioral symptoms had ceased
Sturm *et al*[38]	13, M	Kanner's Autism, ID, infantile cerebral palsy	Self-aggression	Basolateral amygdala	Parental score of 6	Parental score of 2	Decreased in SIB and core symptoms of the autism spectrum in the emotional, social, and cognitive domains
Davis *et al*[36]	44, M	OCD, ASD, MDD, tics, epilepsy	OCD, aggression	Ventral capsule/ventral striatum	Y-BOCS, MADRS, YGTSS	Y-BOCS, MADRS and YGTSS scores decreased by 68%, 66%, and 75% respectively	The clinical improvements were maintained, albeit with fluctuations, after 3 yr. No effect on core symptoms of ASD
Graat *et al*[37]	39, F	OCD, ASD, Depressive episodes	OCD	vALIC	Y-BOCS 33, HAMD 27	Y-BOCS 12, HAMD 7	50% reduction of OCD symptoms following DBS, especially obsessions
	54, F	OCD, ASD	OCD	vALIC, then MFB	Y-BOCS 38, HAMD 30	Y-BOCS 18, HAMD 4	Initially did not benefit from DBS. Thereafter OCD symptoms improved and decreased by more than 50%
	32, M	OCD, ASD, ADHD	OCD, aggressive intrusions	vALIC	Y-BOCS 31, HAMD 18	Y-BOCS 23, HAMD 12	Partial responder probably due to several transient side effects of DBS
	31, F	OCD, ASD, DD, OCPD, AN	OCD	vALIC	Y-BOCS 34, HAMD 30	Y-BOCS 32, HAMD 27	Only some subjective improvements
	51, M	OCD, ASD	OCD	MFB	Y-BOCS 34, HAMD 5	Y-BOCS 0, HAMD 2	Obsessive-compulsive symptoms disappeared entirely. Improved confidence and less social

							shyness
	30, F	OCD, ASD, PDD, GAD, UPD	OCD,	MFB	Y-BOCS 34, HAMD 23	Y-BOCS 22, HAMD 22	35% reduction of OCD symptoms following DBS
Benedetti-Isaac *et al*[41]	27, M	ASD, TBI, epilepsy	Aggressive behavior towards self	PHyp	OAS 9	OAS 1	Improvements in seizures, in aggressive behavior, in quality of life, in daily living skills
	16, M	ASD, epilepsy, Developmental Delay	Self-aggression	PHyp	OAS 8	OAS 1	Aggressive behavior controlled for a month. After 2 mo it reappeared as before surgery

ABC: Antecedent, behavior, consequence; ADHD: Attention deficit hyperactive disorder; ALIC: Anterior limb of internal capsule; AN: Anorexia nervosa; ASD: Autism spectrum disorder; CGI-S: Clinical global impressions-severity; CY-BOCS: Children's Yale-Brown obsessive-compulsive scale; DBS: Deep brain stimulation; DD: Depressive disorder; F: Female; GAD: Generalized anxiety disorder; GAF: Global assessment of functioning; GPi: Globus Pallidus internus; HAMD: Hamilton depression scale; HAS: Hamilton anxiety scale; ID: Intellectual disability; JHMRS: Johns Hopkins motor stereotypy rating scale; K-ARS: Korea attention deficit hyperactive disorder rating scale; KS: Kleefstra syndrome; M: Male; MADRS: Montgomery-Asberg depression rating scale; MDD: Major depressive disorder; MFB: Medial forebrain bundle; NAc: Nucleus accumbens; NR: Not reported; OAS: Overt aggression scale; OCD: Obsessive-compulsive disorder; OCPD: Obsessive-compulsive personality disorder; PDD: Persistent depressive disorder; PHyp: Posterior hypothalamus; SCQ: Social communication questionnaire; SRS: Social responsiveness scale; TBI: Traumatic brain injury; TD: Tardive dyskinesia; UPD: Unspecified personality disorder; vALIC: Ventral anterior limb of the internal capsule; Y-BOCS: Yale-Brown obsessive-compulsive scale; YGTSS: Yale global tic severity scale.

Tardive dyskinesia (TD) is probably the most severe form of extrapyramidal symptoms (EPS) secondary to antipsychotic drugs, manifesting usually after months or years of therapy with involuntary choreiform movements and dystonia, frequently affecting the face and tongue[52]. While there are drug treatments for TD, it is often chronic and irreversible. Furthermore, patients with intellectual disabilities (ID) are more susceptible to EPS[53]. Past literature has shown encouraging evidence of DBS in the treatment of dystonic cerebral palsy in children[54]. GPi DBS in a young adult diagnosed with ASD and ID markedly improved TD symptoms[42]. The anxiety, restlessness, behavioral symptoms, and self-destructive behavior have ceased. Furthermore, the patient's skills, especially communication skills, have returned to the level before the presentation of aggressive seizures.

In 2013 Sturm *et al*[38] treated a 13-year-old boy with ASD and SIB with DBS in the amygdaloid complex and supra-amygdaloid projection system. The implantation of the electrodes in the two areas had been made necessary to testify that possible mechanical irritations, micro-lesions or inflammations in the projections of the amygdala were not effective in controlling the symptoms. Only stimulation of the basolateral nucleus of the amygdala proved effective in improving self-harm and core symptoms of ASD in the emotional, social, communicative, and cognitive domains in a 24-mo follow-up.

Davis *et al*[36] subjected a 44-year-old man with treatment-resistant OCD, major depressive disorder, ASD, and tics to DBS. DBS targets were represented by the ventral capsule and ventral striatum. After 3 years, the clinical improvements obtained within 6 mo of the surgery were maintained, albeit with fluctuations. Indeed, the scores on the Y-BOCS and the Montgomery-Asberg depression rating scale indicated that his symptoms were in the mild range, while the scores on the Yale global tic severity scale were much improved. On the other hand, as expected by the authors, full resolution of symptoms was never achieved and the patient continued to experience the clinical features of ASD.

In 2022, Graat *et al*[37] published the results of six patients with refractory OCD comorbid with ASD who underwent DBS of the vALIC or MFB. The efficacy of DBS on obsessive-compulsive and depressive symptoms was tested with the Y-BOCS and the Hamilton depression rating scale, respectively. Considering Y-BOCS scores, four patients were responders (> 35% decrease Y-BOCS), one patient was a partial responder (25%–35% decrease Y-BOCS) probably due to transient side effects of DBS, and one patient was a non-responder (< 25% decrease Y- BOCS), even though she had subjective symptom improvements.

After considering previously published studies[55,56] on the evidence of surgical treatment of the PHyp in aggressive drug-resistant behaviors, Benedetti-Isaac *et al*[41] published the results of PHyp DBS in 5 patients with DRE associated with intractable aggressive behavior. Only two patients among those recruited were also affected by ASD. A 27-year-old man with ID associated with severe autism, reported improvement in quality of life, better access to special education, and improvements in daily living activities. On the other hand, the aggressive behavior of a 16-year-old boy with ID and severe autism, was partially controlled for a month, but after 2 mo it reappeared as before surgery despite stimulation.

Only one study[37] reported adverse effects of DBS. One patient showed severe transient side effects: an infection of the DBS system that required removal of the system and, at a later stage, a suicide attempt (overdosed of quetiapine). Suicidality resolved without changing stimulation settings. Other

transient adverse effects were represented by restlessness, hypomania, tics, impulsivity, agitation, forgetfulness, cramp/joint pain, headache, memory complaints, agitation, hallucinations, and delusions.

CONCLUSION

The multiple comorbidities associated with ASD and the drug resistance in some patients lead to a decrease in the quality of life of patients and their family members or caregivers. To date, DBS has been used in people with autism solely to treat comorbid conditions. Despite encouraging results for the treatment of drug-resistant diseases, positive effects on core symptoms of ASD have only occasionally been reported. Finding new and innovative treatments is a fundamental aspect for those who take care of people with autism and comorbid conditions resistant to conventional treatments. Among the new therapeutic perspectives, as highlighted by the studies presented in this article, DBS could be a valid option to improve the management of disabling pathologies comorbid with autism and consequently the quality of life. However, further, and more in-depth research is needed in this field.

FOOTNOTES

Author contributions: Marini S and D'Agostino L wrote the article; Ciamarra C performed the research; Gentile A designed the research study. All authors have read and approved the final manuscript.

Conflict-of-interest statement: This paper was entirely funded by the authors, and no pharmaceutical companies were informed of or were involved in the paper. The authors have no potential conflicts of interest that are directly relevant to the contents of the paper.

Country/Territory of origin: Italy

ORCID number: Stefano Marini 0000-0002-1771-6940.

S-Editor: Zhang H
L-Editor: Filipodia
P-Editor: Zhang H

REFERENCES

1 **Speelman JDH**, Schuurman R. The history of deep brain stimulation. In: Temel Y, Leentjens A, de Bie R, Chabardes S, Fasano A. Fundamentals and clinics of deep brain stimulation. Cham: Springer, 2020: 3-13
2 **Benabid AL**, Chabardes S, Torres N, Piallat B, Krack P, Fraix V, Pollak P. Functional neurosurgery for movement disorders: a historical perspective. *Prog Brain Res* 2009; **175**: 379-391 [PMID: 19660668 DOI: 10.1016/S0079-6123(09)17525-8]
3 **Hariz MI**, Blomstedt P, Zrinzo L. Deep brain stimulation between 1947 and 1987: the untold story. *Neurosurg Focus* 2010; **29**: E1 [PMID: 20672911 DOI: 10.3171/2010.4.FOCUS10106]
4 **Aum DJ**, Tierney TS. Deep brain stimulation: foundations and future trends. *Front Biosci (Landmark Ed)* 2018; **23**: 162-182 [PMID: 28930542 DOI: 10.2741/4586]
5 **Nuttin B**, Cosyns P, Demeulemeester H, Gybels J, Meyerson B. Electrical stimulation in anterior limbs of internal capsules in patients with obsessive-compulsive disorder. *Lancet* 1999; **354**: 1526 [PMID: 10551504 DOI: 10.1016/S0140-6736(99)02376-4]
6 **Martinho FP**, Duarte GS, Couto FSD. Efficacy, Effect on Mood Symptoms, and Safety of Deep Brain Stimulation in Refractory Obsessive-Compulsive Disorder: A Systematic Review and Meta-Analysis. *J Clin Psychiatry* 2020; **81** [PMID: 32459406 DOI: 10.4088/JCP.19r12821]
7 **Macerollo A**, Deuschl G. Deep brain stimulation for tardive syndromes: Systematic review and meta-analysis. *J Neurol Sci* 2018; **389**: 55-60 [PMID: 29433807 DOI: 10.1016/j.jns.2018.02.013]
8 **Zhou C**, Zhang H, Qin Y, Tian T, Xu B, Chen J, Zhou X, Zeng L, Fang L, Qi X, Lian B, Wang H, Hu Z, Xie P. A systematic review and meta-analysis of deep brain stimulation in treatment-resistant depression. *Prog Neuropsychopharmacol Biol Psychiatry* 2018; **82**: 224-232 [PMID: 29146474 DOI: 10.1016/j.pnpbp.2017.11.012]
9 **Zhang H**, Wang N, Yu L, Zhao M. Efficacy and feasibility of deep brain stimulation for patients with depression: A protocol for systematic review and meta-analysis. *Medicine (Baltimore)* 2021; **100**: e26044 [PMID: 34011116 DOI:

10.1097/MD.0000000000026044]
10 **Hung YY**, Yang LH, Stubbs B, Li DJ, Tseng PT, Yeh TC, Chen TY, Liang CS, Chu CS. Efficacy and tolerability of deep transcranial magnetic stimulation for treatment-resistant depression: A systematic review and meta-analysis. *Prog Neuropsychopharmacol Biol Psychiatry* 2020; **99**: 109850 [PMID: 31863873 DOI: 10.1016/j.pnpbp.2019.109850]
11 **Servello D**, Zekaj E, Saleh C, Zanaboni Dina C, Porta M. Sixteen years of deep brain stimulation in Tourette's Syndrome: a critical review. *J Neurosurg Sci* 2016; **60**: 218-229 [PMID: 26788742]
12 **Karaszewska D**, Cleintuar P, Oudijn M, Lok A, van Elburg A, Denys D, Mocking R. Efficacy and safety of deep brain stimulation for treatment-refractory anorexia nervosa: a systematic review and meta-analysis. *Transl Psychiatry* 2022; **12**: 333 [PMID: 35970847 DOI: 10.1038/s41398-022-02102-w]
13 **American Psychiatric Association**. Diagnostic and statistical manual of mental disorders. 5th ed. Washington DC: American Psychiatric Publishing, 2013
14 **Lord C**, Elsabbagh M, Baird G, Veenstra-Vanderweele J. Autism spectrum disorder. *Lancet* 2018; **392**: 508-520 [PMID: 30078460 DOI: 10.1016/S0140-6736(18)31129-2]
15 **Kano Y**, Ohta M, Nagai Y, Pauls DL, Leckman JF. Obsessive-compulsive symptoms in parents of Tourette syndrome probands and autism spectrum disorder probands. *Psychiatry Clin Neurosci* 2004; **58**: 348-352 [PMID: 15298645 DOI: 10.1111/j.1440-1819.2004.01266.x]
16 **Gorbis E**. Treatments for obsessive-compulsive disorder comorbid with autism spectrum disorder. Boston: International OCD Foundation, 2011
17 **Tate BG**, Baroff GS. Aversive control of self-injurious behavior in a psychotic boy. *Behav Res Ther* 1966; **4**: 281-287 [PMID: 5978683 DOI: 10.1016/0005-7967(66)90024-6]
18 **Steenfeldt-Kristensen C**, Jones CA, Richards C. The Prevalence of Self-injurious Behaviour in Autism: A Meta-analytic Study. *J Autism Dev Disord* 2020; **50**: 3857-3873 [PMID: 32297123 DOI: 10.1007/s10803-020-04443-1]
19 **Le JF**, Lohr WD. Aggression and self-injury in a patient with severe autism. *Pediatr Ann* 2012; **41**: 1-3 [PMID: 23052137 DOI: 10.3928/00904481-20120924-13]
20 **Adler BA**, Wink LK, Early M, Shaffer R, Minshawi N, McDougle CJ, Erickson CA. Drug-refractory aggression, self-injurious behavior, and severe tantrums in autism spectrum disorders: a chart review study. *Autism* 2015; **19**: 102-106 [PMID: 24571823 DOI: 10.1177/1362361314524641]
21 **Bradley V**, Hiersteiner D, Rotholz D, Maloney J, Li H, Bonardi A, Bershadsky J. Personal characteristics and outcomes of individuals with developmental disabilities who need support for self-injurious behaviour. *J Intellect Disabil Res* 2018; **62**: 1043-1057 [PMID: 30022570 DOI: 10.1111/jir.12518]
22 **Beavers GA**, Iwata BA, Lerman DC. Thirty years of research on the functional analysis of problem behavior. *J Appl Behav Anal* 2013; **46**: 1-21 [PMID: 24114081 DOI: 10.1002/jaba.30]
23 **Leblanc LA**, Patel MR, Carr JE. Recent advances in the assessment of aberrant behavior maintained by automatic reinforcement in individuals with developmental disabilities. *J Behav Ther Exp Psychiatry* 2000; **31**: 137-154 [PMID: 11132117 DOI: 10.1016/s0005-7916(00)00017-3]
24 **Hagopian LP**, Rooker GW, Zarcone JR. Delineating subtypes of self-injurious behavior maintained by automatic reinforcement. *J Appl Behav Anal* 2015; **48**: 523-543 [PMID: 26223959 DOI: 10.1002/jaba.236]
25 **Hagopian LP**, Rooker GW, Zarcone JR, Bonner AC, Arevalo AR. Further analysis of subtypes of automatically reinforced SIB: A replication and quantitative analysis of published datasets. *J Appl Behav Anal* 2017; **50**: 48-66 [PMID: 28032344 DOI: 10.1002/jaba.368]
26 **Oliver C**, Murphy G, Hall S, Arron K, Leggett J. Phenomenology of self-restraint. *Am J Ment Retard* 2003; **108**: 71-81 [PMID: 12564940 DOI: 10.1352/0895-8017(2003)108<0071:POSR>2.0.CO;2]
27 **Malone RP**, Waheed A. The role of antipsychotics in the management of behavioural symptoms in children and adolescents with autism. *Drugs* 2009; **69**: 535-548 [PMID: 19368416 DOI: 10.2165/00003495-200969050-00003]
28 **Sabus A**, Feinstein J, Romani P, Goldson E, Blackmer A. Management of Self-injurious Behaviors in Children with Neurodevelopmental Disorders: A Pharmacotherapy Overview. *Pharmacotherapy* 2019; **39**: 645-664 [PMID: 30793794 DOI: 10.1002/phar.2238]
29 **Ricketts RW**, Goza AB, Ellis CR, Singh YN, Singh NN, Cooke JC 3rd. Fluoxetine treatment of severe self-injury in young adults with mental retardation. *J Am Acad Child Adolesc Psychiatry* 1993; **32**: 865-869 [PMID: 8340311 DOI: 10.1097/00004583-199307000-00024]
30 **McCracken JT**, McGough J, Shah B, Cronin P, Hong D, Aman MG, Arnold LE, Lindsay R, Nash P, Hollway J, McDougle CJ, Posey D, Swiezy N, Kohn A, Scahill L, Martin A, Koenig K, Volkmar F, Carroll D, Lancor A, Tierney E, Ghuman J, Gonzalez NM, Grados M, Vitiello B, Ritz L, Davies M, Robinson J, McMahon D; Research Units on Pediatric Psychopharmacology Autism Network. Risperidone in children with autism and serious behavioral problems. *N Engl J Med* 2002; **347**: 314-321 [PMID: 12151468 DOI: 10.1056/NEJMoa013171]
31 **Greer BD**, Fisher WW, Saini V, Owen TM, Jones JK. Functional communication training during reinforcement schedule thinning: An analysis of 25 applications. *J Appl Behav Anal* 2016; **49**: 105-121 [PMID: 26482103 DOI: 10.1002/jaba.265]
32 **Richman DM**, Barnard-Brak L, Grubb L, Bosch A, Abby L. Meta-analysis of noncontingent reinforcement effects on problem behavior. *J Appl Behav Anal* 2015; **48**: 131-152 [PMID: 25754894 DOI: 10.1002/jaba.189]
33 **Richards C**, Oliver C, Nelson L, Moss J. Self-injurious behaviour in individuals with autism spectrum disorder and intellectual disability. *J Intellect Disabil Res* 2012; **56**: 476-489 [PMID: 22404122 DOI: 10.1111/j.1365-2788.2012.01537.x]
34 **Segar DJ**, Chodakiewitz YG, Torabi R, Cosgrove GR. Deep brain stimulation for the obsessive-compulsive and Tourette-like symptoms of Kleefstra syndrome. *Neurosurg Focus* 2015; **38**: E12 [PMID: 26030700 DOI: 10.3171/2015.3.FOCUS1528]
35 **Doshi PK**, Hegde A, Desai A. Nucleus Accumbens Deep Brain Stimulation for Obsessive-Compulsive Disorder and Aggression in an Autistic Patient: A Case Report and Hypothesis of the Role of Nucleus Accumbens in Autism and Comorbid Symptoms. *World Neurosurg* 2019; **125**: 387-391 [PMID: 30797934 DOI: 10.1016/j.wneu.2019.02.021]
36 **Davis RA**, Winston H, Gault JM, Kern DS, Mikulich-Gilbertson SK, Abosch A. Deep Brain Stimulation for OCD in a

Patient With Comorbidities: Epilepsy, Tics, Autism, and Major Depressive Disorder. *J Neuropsychiatry Clin Neurosci* 2021; **33**: 167-171 [PMID: 33535803 DOI: 10.1176/appi.neuropsych.20060153]

37 **Graat I**, Balke S, Prinssen J, de Koning P, Vulink N, Mocking R, van Rooijen G, Munckhof PVD, Schuurman R, Denys D. Effectiveness and safety of deep brain stimulation for patients with refractory obsessive compulsive disorder and comorbid autism spectrum disorder; A case series. *J Affect Disord* 2022; **299**: 492-497 [PMID: 34952108 DOI: 10.1016/j.jad.2021.12.089]

38 **Sturm V**, Fricke O, Bührle CP, Lenartz D, Maarouf M, Treuer H, Mai JK, Lehmkuhl G. DBS in the basolateral amygdala improves symptoms of autism and related self-injurious behavior: a case report and hypothesis on the pathogenesis of the disorder. *Front Hum Neurosci* 2012; **6**: 341 [PMID: 23346052 DOI: 10.3389/fnhum.2012.00341]

39 **Stocco A**, Baizabal-Carvallo JF. Deep brain stimulation for severe secondary stereotypies. *Parkinsonism Relat Disord* 2014; **20**: 1035-1036 [PMID: 25012696 DOI: 10.1016/j.parkreldis.2014.06.019]

40 **Park HR**, Kim IH, Kang H, Lee DS, Kim BN, Kim DG, Paek SH. Nucleus accumbens deep brain stimulation for a patient with self-injurious behavior and autism spectrum disorder: functional and structural changes of the brain: report of a case and review of literature. *Acta Neurochir (Wien)* 2017; **159**: 137-143 [PMID: 27807672 DOI: 10.1007/s00701-016-3002-2]

41 **Benedetti-Isaac JC**, Torres-Zambrano M, Vargas-Toscano A, Perea-Castro E, Alcalá-Cerra G, Furlanetti LL, Reithmeier T, Tierney TS, Anastasopoulos C, Fonoff ET, Contreras Lopez WO. Seizure frequency reduction after posteromedial hypothalamus deep brain stimulation in drug-resistant epilepsy associated with intractable aggressive behavior. *Epilepsia* 2015; **56**: 1152-1161 [PMID: 26146753 DOI: 10.1111/epi.13025]

42 **Kakko K**, Bjelogrlic-Laakso N, Pihlakoski L, Lehtimäki K, Järventausta K. Tardive Dyskinesia Should Not Be Overlooked. *J Child Adolesc Psychopharmacol* 2019; **29**: 72-74 [PMID: 30388034 DOI: 10.1089/cap.2018.0084]

43 **Yan H**, Siegel L, Breitbart S, Gorodetsky C, Fasano A, Rahim A, Loh A, Kulkarni AV, Ibrahim GM. An open-label prospective pilot trial of nucleus accumbens deep brain stimulation for children with autism spectrum disorder and severe, refractory self-injurious behavior: study protocol. *Pilot Feasibility Stud* 2022; **8**: 24 [PMID: 35109924 DOI: 10.1186/s40814-022-00988-3]

44 **Heiden P**, Weigel DT, Loução R, Hamisch C, Gündüz EM, Ruge MI, Kuhn J, Visser-Vandewalle V, Andrade P. Connectivity in deep brain stimulation for self-injurious behavior: multiple targets for a common network? *Front Hum Neurosci* 2022; **16**: 958247 [PMID: 36092644 DOI: 10.3389/fnhum.2022.958247]

45 **Torres CV**, Blasco G, Navas García M, Ezquiaga E, Pastor J, Vega-Zelaya L, Pulido Rivas P, Pérez Rodrigo S, Manzanares R. Deep brain stimulation for aggressiveness: long-term follow-up and tractography study of the stimulated brain areas. *J Neurosurg* 2020; 1-10 [PMID: 32032944 DOI: 10.3171/2019.11.JNS192608]

46 **Benedetti-Isaac JC**, Camargo L, Cardenas FP, Lo´pezd N. Effectiveness of deep brain stimulation in refractory and drug-resistant aggressiveness in autism spectrum disorder. *Res Autism Spectr Disord* 2023; 102131 [DOI: 10.1016/j.rasd.2023.102131]

47 **Howes OD**, Thase ME, Pillinger T. Treatment resistance in psychiatry: state of the art and new directions. *Mol Psychiatry* 2022; **27**: 58-72 [PMID: 34257409 DOI: 10.1038/s41380-021-01200-3]

48 **Nuttin BJ**, Gabriëls LA, Cosyns PR, Meyerson BA, Andréewitch S, Sunaert SG, Maes AF, Dupont PJ, Gybels JM, Gielen F, Demeulemeester HG. Long-term electrical capsular stimulation in patients with obsessive-compulsive disorder. *Neurosurgery* 2003; **52**: 1263-72; discussion 1272 [PMID: 12762871 DOI: 10.1227/01.neu.0000064565.49299.9a]

49 **Denys D**, Mantione M, Figee M, van den Munckhof P, Koerselman F, Westenberg H, Bosch A, Schuurman R. Deep brain stimulation of the nucleus accumbens for treatment-refractory obsessive-compulsive disorder. *Arch Gen Psychiatry* 2010; **67**: 1061-1068 [PMID: 20921122 DOI: 10.1001/archgenpsychiatry.2010.122]

50 **Harat M**, Rudaś M, Zieliński P, Birska J, Sokal P. Deep Brain Stimulation in Pathological Aggression. *Stereotact Funct Neurosurg* 2015; **93**: 310-315 [PMID: 26227081 DOI: 10.1159/000431373]

51 **Dichter GS**, Richey JA, Rittenberg AM, Sabatino A, Bodfish JW. Reward circuitry function in autism during face anticipation and outcomes. *J Autism Dev Disord* 2012; **42**: 147-160 [PMID: 22187105 DOI: 10.1007/s10803-011-1221-1]

52 **Lerner PP**, Miodownik C, Lerner V. Tardive dyskinesia (syndrome): Current concept and modern approaches to its management. *Psychiatry Clin Neurosci* 2015; **69**: 321-334 [PMID: 25556809 DOI: 10.1111/pcn.12270]

53 **Sheehan R**, Horsfall L, Strydom A, Osborn D, Walters K, Hassiotis A. Movement side effects of antipsychotic drugs in adults with and without intellectual disability: UK population-based cohort study. *BMJ Open* 2017; **7**: e017406 [PMID: 28775195 DOI: 10.1136/bmjopen-2017-017406]

54 **Elia AE**, Bagella CF, Ferré F, Zorzi G, Calandrella D, Romito LM. Deep brain stimulation for dystonia due to cerebral palsy: A review. *Eur J Paediatr Neurol* 2018; **22**: 308-315 [PMID: 29396170 DOI: 10.1016/j.ejpn.2017.12.002]

55 **Jiménez F**, Soto JE, Velasco F, Andrade P, Bustamante JJ, Gómez P, Ramírez Y, Carrillo-Ruiz JD. Bilateral cingulotomy and anterior capsulotomy applied to patients with aggressiveness. *Stereotact Funct Neurosurg* 2012; **90**: 151-160 [PMID: 22508170 DOI: 10.1159/000336746]

56 **Sano K**, Mayanagi Y, Sekino H, Ogashiwa M, Ishijima B. Results of stimulation and destruction of the posterior hypothalamus in man. *J Neurosurg* 1970; **33**: 689-707 [PMID: 5488801 DOI: 10.3171/jns.1970.33.6.0689]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com

0026-895X/88/050605-09$02.00/0

MOLECULAR PHARMACOLOGY, 34:605-613

ACCELERATED COMMUNICATION

Determination and Characterization of a Cannabinoid Receptor in Rat Brain

WILLIAM A. DEVANE, FRANCIS A. DYSARZ III, M. ROSS JOHNSON,[1] LAWRENCE S. MELVIN, and ALLYN C. HOWLETT

Department of Pharmacology, St. Louis University Medical School, St. Louis, Missouri 63104 (W.A.D., F.A.D. and A.C.H.) and Pfizer Central Research, Groton, Connecticut 06340 (M.R.J. and L.S.M.)

Received August 4, 1988; Accepted August 30, 1988

SUMMARY

The determination and characterization of a cannabinoid receptor from brain are reported. A biologically active bicyclic cannabinoid analgetic CP-55,940 was tritium-labeled to high specific activity. Conditions for binding to rat brain P_2 membranes and synaptosomes were established. The pH optimum was between 7 and 8, and specific binding could be eliminated by heating the membranes to 60°. Binding to the P_2 membranes was linear within the range of 10 to 50 μg of protein/ml. Specific binding (defined as total binding displaced by 1 μM Δ^9-tetrahydrocannabinol (Δ^9-THC) or 100 nM desacetyllevonantradol) was saturable. The K_d determined from Scatchard analysis was 133 pM, and the B_{max} for rat cortical P_2 membranes was 1.85 pmol/mg of protein. The Hill coefficient for [^{3}H]CP-55,940 approximated 1, indicating that, under the conditions of assay, a single class of binding sites was determined that did not exhibit cooperativity. The binding was rapid ($k_{on} \approx 2.6 \times 10^{-4}$ pM^{-1} min^{-1}) and reversible ($\kappa_{off} \approx 0.016$ min^{-1}) and ($k_{off}' > 0.06$ min^{-1}). The two K_d values estimated from the kinetic constants approximately 55 pM and exceeded 200 pM, respectively. The binding of the agonist ligand [^{3}H]CP-55,940 was decreased by the nonhydrolyzable GTP analog guanylylimidodiphosphate. The guanine nucleotide induced a more rapid dissociation of the ligand from the binding site, consistent with an allosteric regulation of the putative receptor by a G protein. The binding was also sensitive to $MgCl_2$ and $CaCl_2$. Binding of [^{3}H]CP-55,940 was displaced by cannabinoid drugs in the following order of potency: CP-55,940 ≥ desacetyllevonantradol > 11-OH-Δ^9-THC = Δ^9-THC > cannabinol. Cannabidiol and cannabigerol displaced [^{3}H]CP-55,940 by less than 50% at 1 μM concentrations. The (−)-isomer of CP-55,940 displaced with 50-fold greater potency than the (+)-isomer. This pharmacology is comparable to both the inhibition of adenylate cyclase *in vitro* and the analgetic activity of these compounds *in vivo*. The criteria for a high affinity, stereoselective, pharmacologically distinct cannabinoid receptor in brain tissue have been fulfilled.

Various preparations of *Cannabis sativa* (marihuana) have traditionally been used therapeutically and for their psychological manifestations [see reviews by Hollister (1) and Dewey (2)]. Δ^9-THC is the major compound in extracts of cannabis to have effects on the CNS (3). The predominant CNS responses to Δ^9-THC include analgesia and antiemesis, as well as a "psychological high," drowsiness, alterations in cognition and memory, and a decrement in psychomotor performance in humans (1, 2). Animal behavioral patterns associated with cannabinoid drug actions include altered behavior in monkeys, a characteristic static ataxia in dogs, and hypothermia, analgesia, a typical cannabinoid immobility, and a biphasic change in spontaneous locomotor activity in rodents (3). Although extensive structure-activity relationships have been studied in humans and in these animal models (3), the actions of cannabinoid drugs in the brain remain poorly understood. At the present time, very little is known concerning the neuroanatomical location of cells responsive to cannabinoid drugs, the classical neurotransmitter pathways that may interact with cannabinoceptive cells, or the effects that cannabinoid drugs have on neurons in the CNS.

One reason for our lack of insight concerning the actions of cannabinoid drugs in the CNS is that a clearly defined cellular mechanism(s) for this class of drugs has remained elusive [see Ref. 4 for a thorough evaluation). Our recent studies have overcome this obstacle by demonstrating that the centrally active cannabinoid drugs inhibit adenylate cyclase activity in a model neuronal system (5, 6). The ability of cannabinoid drugs

This work was supported in part by National Institutes of Health Grant R01-DA03690 and Research Career Development Award K04-NS00868 to A.C.H. W.A D. was supported by a Neuropharmacology Training Grant T32-NS07254. F.A.D. was supported by a Medical Student Summer Fellowship from St. Louis University Medical School.

[1] Present Address: Glaxo, Inc., 5 Moore Drive, Research Triangle Park, NC 27709.

ABBREVIATIONS: THC, tetrahydrocannabinol; DALN, desacetyllevonantradol; Gpp(NH)p, guanyl (β,γ)-imidodiphosphate; CNS, central nervous system; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

to regulate adenylate cyclase was determined to be related to the ability of these compounds to produce CNS effects in humans and animal models (7, 8). The response could be produced at submicromolar concentrations (7, 8) and thus would be consistent with drug levels that might be expected to be present in the brain during peak activity (9–11). Using a series of cannabinoid compounds, developed at Pfizer Central Research for their analgetic activity (12), enantioselectivity was demonstrated for the inhibition of adenylate cyclase that paralleled the isomeric selectivity exhibited in analgetic tests in animals (8). The inhibition of adenylate cyclase occurred only in certain cell types (13, 14), arguing that the effect on adenylate cyclase was not a universal phenomenon such as would be expected of cannabinoid-induced membrane fluidity changes. Further studies clearly demonstrated the requirement for G_i (6, 13), a guanine nucleotide regulatory protein that mediates the responses of hormone receptors to ultimately decrease adenylate cyclase activity. The evidence accrued from these studies strongly suggested the presence of pharmacologically unique cannabinoid receptors on the cultured neuronal cells. Logically, neurons in the CNS should also possess cannabinoid receptors.

The tools to search for a cannabinoid receptor in the brain were not available until recently. The relatively low potency and tendency to partition into biological membranes suggest that Δ^9-THC is a poor candidate for a radiolabeled ligand for the detection and characterization of cannabinoid receptors. Δ^9-THC was able to inhibit adenylate cyclase with a K_{inh} of 430 nM (7). It might be expected that radioactively labeled Δ^9-THC would have an affinity for cannabinoid receptors in the nanomolar range and would bind to receptors estimated to be present in the brain in the range of fmoles per milligram of tissue. Reports of the membrane/buffer partition coefficient for Δ^9-THC have ranged from 400 (15) to 12,500 (16). It can be calculated that the amount of labeled Δ^9-THC binding to receptors could potentially be 5 or 6 orders of magnitude smaller than the amount that would be expected to partition into membranes.

A collaborative interaction between our laboratories has allowed the investigation of cannabinoid receptors in the brain using a highly potent analgetic bicyclic cannabinoid compound, CP-55,940 (8, 12). This structure is one of a series of compounds that conform to a postulated three-point agonist-receptor interaction model proposed for the cannabinoid association with the CNS receptor that mediates analgesia (12). The important functional groups for agonist-receptor interaction were proposed to be 1) the C-ring hydroxyl, 2) the phenolic A-ring hydroxyl, and 3) the A-ring alkyl side chain. These same functional groups were found to be required for the inhibition of adenylate cyclase *in vitro* (8). The regulation of adenylate cyclase by CP-55,940 was found to exhibit a K_{inh} of 25 nM, and the (−)-isomer was found to be 200-fold more potent than the poorly analgetic (+)-isomer (8). The high affinity and enantioselectivity exhibited by CP-55,940 made it a potentially useful radioligand for binding studies to characterize the cannabinoid receptor. The studies reported here describe the binding site for [^{3}H]CP-55,940 and provide convincing evidence that this binding site is the elusive cannabinoid receptor.

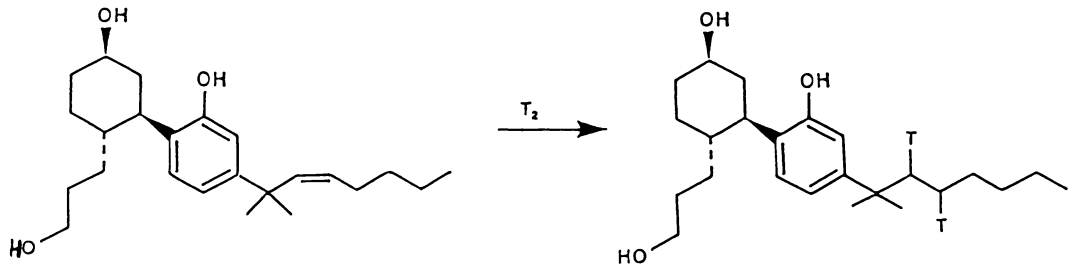
Experimental Procedures

Materials. The natural cannabinoid drugs were provided by the National Institute on Drug Abuse. DALN and the isomers of CP-55,940 were synthesized at Pfizer Central Research. Cannabinoid drugs were stored as 10 mM stock solutions in absolute ethanol at −20°. Drugs were initially diluted to 20 μM in 9.4 mg/ml fatty acid-deficient bovine serum albumin using Regisil-treated glassware. All subsequent dilutions were made into a vehicle containing 5 mg/ml bovine serum albumin.

[^{3}H]CP-55,940 was radiolabeled at DuPont NEN by tritium reduction, in the presence of a Pd catalyst, of a double bond between carbons 2 and 3 of the A-ring alkyl side chain (Fig. 1). Labile tritium was removed by several washes with methanol. Product was purified by high performance liquid chromatography on a 25-cm Zorbax ODS column using the solvent system CH_3CN/25 mM NaH_2PO_4, pH 4.3 (65:35). Purified material was judged by high performance liquid chromatography to be greater than 97% chemically pure. The specific activity was determined to be 93.4 Ci/mmol, using the UV absorbance to quantitate the yield of product. Tritium exchange with labile hydrogens probably accounts for the labeling in excess of the theoretical specific activity. [^{3}H]CP-55,940 was stored at 1 mCi/ml in ethanol at −80° for long term storage and at −20° for routine usage. Purity of the stored material was monitored by thin layer chromatography on silica gel GHLF plates using the solvent system ether/isopropanol (98:2). Biological activity of the radioligand was also monitored. [^{3}H]CP-55,940 was able to inhibit the adenylate cyclase activity of N18TG2 membranes in a dose-dependent manner, using the protocol previously described (6) (data not shown).

Membrane preparations. Male Sprague-Dawley rats weighing 250 to 370 g were decapitated, and the brains were rapidly removed and dissected on ice. Unless indicated, all results reported were obtained with a washed P_2 preparation prepared as follows. The entire cortices of two or three rats were homogenized with a Dounce glass homogenizer in 45 ml of a solution consisting of 320 mM sucrose, 2 mM Tris·EDTA, and 5 mM $MgCl_2$. The homogenate was centrifuged at 1600 × *g* for 10 min. The supernatant was saved, and the pellets were washed twice as above. The combined supernatant fractions were then centrifuged at 39,000 × *g* for 15 min. The pellet was resuspended in 90 ml of buffer A (50 mM Tris·HCl, pH 7.0 at 30°, 2 mM Tris·EDTA, 5 mM $MgCl_2$), incubated at 37° for 10 min, and centrifuged at 23,000 × *g* for 10 min. The membranes were resuspended in buffer A, incubated at 30° for 40 min, and centrifuged at 11,000 × *g* for 15 min. These two washing steps were found to be important for observing a single homogeneous binding site in equilibrium studies (see Results). The final pellet was resuspended in buffer B (50 mM Tris·HCl, pH 7.4 at 30°, 1 mM Tris·EDTA, 3 mM $MgCl_2$) at a protein concentration of 4 to 5 mg/ml and stored at −80°. Storage for up to 6 weeks had no noticeable effect on binding. Protein values were determined by the method of Bradford (17) using bovine γ-globulin as the standard. Some of the studies were also performed using a synaptosomal preparation derived from the hippocampus plus prefrontal cortex of the rat. The synaptosomal preparation was made following the protocol of Dodd *et al.* (18) with several modifications. The initial homogenization was performed using a 50-ml Dounce glass homogenizer and the homogenate was centrifuged in 50-ml tubes at 1600 × *g* for 10 min. The differential sedimentations over 1.2M and 0.8M sucrose were performed at 50,000 rpm in a Beckman Ti50 rotor for 12 min. The final synaptosomal pellet was resuspended to 5 mg/ml protein in a buffer containing 25 mM Tris·HCl, pH 7.4, 1 mM Tris·EDTA, and 16.6 mM sucrose and was stored at −80°.

Ligand binding assays. Ligand binding assays were performed in Regisil-treated test tubes in a volume of 1 ml containing buffer B, radioligand, and cannabinoid drugs as specified. The incubation was started by the addition of 20 to 50 μg of membrane protein. With the exception of the kinetic experiments, all reactions were carried out at 30° for 50 min. After incubation, the samples were transferred to 1.5-ml polypropylene microfuge tubes and immediately centrifuged for 9 min at 13,000 × *g*. After centrifugation, the supernatant was aspirated and counted to determine the concentration of free [^{3}H]CP-55,940. The microfuge tubes were drained on drying pins for 30 min, after



1

$^3H_2$ -CP-55,940

Fig. 1. The synthesis of [^{3}H] CP-55,940 by the tritium reduction of compound 1.

which the tips of the tubes were sliced, using a heated spatula and a cutting block designed to ensure that the cut tips were of identical size. The tips were then placed in scintillation vials and submersed in 2 ml of a solubilizing solution (5% ethanol, 5% Triton X100, 0.2 N NaOH). The vials were then shaken for at least 4 hr in order to dissolve the pelleted membranes. Scintillation cocktail (10 ml) was added to each vial and the radioactivity was determined using a Beckman LS1800 with an efficiency for tritium of 30%. Nonspecific binding to the microfuge tip was assessed in control tubes having radioligand but no protein (typically 2% to 3% of the total radioactivity available in the incubation medium). Subtracting this value from total binding gave the total binding in the pelleted membranes. Specific binding was defined as the difference between total binding to the membranes in the absence and presence of either 100 nM DALN or 1 μM Δ^9-THC. Nonspecific binding to the membranes was typically 15% to 30% of the total binding to the membranes, dependent upon the membrane and ligand concentrations (see Fig. 2). Assays were carried out in triplicate with an average coefficient of variation for the samples of 2.5%, and experiments were repeated at least three times.

Metabolism of [^{3}H]CP-55,940. To determine whether [^{3}H]CP-55,940 was metabolized during the binding assay, 40 μg of membrane protein were incubated with 70 pM [^{3}H]CP-55,940 for 90 min at 30° in the standard assay buffer described above. After centrifugation, the supernatant and the pellet were separated and the radioactivity was extracted from each using ether. After drying with N_2 gas, the samples and an unincubated [^{3}H]CP-55,940 control were resuspended in absolute ethanol, and thin layer chromatography was performed using the procedure described above. The plate was sprayed with EN^{3}HANCE (NEN, Boston, MA), placed against Kodak X-Omat film and stored at −80° for 6 days. Upon developing, a single band was observed for the samples which comigrated with the control. These studies demonstrated that neither the free nor bound [^{3}H]CP-55,940 was metabolized or chemically modified during the assay procedure.

Results

Conditions for cannabinoid receptor binding. Initial studies addressed the separation of unbound ligand from bound radiolabeled ligand. In agreement with the experience of Roth and Williams (16) and Harris *et al.* (19) using THC as the labeled ligand, separation of free [^{3}H]CP-55,940 using a filtration technique met with little success. The binding of ligand to glass fiber and cellulose nitrate or cellulose acetate filters was excessive and varied with the concentration of radioligand added. Treatment of the filters with various organic solvents, detergents, polyethyleneimine, or bovine serum albumin did not provide acceptable conditions for separation. Separating the free [^{3}H]CP-55,940 by adsorption onto dextran-coated charcoal was also unsuccessful (see also Ref. 19). Optimal conditions were achieved using the sedimentation procedure described above. The incubations were performed in Regisil-treated glass tubes in an effort to minimize the adsorption of cannabinoid compounds to the surface (20). Similarly, the presence of bovine serum albumin in the incubation mixture would also effectively decrease the amount of cannabinoid drug bound to the glassware (16). Any alterations in the free concentration of [^{3}H]CP-55,940 resulting from adherence to the glassware during the incubation were accounted for by determining the exact amount of radiolabeled ligand in the supernatant and adhering to the microfuge tube after the sedimentation for each assay.

To determine the optimal incubation conditions for binding, several different buffers were tried at various concentrations and pH values. Of the buffers tested, including K^+ HEPES, *K^+N-tris[Hydroxymethyl]methyl-2-aminoethanesulfonate, K^+ phosphate, and imidazole Cl^-, none performed any better than 50 mM Tris Cl^-. Optimal binding was observed between pH 7 and 8, with specific binding of only 60% of optimal at pH 6 or 9 (data not shown). The phenolic moiety of [^{3}H]CP-55,940 might be expected to have a pK in the vicinity of pH 10. The 40% loss of specific binding observed between pH 8 and 9 cannot be entirely accounted for by a modification of the ligand. The pH requirement can be postulated to also reflect the optimal pH for amino acids that would interact with the ligand or be required to maintain the optimal protein conformation.*

Under the experimental conditions used, linear binding extended from 10 to 50 μg/ml of protein for the P_2 membrane preparation (data not shown). Experiments were routinely conducted using 20 to 40 μg/ml P_2 protein, and in this range specific binding typically represented 85% of the total binding. Specific binding was linear through 120 to 150 μg/ml of protein for the synaptosomal preparation (data not shown). As the concentration of membranes was increased, there was a decline in the percentage of the total binding that could be described as specific.

Thermolability of the specific binding was demonstrated. Membrane preparations that were incubated at 60° for 12 min before assay failed to show significant specific binding (data not shown). These findings would be consistent with the binding of [^{3}H]CP-55,940 to a protein component of the membranes that is subject to thermal denaturation.

Characterization of the [^{3}H]CP-55,940 binding site. [^{3}H]CP-55,940 binding to cortical membranes was saturable, whereas nonspecific binding continued to increase with increasing concentrations of [^{3}H]CP-55,940 (Fig. 2A, *inset*). An example of a saturation binding isotherm and the Scatchard plot obtained therefrom are depicted in Fig. 2. A K_d of 133 ± 11 pM was obtained by Scatchard transformation (21) of the data from four experiments (mean ± standard error). The density of binding sites for the P_2 cortical preparation was 1.85 ± 0.26 pmol/mg of protein (four experiments). The data were analyzed by the Hill transformation (21), yielding a straight line (Fig.

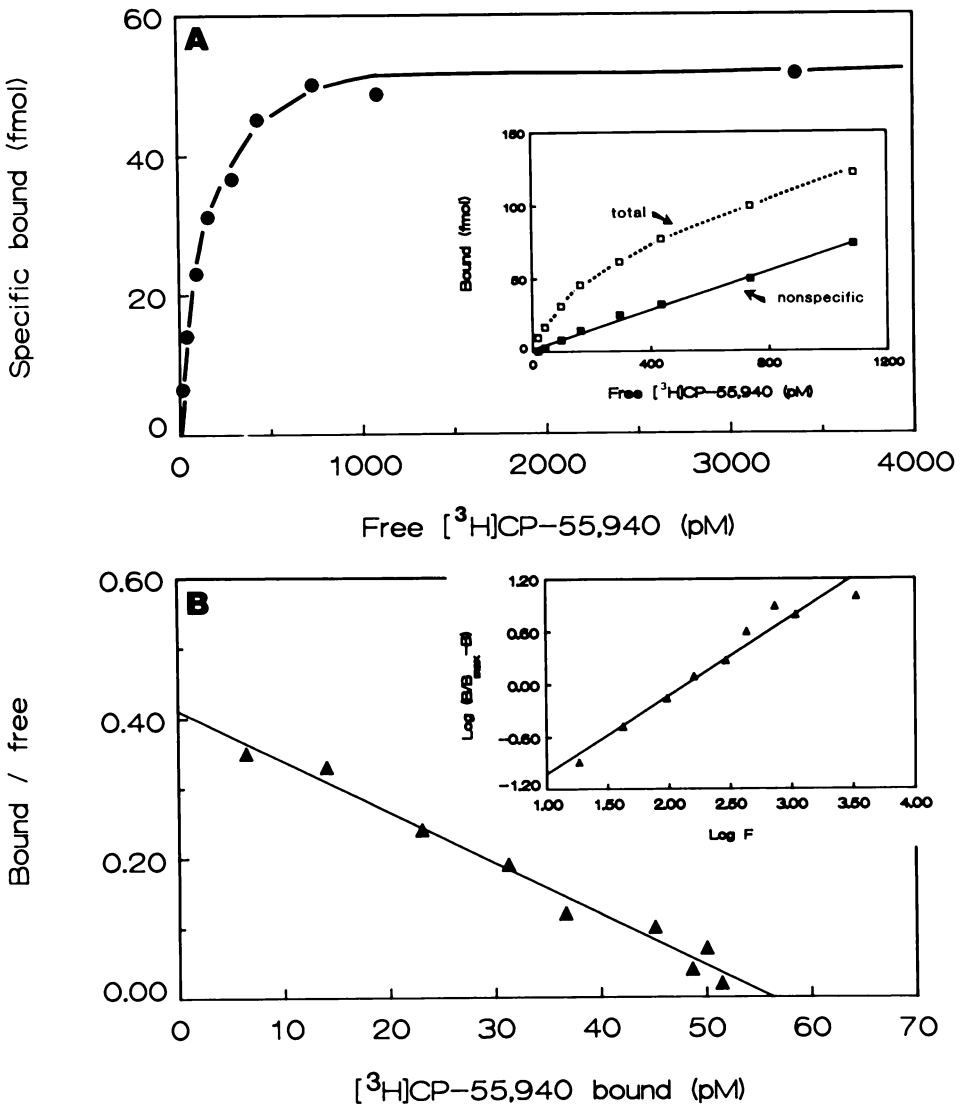


Fig. 2. Equilibrium binding of [^{3}H]CP-55,940. Membranes (43 μg of protein) were incubated with various concentrations of [^{3}H]CP-55,940. A, The saturation isotherm of specific binding. *Inset;* binding of [^{3}H]CP-55,940 in the absence (□) or presence (■) of 1 μM DALN. B, Scatchard transformation of [^{3}H]CP-55,940 binding data from A with the bound ligand being expressed in terms of concentration (pM). This experiment exhibited K_d and B_{max} values of 139 pM and 1.3 pmol/mg of protein, respectively. *Inset;* The Hill transformation of data from A. *F*; free drug concentration; *B*; specifically bound drug. The Hill coefficient (n_H) was calculated to be 0.90 for this experiment. The *lines* drawn represent the best fit as determined by least squares linear regression analysis.

2B, *inset*). The K_d derived from such analysis was 116 ± 12 pM and the n_H was 0.88 ± 0.08 (four experiments). The observation that the n_H approaches one suggests that a single class of binding sites is being labeled by [^{3}H]CP-55,940 under the assay conditions described and that no significant cooperativity exists among binding sites.

Kinetic analysis of the binding of [^{3}H]CP-55,940 to P_2 membranes indicates a rapid association of the ligand with the receptor (Fig. 3A). Equilibrium was reached rapidly, with greater than 90% of maximal specific binding attained within 50 min at 30°. The binding plateau remained stable for at least 2 hr. This is consistent with the determination that the radioligand is not being metabolized or chemically altered during the incubation (see Experimental Procedures). The nonspecific binding component reached steady state at the earliest time point measurable and underwent no further change through 3 hr.

The dissociation of the [^{3}H]CP-55,940-receptor complex initiated by the addition of 100 nM DALN is depicted in Fig. 3B. These studies were performed by establishing equilibrium directly in the microfuge tubes rather than by transferring the reaction mixture before sedimentation. Semilog plots suggest that dissociation was not monophasic (Fig. 3B). When the microfuge tubes were centrifuged immediately upon addition of 100 nM DALN, 20% of the specific binding at equilibrium was already displaced. It should be noted that the time for complete sedimentation is 10 min. However, one would expect that the major fraction of membranes would have sedimented within the first 3 min of centrifugation. Thus, the data obtained for the earliest time points depicted may represent the displacement occurring during the period of manipulation. Assuming first-order dissociation (21), the k_{-1} for the slower component was 0.016 ± 0.001 min^{-1} (three experiments) ($t_{1/2}$ = 45 min). A more rapid dissociation could also be discerned, having at $t_{1/2} \leq$ 11 min ($K_{-1} \geq 0.06$ min^{-1}). It is possible that these two kinetic states may represent interchangeable forms of the receptor. One mechanism for this may be the transient interaction of the receptor with G proteins either possessing tightly bound GDP or free of guanine nucleotides. Evidence for such an interaction is described below. It is of interest that two binding affinities were not discernible in the equilibrium binding studies. In preliminary studies using unwashed P_2 membranes, it was observed that multiple affinity states could be discerned in equilibrium binding studies (data not shown). An explanation

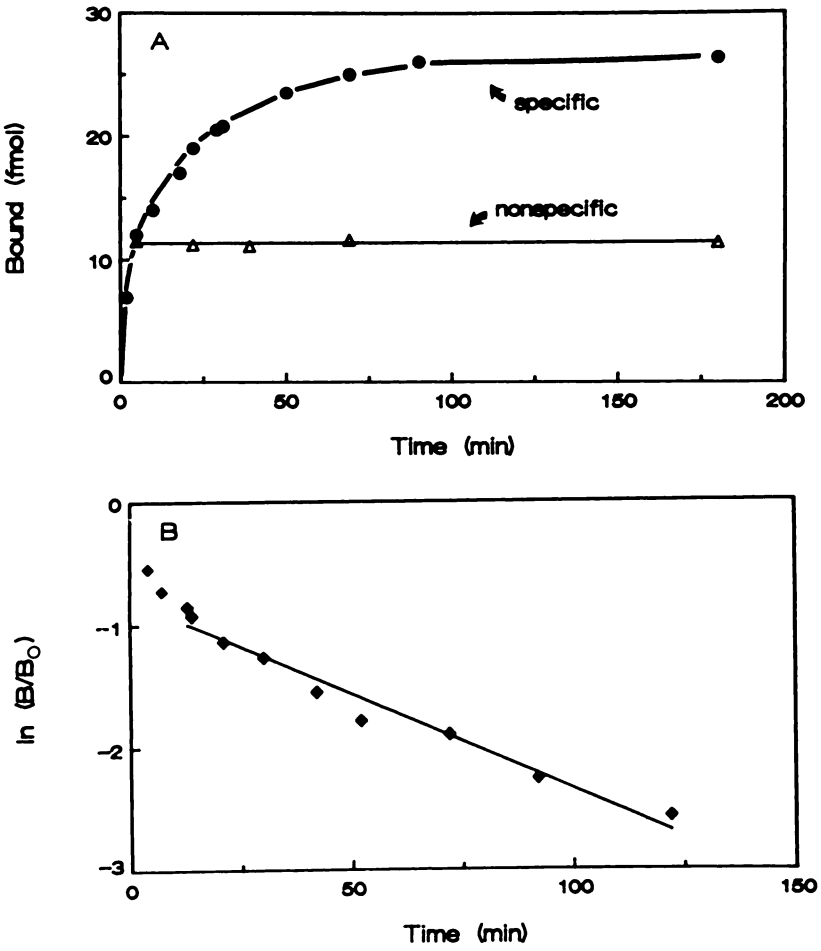


Fig. 3. Time course of association (A) and dissociation (B) of [^{3}H]CP-55,940. [^{3}H]CP-55,940 (81 pM) was incubated with 28 μg of P_2 membranes at 30°. A, The times indicated are those that elapsed between the addition of protein (start of the reaction) and the start of centrifugation of the microfuge tubes. Specific and nonspecific binding were determined with 100 nM DALN as described in Experimental Procedures. B, After equilibrium binding of [^{3}H]CP-55,940 had been reached (70 min), 100 nM DALN was added (t = 0) and dissociation was monitored. Data presented are a first-order representation with B/B_0 denoting the specific binding at the time indicated/specific binding at t = 0. The results are means of triplicate determinations from a single representative experiment, which was performed three times.

for these results might be that the population of G proteins possessing tightly bound GDP could be greater in unwashed membranes.

The K_d for binding may be calculated from the association and dissociation rates. The initial rate of association was estimated by assuming that pelleting of the membranes required 3 min and that the dissociation would not contribute appreciably to the reaction until after 5 min of incubation. The k_{+1} estimated from the initial rate of binding (22) was 3.4 ± 0.76 × 10^{-4} pM^{-1} min^{-1} (three experiments). Using this value, the K_d calculated for the slowly displacing site was 47 pM. The K_d for the rapidly dissociating site would have to exceed 180 pM. An alternative treatment of the data would be to estimate a k_{obs} as the reaction proceeds to equilibrium using a pseudo-first-order method (21, 22). Using the slower dissociation rate, the k_{+1} may be calculated to be 2.6 ± 0.2 × 10^{-4} pM^{-1} min^{-1} and the K_d would be 62 pM. Although both of these methods for estimating the k_{+1} (and thus the K_d values) have theoretical and methodological limitations, the kinetic estimates of the K_d are similar to the values calculated using linear transformation of the equilibrium binding data. Thus, an internal consistency for the methodology has been demonstrated.

Allosteric regulation of binding. One would hypothesize that a receptor that transmits its signal to the adenylate cyclase system via a G protein would be regulated by allosteric mechanisms similar to those that have been demonstrated for other functionally homologous receptors. Our current understanding of the influence of G proteins on agonist-receptor interactions has been reviewed by Birnbaumer and colleagues (23) and Casey and Gilman (24). To summarize briefly, G proteins are believed to exist in a heterotrimer form ($\alpha\beta\gamma$) possessing tightly bound GDP in the presence of Mg^{2+}. Upon interaction with a receptor-agonist complex, a conformational change confers sufficient energy to the system such that the GDP dissociates. In the absence of guanine nucleotides, the receptor-hormone-G protein intermediate complex exhibits a relatively high affinity for the agonist. Upon binding of GTP or a nonhydrolyzable analog to this complex, the affinity of the agonist ligand for the receptor is decreased, and the G protein dissociates from the receptor and separates into α and $\beta\gamma$ subunits. The effector (e.g., adenylate cyclase) interacts with the GTP-bound α subunit. Dissociation of the hormone from the receptor and hydrolysis of the GTP on the α subunit allow the system to perpetually respond to altered concentrations of hormone.

For the cannabinoid receptor, equilibrium binding studies indicated that the presence of 100 μM Gpp(NH)p resulted in a 40% decrease in specific binding of [^{3}H]CP-55,940 (data not shown). The kinetics of dissociation were analyzed in the presence or absence of guanine nucleotide (Fig. 4). The addition of Gpp(NH)p reduced the $t_{1/2}$ from 45 min to 12 min. The k_{-1} calculated for dissociation in the presence of Gpp(NH)p was 0.059 ± 0.009 min^{-1} (three experiments) and the K_d was 176 pM. This value is similar to the K_d estimated for the rapidly dissociating component described above. An in-depth analysis

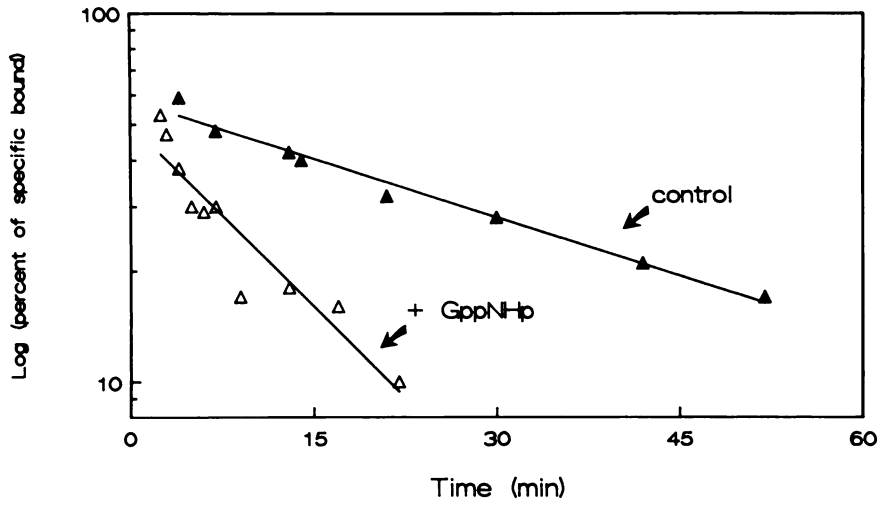


Fig. 4. Effect of Gpp(NH)p on the dissociation rate of [^{3}H]CP-55,940. [^{3}H]CP-55,940 (81 pM) was incubated with 28 μg of P_2 preparation membranes for 70 min at 30° and dissociation was monitored after addition of 100 nM DALN (t = 0). *Control*, addition of 100 nM DALN; *+Gpp(NH)p*, simultaneous addition of 100 nM DALN plus 100 μM Gpp(NH)p. The x axis indicates the time that elapsed between the addition of the above compounds and 2 min after the start of centrifugation. The y axis is a log-scale presentation of the percentage of specific binding at the indicated time/specific binding at t = 0. The data are the means of triplicate determinations from a single representative experiment, which was repeated three times.

of the interaction of the [^{3}H]CP-55,940 binding site with G proteins in various states is beyond the scope of this study. The most facile interpretation of the results presented here is that the [^{3}H]CP-55,940 binding site can be influenced by guanine nucleotides in a manner consistent with the interaction of a receptor with a G protein.

Divalent cations have been reported to influence the affinity of agonists for their receptors. It is believed that the G protein possesses at least one site for Mg^{2+} (23, 24). A role for this divalent cation has been shown for the dissociation of GDP in the presence of the receptor-hormone complex, in addition to other functions associated with a site having a much higher affinity for Mg^{2+} (23, 24). The effects of Mg^{2+} to increase the affinity of agonist ligands for receptors associated with adenylate cyclase have been discussed by Maguire (25). In the present investigation, concentrations of $MgCl_2$ as low as 1 mM stimulated specific binding of the agonist ligand [^{3}H]CP-55,940 by greater than 50% (Fig. 5). Qualitatively similar effects were observed with $CaCl_2$. $MnCl_2$ also stimulated specific binding in a manner similar to $MgCl_2$ (data not shown).

Studies of the opioid receptor, which is coupled to adenylate cyclase in an inhibitory manner, indicated that Na^+ may act as an allosteric regulator (26, 27). Na^+ has been demonstrated to decrease the affinity of agonist ligands for the opioid receptor (see Ref. 26 and references therein). In an effort to determine whether regulation of the binding of [^{3}H]CP-55,940 by monovalent cations could be observed, the effects of various chloride salts were determined (Fig. 5). Monovalent cations were tested at concentrations that might be expected to be present intracellularly or extracellularly. At 20 mM, Na^+ reduced specific binding by about 40%. Low concentrations of K^+ had minimal effects. Concentrations of 120 mM NaCl and 100 mM KCl inhibited specific binding by about 80%. The selectivity of this response to Na^+ does not appear to be great, suggesting that this inhibition may not be the result of a specific interaction with a Na^+ site. It is possible that a chaotropic effect of higher salt concentrations is altering the ability of the ligand to bind to the receptor.

Pharmacology of the cannabinoid receptor. The specificity of [^{3}H]CP-55,940 binding was established by determining the ability of related synthetic compounds and several natural cannabinoid compounds to compete with [^{3}H]CP-55,940 for occupancy of the specific binding sites in the cortical membranes (Fig. 6). Unlabeled CP-55,940 had a K_i of 68 ± 6.2 pM and B_{max} of 1.75 ± 0.20 pmol/mg protein (three experiments) as determined by computer analysis of homologous displacement data using the LIGAND program (version 2.3.11; May, 1987) (28). A one-site model fit the data better than a two-site model for both CP-55,940 and other cannabinoid drugs tested using an F test criterion on the residual variances at the level

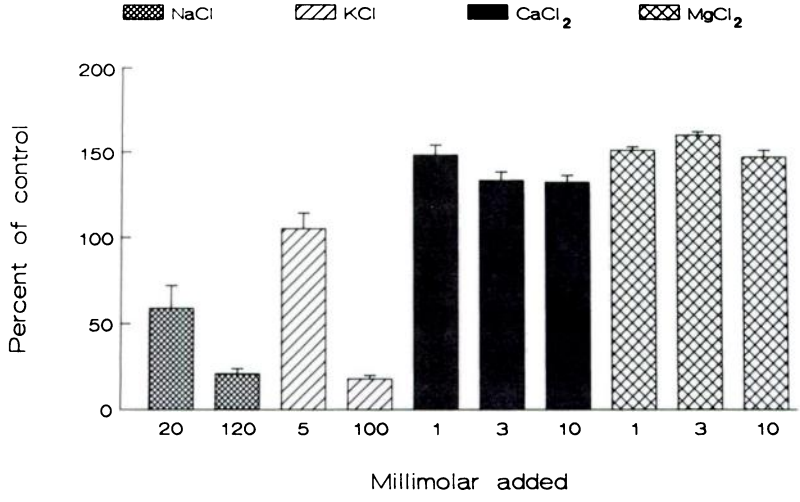


Fig. 5. The influence of cations on specific binding of [^{3}H] CP-55,940. Control samples contained 50 mM Tris·HCl, 1 mM Tris·EDTA, and 0.1 mM $MgCl_2$. Experimental samples contained the same buffer plus the indicated concentrations of salts. The data are the means ± standard error of triplicate determinations from a single representative experiment. All values were different from control at $p < 0.05$ except 20 mM NaCl and 5 mM KCl. Similar results were observed in two other experiments.

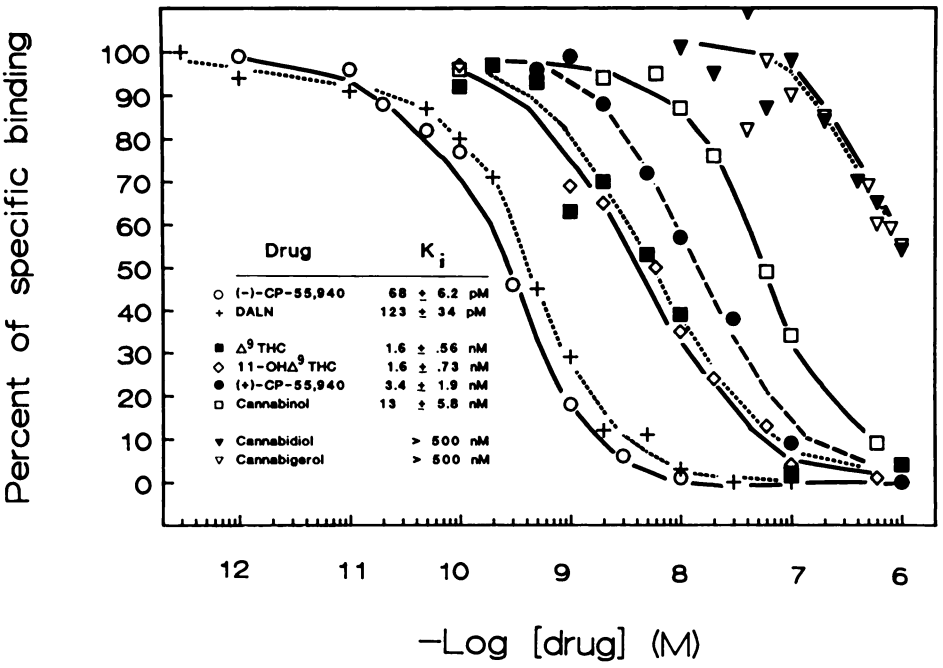


Fig. 6. Competitive inhibition of [^{3}H]CP-55,940 binding by various synthetic and natural cannabinoid drugs. [^{3}H]CP-55,940 (50–70 pM) was incubated with P_2 membranes (16–30 μg) for 50 min at 30° with either the indicated concentrations of drug or vehicle alone. The results were normalized to 100% of specific binding, which was determined with 100 nM DALN as described in Experimental Procedures. Data points represent the averages of triplicate determinations from single representative experiments. The K_i values listed in the *inset table* were determined using the LIGAND program and represent the mean ± standard error of three independent experiments for each drug.

of $p = 0.05$. Using 68 pM as the K_d for [^{3}H]CP-55,940, the K_i values of the various compounds were determined by LIGAND analysis of heterologous displacement data. The (+)-isomer of CP-55,940 was 50-fold less potent than the (−)-isomer, having a K_i of 3.4 nM. DALN was nearly equipotent with CP-55,940, having a K_i of 123 pM. Δ^9-THC and 11-OH-Δ^9-THC both exhibited high affinity binding with K_i values of 1.6 nM. Cannabinol was 8-fold less potent than Δ^9-THC, having a K_i of 13 nM. This order of potency generally parallels the order of potency for both CNS activity *in vivo* (1, 3, 12, 29) and inhibition of adenylate cyclase *in vitro* (7, 8).

Cannabidiol and cannabigerol were much less potent, binding the [^{3}H]CP-55,940 site with K_i values estimated to be greater than 500 nM. Cannabidiol and cannabigerol were unable to fully displace the specifically bound [^{3}H]CP-55,940 at the highest concentrations tested (1 μM). Concentrations greater than this were not tested due to the limited solubility of cannabinoid drugs above 1 μM and the confounding aspects of increasing the solvent or bovine serum albumin concentration, which would be necessary to maintain higher concentrations of cannabinoid compounds in solution. These latter two compounds fail to exhibit cannabinoid activity in humans or animal models (1–3). One of several explanations for the binding results could apply. 1) The two compounds could bind to the receptor with low affinity, but the concentrations required to observe a biological response may not be possible to achieve *in vivo*. 2) The cannabinoid response to these two compounds may be masked by drug effects at high concentrations, such as membrane perturbation in *in vitro* studies and CNS depression in *in vivo* studies. 3) The observed binding displacement may be the result of a contaminant in the drug preparation. The latter explanation is a possible artifact that must be dealt with in future investigations. These drugs were isolated from organic extracts of cannabis that originally contained a variety of cannabinoid compounds, including Δ^9-THC.

Discussion

Studies suggestive of a cannabinoid receptor were based on the ability of centrally active cannabinoid drugs to interact with a well characterized, cellular second messenger system. The ability of cannabinoid compounds to inhibit adenylate cyclase in a reversible, cell type-specific, potent, and enantioselective manner (5–8) would support the hypothesis that these compounds interact with a biological membrane-bound receptor. Additional arguments in favor of a receptor mediating the interaction of cannabinoid drugs with adenylate cyclase are the characteristic guanine nucleotide and divalent cation requirements for this interaction and the demonstrated pertussis toxin sensitivity characteristic of G_i-linked receptors (6, 13).

The findings presented in this study provide the strongest argument currently available for a cannabinoid receptor. The binding site described here is entirely consistent with a receptor that would be associated with a second messenger system via a G protein. The pH sensitivity and thermolability are consistent with a protein structure for this binding site. The rapid and reversible binding are properties expected of a neuromodulator receptor. The binding saturability and the B_{max} determined in the rat cortex are consistent with values reported for CNS neuromodulator receptors (30). The K_d for binding of [^{3}H]CP-55,940 derived from the kinetic constants agrees remarkably with the K_d obtained from equilibrium binding studies. The affinity determined for this agonist ligand is consistent with what would be expected for a neuromodulatory receptor in the CNS (30).

It may be hypothesized that the binding site for [^{3}H]CP-55,940 is linked to adenylate cyclase in the brain. Previous investigations of the inhibition of adenylate cyclase by cannabinoid drugs have utilized a cloned neuroblastoma cell model system. To strengthen the putative association of the cannabinoid receptor with adenylate cyclase in the CNS, we now have evidence using brain slice preparations. Cyclic AMP production in several rat brain regions is decreased in response to cannabinoid drugs (31, 32). The affinity of the agonist [^{3}H]CP-55,940 for its cortical binding site in the absence of guanine nucleotides was more than 2 orders of magnitude greater than its K_{inh} for regulation of adenylate cyclase in the neuronal cell

model (8). However, the affinity state promoted by the addition of Gpp(NH)p would be the prevalent state concurrent with adenylate cyclase regulation (23, 24). The order of potencies for ligand interaction and the enantioselectivity described for this binding site are consistent with previously reported data for the inhibition of adenylate cyclase (7, 8).

One of the responses that the [^{3}H]CP-55,940 receptor site may regulate *in vivo* is analgesia. This ligand was specifically designed to possess potent analgetic activity (12). The analgetic activity for CP-55,940 was demonstrated in the tail flick, hot plate, phenylbenzylquinone writhing, tail clamp, and flinch jump tests in rodents (8, 12). The ratio of the activities of the (−)- to the (+)-isomer in the analgetic tests was 200-fold. This agrees reasonably well with the 50-fold enantioselectivity demonstrated here for the [^{3}H]CP-55,940 binding site. The order of potency for analgetic activity is mimicked by the order of potency reported here for the binding to the receptor. Other functions typical of the cannabinoid class of drugs, including changes in spontaneous locomotor activity, hypothermia, and immobility, have also been demonstrated for CP-55,940 and have been shown to be enantioselective (29). Thus, this receptor site appears to be associated with certain of the typical cannabinoid responses observed in animals in addition to analgesia.

Previous attempts to find and characterize a cannabinoid receptor associated with *in vitro* or *in vivo* functions have not met with success. Harris and colleagues (19) and Roth and Williams (16) demonstrated binding of [^{3}H]Δ^8-THC and [^{3}H]Δ^9-THC, respectively, to crude or purified synaptosomal membranes from rat brains. The former group were able to displace up to 10% of the binding with 1 μM Δ^8-THC; however, the binding was not saturable and pharmacological displacement by other cannabinoid ligands was not performed (19). The latter investigators were unable to discern a high affinity component of binding other than membrane adsorption, which was not dependent upon the concentration of free Δ^9-THC (16).

A high affinity binding site in brain membranes was described by Nye and colleagues (33, 34) using the [^{3}H]5′-trimethylammonium analog of Δ^8-THC. The ligand used for binding to this site does not exhibit biological activity in typical animal behavioral models of cannabinoid action, with the exception of CNS depression (35). This poor biological activity is consistent with our previous demonstration of the importance of maintaining the hydrophobic nature of the alkyl side chain extending from the A-ring (8). The pharmacological profile for displacement of [^{3}H]5′-trimethylammonium Δ^8-THC indicated that cannabinoid compounds having greatest affinity in several brain regions (e.g., cannabigerol and cannabidiol) are neither agonists nor antagonists in *in vivo* animal models or in humans (33). Thus, the pharmacological relevance of this binding site to cannabinoid effects *in vivo* may be questioned. The selectivity of the [^{3}H]trimethylammonium Δ^8-THC binding site for stereoisomers of Δ^9-THC and Δ^8-THC was less than 2-fold, and no stereoselectivity was observed for levonantradol and dextronantradol (33). The kinetic constants derived for the binding of [^{3}H]trimethylammonium Δ^8-THC yielded a K_d that was 3 orders of magnitude lower than the K_d determined by Scatchard analysis of the equilibrium binding data (34). This unusual finding may in part be explained by the observation that, throughout these experiments, the aqueous solubility and adsorption to glass of the ligands were not considered (33). Evidence suggests that this binding site is not linked to a G protein inasmuch as binding of [^{3}H]trimethylammonium Δ^8-THC was enhanced rather than decreased by nonhydrolyzable analogs of guanine nucleotides (34). It is clear that the [^{3}H]trimethylammonium Δ^8-THC binding site is definitely different from the cannabinoid receptor described here using [^{3}H]CP-55,940 as the ligand.

Other laboratories have suggested that cannabinoid drugs alter the binding of other neuromodulators to their receptors. Hillard and Bloom (36) reported that concentrations in excess of 3 μM Δ^9-THC or 11-OH-Δ^9-THC in the presence of a detergent vehicle increased the specific binding of the β-adrenergic antagonist ligand [^{3}H]dihydroalprenolol in mouse cortical homogenates. The interpretation of this finding was that the cannabinoid drugs altered membrane properties such that the binding behavior was modified (36). This mechanism is supported by additional studies from that laboratory, which demonstrated that similarly high concentrations of cannabinoid drugs altered fluidity of synaptic plasma membranes as detected by fluorescence polarization (37). Vaysse and colleagues (38) reported a similar interference with certain binding assays for opioid receptors by addition of high concentrations of several cannabinoid drugs in the presence of 100 mM ethanol. We have previously demonstrated that the site of cannabinoid action in neuroblastoma cells is not related to the binding of agonists to the δ opioid receptor or to subsequent signal transduction (14). The studies reported in the present work clearly indicate the presence of a pharmacologically selective, high affinity binding site for cannabinoid drugs. However, membrane perturbation may be the mechanism by which high concentrations of cannabinoid drugs may interfere with binding determinations made for a variety of other receptor types.

The development of a ligand binding assay for the cannabinoid class of drugs will allow investigations of cannabinoid actions that have previously not been possible. Pathways in the brain that may be involved in cannabinoid action can be examined. The cellular regulation of the receptor can be more fully characterized and its interaction with alternative second messenger systems can be assessed. Perhaps an antagonist for the cannabinoid drugs can be developed now that a binding site has been found that correlates with a cellular function (inhibition of adenylate cyclase). Furthermore, efforts to search for a putative endogenous ligand can now proceed. Thus, the importance of the characterization of a cannabinoid receptor will make a major impact on research in this field.

Acknowledgments

The excellent clerical assistance of Maggie Klevorn is gratefully appreciated.

References

1. Hollister, L. E. Health aspects of cannabis. *Pharmacol. Rev.* **38**:1–20 (1986).
2. Dewey, W. L. Cannabinoid pharmacology. *Pharmacol. Rev.* **38**:151–178 (1986).
3. Razdan, R. K. Structure-activity relationships in cannabinoids. *Pharmacol. Rev.* **38**:75–149 (1986).
4. Martin, B. R. Cellular effects of cannabinoids. *Pharmacol. Rev.* **38**:45–74 (1986).
5. Howlett, A. C., and R. M. Fleming. Cannabinoid inhibition of adenylate cyclase: pharmacology of the response in neuroblastoma cell membranes. *Mol. Pharmacol.* **26**:532–538 (1984).
6. Howlett, A. C. Cannabinoid inhibition of adenylate cyclase: biochemistry of the response in neuroblastoma cell membranes. *Mol. Pharmacol.* **27**:429–436 (1985).
7. Howlett, A. C. Cannabinoid inhibition of adenylate cyclase: relative activity of constituents and metabolites of marihuana. *Neuropharmacology* **26**:507–512 (1987).
8. Howlett, A. C., M. R. Johnson, L. S. Melvin, and G. M. Milne. Nonclassical cannabinoid analgetics inhibit adenylate cyclase: development of a cannabinoid receptor model. *Mol. Pharmacol.* **33**:297–302 (1988).

9. Gill, E. W., and G. Jones. Brain levels of Δ^1-tetrahydrocannabinol and its metabolites in mice— Correlation with behaviour, and the effect of the metabolic inhibitors SKF 525A and piperonyl butoxide. *Biochem. Pharmacol.* **21**:2237–2248 (1972).
10. Ho, B. T., V. S. Estevez, and L. F. Englert. The uptake and metabolic fate of cannabinoids in rat brains. *J. Pharm. Pharmacol.* **25**:488–490 (1973).
11. Ohlsson, A., M. Widman, S. Carlsson, T. Ryman, and C. Strid. Plasma and brain levels of Δ^6-THC and seven monooxygenated metabolites correlated to the cataleptic effect in the mouse. *Acta Pharmacol. Toxicol.* **47**:308–317 (1980).
12. Johnson, M. R., and L. S. Melvin. The discovery of nonclassical cannabinoid analgetics, in *Cannabinoids as Therapeutic Agents* (R. Mechoulam, ed.). CRC Press, Boca Raton, FL, 121–145 (1986).
13. Howlett, A. C., J. M. Qualy, and L. K. Khachatrian. Involvement of G_i in the inhibition of adenylate cyclase by cannabimimetic drugs. *Mol. Pharmacol.* **29**:307–313 (1986).
14. Devane, W. A., J. W. Spain, C. J. Coscia, and A. C. Howlett. An assessment of the role of opioid receptors in the response to cannabimimetic drugs. *J. Neurochem.* **46**:1929–1935 (1986).
15. Seeman, P., M. Chau-Wong, and S. Moyyen. The membrane binding of morphine, diphenylhydantoin and tetrahydrocannabinol. *Can. J. Physiol. Pharmacol.* **50**:1193–1200 (1972).
16. Roth, S. H., and P. J. Williams. The non-specific membrane binding properties of Δ^9-tetrahydrocannabinol and the effects of various solubilizers. *J. Pharm. Pharmacol.* **31**:224–230 (1979).
17. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248–254 (1976).
18. Dodd, P. R., J. A. Hardy, A. E. Oakley, J. A. Edwardson, E. K. Perry, and J.-P. Delaunoy. A rapid method for preparing synaptosomes: Comparison with alternative procedures. *Brain Res.* **226**:107–118 (1981).
19. Harris, L. S., R. A. Carchman, and B. R. Martin. Evidence for the existence of specific cannabinoid binding sites. *Life Sci.* **22**:1131–1138 (1978).
20. Garrett, E. R., and C. A. Hunt. Physicochemical properties, solubility, and protein binding of Δ^9-tetrahydrocannabinol. *J. Pharm. Sci.* **63**:1056–1064 (1974).
21. Limbird, L. E. *Cell Surface Receptors: A Short Course on Theory and Methods.* Martinus Nijhoff Publishing, Boston, 51–96 (1986).
22. Bennett, J. P., and H. I. Yamamura. Neurotransmitter, hormone, or drug receptor binding methods, in *Neurotransmitter Receptor Binding* (H. I. Yamamura, S. J. Enna, and M. J. Kuhar, eds.). Raven Press, New York 61–89 (1985).
23. Birnbaumer, L., J. Codina, R. Mattera, R. A. Cerione, J. D. Hildebrandt, T. Sunyer, F. J. Rojas, M. G. Caron, R. J. Lefkowitz, and R. Iyengar. Regulation of hormone receptors and adenylyl cyclases by guanine nucleotide binding N proteins. *Recent Prog. Horm. Res.* **41**:41–99 (1985).
24. Casey, P. J., and A. G. Gilman. G protein involvement in receptor-effector coupling. *J. Biol. Chem.* **263**:2577–2580 (1988).
25. Maguire, M. E. Hormone-sensitive magnesium transport and magnesium regulation of adenylate cyclase. *Trends Pharmacol. Sci.* **5**:73–77 (1984).
26. Blume, A. J., D. Lichtshtein, and G. Boone. Coupling of opiate receptors to adenylate cyclase: requirement for Na^+ and GTP. *Proc. Natl. Acad. Sci. USA* **76**:5626–5630 (1979).
27. Koski, G., R. A. Streaty, and W. A. Klee. Modulation of sodium-sensitive GTPase by partial opiate agonist. An explanation for the dual requirement for Na^+ and GTP in inhibitory regulation of adenylate cyclase. *J. Biol. Chem.* **257**:14035–14040 (1982).
28. Munson, P. J., and D. Rodbard. LIGAND: a versatile computerized approach for characterization of ligand-binding systems. *Anal. Biochem.* **107**:220–239 (1980).
29. Little, P. J., D. R. Compton, M. R. Johnson, L. S. Melvin, and B. R. Martin. Pharmacology and stereoselectivity of structurally novel cannabinoids in mice. *J. Pharmacol. Exp. Ther.*, in press.
30. Burt, D. R. Criteria for receptor identification, in *Neurotransmitter Receptor Binding* (H. I. Yamamura, S. J. Enna, and M. J. Kuhar, eds.). Raven Press, New York, 41–60 (1985).
31. Bidaut-Russell, M., and A. C. Howlett. Cannabinoid drugs regulate cyclic AMP metabolism in rat brain. *Trans. Am. Soc. Neurochem.* **19**:163 (1988).
32. Bidaut-Russell, M., and A. Howlett. Opioid and cannabinoid analgetics both inhibit cyclic AMP production in the rat striatum. *Adv. Biosci.* in press.
33. Nye, J. S., H. H. Seltzman, C. G. Pitt, and S. H. Snyder. High-affinity cannabinoid binding sites in brain membranes labeled with [^{3}H]-5′-trimethylammonium Δ^8-tetrahydrocannabinol. *J. Pharmacol. Exp. Ther.* **234**:784–791 (1985).
34. Nye, J. S., and S. H. Snyder. The high-affinity cannabinoid binding site in brain: regulation by guanine nucleotides and isolation of an endogenous inhibitor. *Natl. Inst. Drug Abuse Res. Monogr.* **79**:134–147 (1987).
35. Compton, D. R., P. J. Little, and B. R. Martin. Cannabinoid behavioral effects: specific versus non-specific actions, in *Marijuana '87: Proceedings of the Melbourne Symposium on Cannabis* (G. B. Cheshire, P. Consroe, and R. Musty, eds.). NCADA Monograph, Australian Government Publishing Service, Canberra, 213–218 (1988).
36. Hillard, C. J., and A. S. Bloom. Δ^9-Tetrahydrocannabinol-induced changes in β-adrenergic receptor binding in mouse cerebral cortex. *Brain Res.* **235**:370–377 (1982).
37. Hillard, C. J., R. A. Harris, and A. S. Bloom. Effects of the cannabinoids on physical properties of brain membranes and phospholipid vesicles: fluorescence studies. *J. Pharmacol. Exp. Ther.* **232**:579–588 (1985).
38. Vaysse, P. J.-J., E. L. Gardner, and R. S. Zukin. Modulation of rat brain opioid receptors by cannabinoids. *J. Pharmacol. Exp. Ther.* **241**:534–539 (1987).

Send reprint requests to: Dr. Allyn C. Howlett, Department of Pharmacology, St. Louis University School of Medicine, 1402 South Grand Boulevard, St. Louis, MO 63104.

Short Report

autism

Autism
2015, Vol. 19(1) 102–106

DOI: 10.1177/1362361314524641
aut.sagepub.com
SAGE

Drug-refractory aggression, self-injurious behavior, and severe tantrums in autism spectrum disorders: A chart review study

Benjamin A Adler[1], Logan K Wink [2], Maureen Early[3,4], Rebecca Shaffer[3,4], Noha Minshawi[3,4], Christopher J McDougle[5] and Craig A Erickson[2]

Abstract

Aggression, self-injurious behavior, and severe tantrums are impairing symptoms frequently experienced by individuals with autism spectrum disorders. Despite US Food and Drug Administration approval of two atypical antipsychotics targeting these symptoms in youth with autistic disorder, they remain frequently drug refractory. We define drug-refractory aggression, self-injurious behavior, and severe tantrums in people with autism spectrum disorders as behavioral symptoms requiring medication adjustment despite previous trials of risperidone and aripiprazole or previous trials of three psychotropic drugs targeting the symptom cluster, one of which was risperidone or aripiprazole. We reviewed the medical records of individuals of all ages referred to our clinic for autism spectrum disorder diagnostic evaluation, as well as pharmacotherapy follow-up notes for all people meeting autism spectrum disorder criteria, for drug-refractory symptoms. Among 250 consecutively referred individuals, 135 met autism spectrum disorder and enrollment criteria, and 53 of these individuals met drug-refractory symptom criteria. Factors associated with drug-refractory symptoms included age 12 years or older ($p < 0.0001$), diagnosis of autistic disorder ($p = 0.0139$), and presence of intellectual disability ($p = 0.0273$). This pilot report underscores the significance of drug-refractory aggression, self-injurious behavior, and severe tantrums; suggests the need for future study clarifying factors related to symptom development; and identifies the need for focused treatment study of this impairing symptom domain.

Keywords

aggression, atypical antipsychotics, autism, autism spectrum disorders, self-injurious behavior, severe tantrums

Aggression, self-injurious behavior (SIB), and severe tantrums are common targets of pharmacotherapy in people with autism spectrum disorders (ASDs; Posey et al., 2008). The triggers for these behavioral challenges can be many, and include environmental demands, medical illness, sensory sensitivities, and routine change, among others. These challenges are often sufficiently severe to place patients and caregivers at risk of physical injury (Bronsard et al., 2010) and to limit the efficacy of therapeutic, educational, and vocational interventions (Stigler and McDougle, 2008).

Given the significant impact and frequent occurrence of aggression, SIB, and severe tantrums in people with ASDs, this target symptom cluster has been the focus of substantial pharmacotherapy research. Among drug classes, atypical antipsychotics are most commonly used as first-line pharmacotherapy for the treatment of irritability in people with ASDs (Stigler and McDougle, 2008) (irritability defined by the US Food and Drug Administration (FDA, 2006) as a symptom cluster, including aggression, SIB, and severe tantrums). Alpha 2 agonists, mood stabilizers, and anticonvulsants are also employed to target aggression, SIB, and severe tantrums in ASDs, but less convincing evidence supports the effectiveness of these drug classes (Stigler and McDougle, 2008). Following large placebo-controlled trials which demonstrated relatively

[1]Seattle Children's Hospital, USA
[2]Cincinnati Children's Hospital Medical Center, USA
[3]Riley Children's Hospital, USA
[4]Indiana University School of Medicine, USA
[5]Lurie Center for Autism, USA

Corresponding author:
Craig A Erickson, Department of Pediatrics, Division of Child and Adolescent Psychiatry, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue MLC 4002, Cincinnati, OH 45229, USA.
Email: Craig.Erickson@cchmc.org

robust reduction in irritability with treatment as measured by the Aberrant Behavior Checklist–Irritability subscale (ABC-I), which measures the severity of aggressive, self-injurious, and tantrum behaviors, risperidone and aripiprazole have been FDA-approved for treatment of irritability in youth with autistic disorder (Blankenship et al., 2010; McCracken et al., 2002; Owen et al., 2009). Additionally, positive and negative predictors of medication treatment response have recently been reported on by Arnold et al. (2010) using data compiled during the Research Units on Pediatric Psychopharmacology (RUPP) Autism Network placebo-controlled trial of risperidone in youth with autistic disorder (Arnold et al., 2010; McCracken et al., 2002). However, to date, no reports have endeavored to describe the prevalence of individuals suffering from aggression, SIB, and severe tantrums refractory to first-line treatments.

In our clinical experience, we frequently work with people with ASDs who display aggression, SIB, and severe tantrums that have been refractory to drug treatment, including first-line treatment with risperidone and/or aripiprazole. Given our anecdotal clinical experience, we hypothesize that drug-refractory aggression, SIB, and severe tantrums occur frequently in people with ASDs. Many individuals have histories of repeated suboptimal medication trials defined by partial response, nonresponse, and, at times, treatment-limiting adverse effects. Prior to embarking on a systematic prospective study of this refractory symptom cluster in ASDs, we sought first to define the cluster within our tertiary-care clinical setting, to estimate the frequency of these refractory behavioral symptoms in people with ASDs, and to begin identifying potential predictors associated with the presentation of symptoms refractory to first-line drug treatment.

Methods

For the purpose of this analysis, we defined drug-refractory behaviors as clinician report of aggression, deliberate self-injury, and temper tantrums remaining a primary target of active treatment despite a history of previous trials of (a) risperidone (at least 2 mg/day) and aripiprazole (at least 5 mg/day) dosed within FDA-approved dose ranges when tolerated, *or* (b) three or more psychotropic drugs targeting aggression, SIB, and severe tantrums, including either risperidone or aripiprazole. Cutoff doses of aripiprazole and risperidone are based on doses demonstrated effective in the pivotal studies leading to FDA approval of each medication for this indication (McCracken et al., 2002; Marcus et al., 2009). For individuals without history of previous trials of both risperidone and aripiprazole, we felt that it was imperative to have been treated with at least one of the FDA-approved medications for their symptoms to be considered drug refractory. Furthermore, we felt that a significant effort to reduce symptoms must be demonstrated prior to describing symptoms as drug refractory and that in addition to one of the FDA-approved medications, two other medications clinically targeting this symptom domain was adequate.

This project took place at the Christian Sarkine Autism Treatment Center (CSATC) in Indianapolis, Indiana. The CSATC is an autism treatment and research clinic with strong psychopharmacology and clinical trial focus. The majority of patients followed longitudinally for treatment at CSATC receive ongoing medication management for behavioral concerns provided by child psychiatrists with expertise in ASDs. Individuals treated at CSATC are also frequently referred for Parent Management Training based on Applied Behavioral Analysis with a psychologist or social worker with expertise in ASDs. However, due to the wide referral base of the clinic, we estimate that less than half of the sample reviewed in this project was receiving ongoing therapy services at our clinic. The majority of individuals were likely receiving some educational or community services; however, the intensity and frequency of these services varied widely. Considering the pharmacotherapy focus of CSATC and the resulting consistency of pharmacotherapy records, in this project, we chose to focus primarily on medication treatment of refractory behavioral concerns without focus on behavioral intervention.

We systematically reviewed the medical records of 250 consecutive individuals of all ages referred for initial evaluation of ASD at the CSATC between April 2007 and April 2009 for presence of ASDs and aggression, SIB, and severe tantrums refractory to drug treatment. In addition to diagnostic evaluation records, pharmacotherapy clinical notes (through April 2012) of patients diagnosed with ASDs were reviewed for the development of drug-refractory aggression, SIB, and severe tantrums. As the goal of this project was to evaluate the incidence of this drug-refractory symptom domain in our patient population, we included subjects who presented with drug-refractory symptoms and those who developed drug-refractory symptoms despite treatment at our center. People whose initial presentation was consistent with this symptom domain provided historical information consistent with our definition (including information regarding doses of previous aripiprazole and risperidone trials). Those who developed drug-refractory aggression, SIB, and severe tantrums while in treatment at our center did so despite treatment meeting our proposed definition. Patient characteristics, including age, gender, specific ASD diagnosis, presence/absence of comorbid intellectual disability, and data describing current and past pharmacotherapy trials, including drug name, dosing, and target symptoms of specific drug treatment, were gathered. Due to inconsistency of records and varied intensity and frequency of behavioral interventions received by individuals reviewed in this project, we did not include this information in our analysis

Table 1. Descriptive data.

	Individuals with ASDs and drug-refractory aggression, self-injurious behaviors, and severe tantrums (n = 53)	
Age	17, ages 2–11 years	36, ages ≥ 12 years
Gender	42 males	11 females
Diagnosis	35 autistic disorder	18 PDD-NOS or Asperger's disorder
ID	41 with ID	12 without ID

ASD: autism spectrum disorder; PDD-NOS: pervasive developmental disorder–not otherwise specified; ID: intellectual disability.

despite our recognition of the important role behavioral treatment may have in modifying the behaviors we are describing.

ASD diagnosis (autistic disorder, pervasive developmental disorder–not otherwise specified (PDD-NOS), or Asperger's disorder) was made by a clinician with expertise in ASD diagnosis (CAE) using diagnostic criteria from the *Diagnostic and Statistical Manual of Mental Disorders* (4th ed.; text rev.; *DSM-IV-TR*; American Psychiatric Association (APA), 2000). Intellectual disability diagnosis was based upon review of neuropsychological testing and school reports when available, combined with clinical interview focused on adaptive functioning. Due to inconsistency in whether degree of intellectual disability (mild, moderate, severe, and profound) was specified in evaluation notes and lack of direct standardized assessment, we chose to describe only whether intellectual disability was present or absent.

Between-group comparisons assessing the impact of age, gender, specific ASD diagnosis, and presence of intellectual disability on drug-refractory aggression, SIB, and severe tantrum rates were made using Fisher's exact test, two-tailed. With regard to age, we divided the sample into two groups above and below age 12 years in an effort to capture pre- and postpubertal groups, as Tanner staging was not available. We chose this age division, as maladaptive behaviors in adolescents with autism have been demonstrated to trend toward improvement with age (Anderson et al., 2011). Correction for multiple comparisons was not utilized, given the pilot nature of this initial report. This study was reviewed and approved by our local Institutional Review Board.

Results

Of 250 consecutively referred patients, 140 met *DSM-IV-TR* criteria for ASDs. Five patient records were excluded from further analysis due to lack of follow-up documentation beyond the initial evaluation. Thus, medical records from 135 people with ASDs were included in our final analysis. Ages of included individuals ranged from 2 to 54 years at the time of initial evaluation, 80% were male, 52% were diagnosed with autistic disorder, and 67% had intellectual disability.

Aggression, SIB, and severe tantrums were common targets of treatment among people with ASDs included in this analysis. In all, 94 people presented with a chief complaint of aggression, SIB, and severe tantrums on initial evaluation. In addition, seven people developed this symptom cluster through the treatment period reviewed. In all cases, people with aggression, SIB, and severe tantrums were receiving or received pharmacotherapy trials targeting this symptom cluster.

Drug-refractory aggression, SIB, and severe tantrums were noted in 53 people with ASDs (39.5% of all people with ASDs; 52.5% of people who were treated for aggression, SIB, and severe tantrums). Of these 53 people with drug-refractory symptoms, 32 people (60.4%) met criteria on initial evaluation, and 21 people (39.6%) developed drug-refractory symptoms while followed at our clinic (through April 2012). In all, 36 (67.9%) people meeting criteria met the definition by having ongoing aggression, SIB, and severe tantrums requiring active treatment adjustment despite previous trials of both aripiprazole and risperidone. In total, 17 (32.1%) people met criteria based on a history of ongoing aggression, SIB, and severe tantrums despite previous trials of at least three psychoactive drugs targeting aggression, SIB, and severe tantrums, including either aripiprazole or risperidone. Descriptive characteristics of the drug-refractory treatment group are listed in Table 1.

People meeting drug-refractory criteria had received an average of 6.42 previous drug trials targeting aggression, SIB, and severe tantrums, with many medications often prescribed concurrently. At the time of labeling subjects with drug-refractory symptoms, people meeting criteria were taking an average of two medications targeting this symptom cluster. Four people with ASDs and drug-refractory symptoms were not taking any psychotropic drugs upon initial presentation due to history of intolerable adverse effects and/or lack of drug efficacy. The FDA-approved agents risperidone and aripiprazole were the most commonly utilized drugs, with atypical antipsychotics being the most frequently used drug class (n = 155 drug trials; 46% of all drug trials). In all, 46 (86.8%) people with drug-refractory symptoms had a trial of risperidone in their lifetime, and 44 (83.0%) people had received a trial of aripiprazole. Quetiapine (n = 23; 43.4%), sertraline (n = 22, 41.5%), and clonidine (n = 17; 32.1%) were the next most

frequently utilized drugs targeting aggression, SIB, and severe tantrums in this group. Antidepressants (primarily selective serotonin reuptake inhibitors; n = 80 drug trials; 24% of all drug trials), alpha 2 agonists (n = 25 drug trials; 7.4% of all drug trials), benzodiazepines (n = 18 drug trials; 5.3% of all drug trials), typical antipsychotics (n = 17 drug trials; 5% of all drug trials), and mood stabilizer/anticonvulsants (n = 16 drug trials; 4.7% of all drug trials) were also commonly used drug classes. Use of stimulant medications in this group was not assessed, as the target symptoms of stimulant medications are not typically aggression, SIB, and severe tantrums.

In assessing our sample of people with ASDs with drug-refractory aggression, SIB, and severe tantrums, we looked at the impact of patient age, gender, specific ASD diagnosis, and presence of comorbid intellectual disability on the development of drug-refractory symptoms. People 12 years of age and older were more likely to meet criteria, with 36 (73.5%) of 49 people 12 years and older exhibiting drug-refractory symptoms ($p < 0.0001$) versus 17 (19.8%) of 86 people aged 2–11 years. Diagnosis of autistic disorder was also associated with an increased risk of drug-refractory symptoms. Of 71 people with autistic disorder, 35 (49.3%) exhibited drug-refractory symptoms compared to only 18 (28.1%) of 64 people with PDD-NOS or Asperger's disorder ($p = 0.01$). Regarding intellectual disability, 41 (45.1%) of 91 people with comorbid intellectual disability met criteria versus 12 (27.3%) of 44 people without intellectual disability ($p = 0.02$). Gender was not a significant differentiating factor with 42 (38.5%) of 109 males and 11 (42.3%) of 26 females exhibiting drug-refractory symptoms ($p = 0.82$). Overall, in this pilot analysis, age 12 years and above, presence of autistic disorder and presence of intellectual disability were identified as potential risk factors for the development of drug-refractory aggression, SIB, and severe tantrums.

Discussion

In our sample of 135 individuals with ASDs treated longitudinally at a tertiary-care center, over half of those with a primary complaint of aggression, SIB, and severe tantrums presented with or became refractory to first-line drug treatment. These preliminary data suggest that drug-refractory aggression, SIB, and severe tantrums may be clinically significant concerns in this population, given that 39.5% of our sample of individuals with ASDs presented with or developed drug-refractory symptoms over 3–5 years of treatment. In our sample, potential risk factors associated with increased risk of developing drug-refractory symptoms included age 12 years or older, diagnosis of autistic disorder, and the presence of intellectual disability. Our age finding is counter to previous reported reduction in irritability symptoms with increasing age (Anderson et al., 2011). This indicates that drug-refractory symptoms may be unique from the general presentation of irritability in youth with ASDs. Another explanation for this difference may be related to the referral bias of our clinical population. As a tertiary-care facility, our population may represent a subset of individuals with more severe and more frequent aggression, SIB, and severe tantrums.

Limitations

The findings of this report must be taken in the context of the limitations of the study. Most strikingly, our report is based on a retrospective review of prospectively acquired data collected at a single tertiary-care clinic with specific ASD expertise. Our patient sample is biased toward individuals with potentially more severe pathology who may have been difficult to manage in a community setting prompting referral to our center. Given this bias, our report of drug-refractory aggression, SIB, and severe tantrum frequency may not be generalizable to the greater community ASD population. Furthermore, our report attempts to capture information regarding the frequency of this drug-refractory symptom domain in our patient population, and therefore included those individuals who presented to our clinic with these symptoms, as well as those who developed drug-refractory symptoms while in treatment with us. This design does not allow us to comment on the outcomes of these patients once receiving treatment at a specialty clinic, particularly information regarding those individuals who no longer have symptoms of significant aggression, SIB, and severe tantrums following treatment at our center. Additionally, the severity of the symptoms suffered by our patient population may have contributed to a clinician and caregiver bias to continue treatment even if symptoms remained refractory, thus creating the potential to underestimate the true occurrence of treatment nonresponse.

Other limitations include the lack of standardized assessment measures for both diagnoses and symptoms. The diagnosis of ASDs was made by experts in ASD diagnosis and treatment based upon *DSM-IV-TR* criteria without use of diagnostic measures such as the Autism Diagnostic Interview–Revised or the Autism Diagnostic Observation Schedule. These measures are considered gold standard diagnostic instruments utilized commonly in research; however, it is widely agreed that clinical experience and expert clinical judgment are integral to diagnostic competence in ASDs (Gotham et al., 2011). Intellectual functioning was determined through chart review and clinical evaluation, rather than by standardized testing. We defined aggression, SIB, and severe tantrums in line with the definition of irritability used by the FDA in drug trials targeting this symptom domain (US FDA, 2006); however, without use of standardized measures of symptoms and symptom severity such as the ABC, there remains likely wide variability in our treatment sample. Additionally, our reliance on clinician report of ongoing aggression, SIB,

and severe tantrums as the primary symptomatic measure does not allow for precise description of specific behaviors that are refractory to treatment in this population, making application of our findings to a more general ASD community population difficult. Furthermore, detailed data regarding dosages and duration of previous drug trials, presence of comorbid psychiatric diagnoses, socioeconomic factors, adverse medication effects, and non-pharmacologic behavioral and educational intervention which may have impacted treatment response were also not thoroughly evaluated.

Clinical significance

Despite these limitations, we believe these data underscore the need for additional study. It is clear from this pilot report that drug-refractory aggression, SIB, and severe tantrums may be of clinical significance in people with ASDs, thus requiring focused future treatment development. Drug-refractory symptoms also warrant future large-scale prospective study to better clarify factors related to their development, including drug tolerability and partial response to treatment. Such prospective future study would benefit from being multisite to assess drug-refractory symptoms in multiple settings. Future study will also benefit from further development of specific drug-refractory criteria (i.e. more detailed designation of "adequate" previous medication trials based on drug, dose, and duration of treatment). Further investigation of the impact of age would also be appropriate, with attention to puberty and its potential influence on behaviors. Further study analyzing the impact of factors such as comorbid mood and anxiety symptoms, seizure disorder, presence of known genetic cause of ASD, and availability of/engagement with intensive behavioral therapy on the development of drug-refractory symptoms also should be undertaken. Greater understanding of these symptoms in people with ASDs, including further understanding of the mechanism of action of failed medications along with impact of non-pharmacologic interventions, will guide the development of treatments potentially targeting different neurotransmitter pathways or utilizing novel behavioral techniques. Such future efforts will likely require prospective longitudinal tracking to follow the development of drug-refractory aggression, SIB, and severe tantrums in a large group of persons with ASDs beginning at a young age.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

American Psychiatric Association (APA) (2000) *Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR)*. 4th ed. Text revision. Washington, DC: APA.

Anderson DK, Maye MP and Lord C (2011) Changes in maladaptive behaviors from midchildhood to young adulthood in autism spectrum disorder. *American Journal on Intellectual and Developmental Disabilities* 116: 381–397.

Arnold LE, Farmer C, Kraemer HC, et al. (2010) Moderators, mediators, and other predictors of risperidone response in children with autistic disorder and irritability. *Journal of Child and Adolescent Psychopharmacology* 20: 83–93.

Blankenship K, Erickson CA and McDougle CJ (2010) Pharmacotherapy of autism and related disorders. *Psychiatric Annals* 40: 203–209.

Bronsard G, Botbol M and Tordjman S (2010) Aggression in low functioning children and adolescents with autistic disorder. *PLoS One* 5: e14358.

Gotham K, Bishop S and Lord C (2011) Diagnosis of autism spectrum disorders. In: Amaral DG, Dawson G and Geschwind D (eds) *Autism Spectrum Disorders*. Oxford: Oxford University Press, pp. 30–43.

McCracken JT, McGough J, Shah B, et al.; Research Units on Pediatric Psychopharmacology Autism Network (2002) Risperidone in children with autism and serious behavioral problems. *The New England Journal of Medicine* 347: 314–321.

Marcus RN, Owen R, Kamen L, et al. (2009) A placebo-controlled, fixed-dose study of aripiprazole in children and adolescents with irritability associated with autistic disorder. *Journal of the American Academy of Child and Adolescent Psychiatry* 48: 1110–1119.

Owen R, Sikich L, Marcus RN, et al. (2009) Aripiprazole in the treatment of irritability in children and adolescents with autistic disorder. *Pediatrics* 124: 1533–1540.

Posey DJ, Stigler KA, Erickson CA, et al. (2008) Antipsychotics in the treatment of autism. *The Journal of Clinical Investigation* 118: 6–14.

Stigler KA and McDougle CJ (2008) Pharmacotherapy of irritability in pervasive developmental disorders. *Child and Adolescent Psychiatric Clinics of North America* 17: 739–752.

US Food and Drug Administration (2006) FDA approves the first drug to treat irritability associated with autism, risperdal. Available at: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2006/ucm108759.htm

Published in final edited form as:

J Dev Behav Pediatr. 2022 April 01; 43(3): 140–148. doi:10.1097/DBP.0000000000001028.

Emotional and behavioral problems in youth with autism: high prevalence and impact on functioning

Eric Fombonne, MD[a],

Lisa A. Croen, PhD[b],

Joanna E. Bulkley, PhD[c],

Alexandra M. Varga, MPH[c],

Yihe G. Daida, PhD[d],

Brigit A. Hatch, MD[a,e],

John F. Dickerson, PhD[c],

Frances L. Lynch, PhD[c]

[a]Oregon Health & Science University, Portland, OR

[b]Kaiser Permanente Northern California, Oakland, CA

[c]Center for Health Research, Kaiser Permanente Northwest, Portland, OR

[d]Center for Integrated Health Care Research, Kaiser Permanente, Honolulu, HI

[e]OCHIN, Inc., Portland, OR.

Abstract

Objective: Emotional and behavioral problems (EBP) may co-occur with autism spectrum disorder (ASD) and impair children's functioning beyond autism symptomatology. We compared the prevalence of EBP in youths with or without ASD and evaluated their unique contribution to impairment in ASD.

Method: We surveyed 1,267 children (79.4% males, mean age: 9.2, range:3-17) recruited at 3 sites in Kaiser-Permanente and OCHIN primary care clinical networks, with confirmed ICD diagnosis of either ASD (N=564), asthma (N=468), or neither (N=429). Children from the two comparison groups were age- and sex-matched to the ASD group. EBP and impairment were measured by the Strengths and Difficulties Questionnaire (SDQ), and autism symptomatology by the Social Responsiveness Scale (SRS) in the ASD group only.

Results: EBP and impairment mean scores were significantly ($p<.001$) higher in participants with ASD compared to children from the two comparison groups, across sexes and age groups, with no significant difference between the asthma and control groups. Among children with ASD, both EBPs and autistic symptoms were significantly correlated with impairment ($r=.64$ and $r=.65$, respectively) and explained a significant proportion of impairment variance ($R^2=.525$; $p<.001$)

Address correspondence to: Eric Fombonne, Departments of Psychiatry & Pediatrics, Oregon Health & Science University, 3198 SW Sam Jackson Park Road, Portland, OR, 97239, fombonne@ohsu.edu, 503-494-7448 (w) 503-701-0132 (cell).

Conflict of Interest Disclosures: The authors have no conflicts of interest to disclose.

in multiple linear regression. In the relative importance analysis, EBPs and autistic symptoms explained comparable proportions of impairment variance (46% and 52%, respectively) with no significant difference between their relative weights (mean difference: 0.03; 95%CI: −.049-.114).

Conclusion: Among youth with ASD, high levels of EBP impair daily functioning as much as autistic symptoms. Systematic detection and management of EBP may improve functioning and outcomes in youth with ASD.

Introduction

Autism spectrum disorder (ASD) is characterized by developmental impairments in communication and reciprocal social interactions and by atypical patterns of play, behavior, and sensorimotor responses.[1] Beyond core ASD symptoms, youths with ASD have an elevated incidence of co-occurring medical and mental health problems.[2] Because many of these problems can be effectively treated, prompt identification and management of comorbid symptoms and disorders is necessary to improve outcomes and quality of life for children with ASD.

Surveys of psychiatric problems in children with ASD have employed two levels of definition. Studies using categorical diagnostic approaches have reported high prevalence of ICD or DSM psychiatric disorders with up to 80% of children meeting criteria for at least one psychiatric disorder and about 40% of individuals with ASD meeting criteria for more than one psychiatric disorder. A recent meta-analysis of 96 such surveys reported high pooled prevalence estimates for attention-deficit hyperactivity disorder (28%), anxiety disorders (20%), sleep-wake disorders (13%), disruptive disorders (12%) and depressive disorders (11%).[3] Other studies have relied on dimensional measures of psychopathology and have evaluated the prevalence of individual emotional and behavioral problems (EBP) using parent or teacher standardized behavioral checklists. Results have consistently shown elevated prevalence estimates of EBP in clinical samples,[4] registries[5] and population-based samples.[6]

Interpretation of findings has been hampered by methodological variability across surveys including reliance on clinical versus population-based samples, variable age ranges and sample sizes, and inconsistent use of comparison groups of typically developing children[7]. Additionally, with few exceptions,[4,6,8] lack of a non-ASD clinical control group also prevented testing the specificity of findings to autism.

Assessing psychopathology in the context of autism is challenging. First, some psychiatric symptoms are not easily differentiated from autistic symptoms (e.g. social anxiety versus aloofness, obsessional rituals versus autistic repetitive behaviors, inattention versus social disengagement).[7,9] Second, existing diagnostic interviews need to be modified for use with autistic individuals[10] or new instruments must be developed[11,12]. Third, there are particular difficulties in evaluating symptoms in those individuals with ASD and limited or no language, or associated intellectual disability.[13,14] Fourth, most psychiatric diagnoses require identification of a symptom pattern together with a duration criterion and evidence of impairment attributable to the psychiatric symptoms. No clinical or research rules have been developed allowing to link co-occurring psychiatric problems specifically to impairment

in the presence of autistic symptomatology, itself a concomitant source of impairment. Moreover, few studies[9] of psychiatric disorders or EBPs have included separate measures of impairment, and no research on impairment exists that has partitioned the respective contributions of EBPs and autism symptoms to overall function in a person with ASD.

This study was precisely set to examine the relative contribution of co-occurring behavioral problems and autistic symptomatology to overall impairment in functioning among participants with ASD. The data were collected as part of a longitudinal study examining a broad array of financial and economic costs to families with a child with ASD, a different chronic medical condition (asthma), or neither. Data required for our study were available only at baseline; thus, we used cross-sectional data obtained at baseline of the main health economics study. Results from health economics analyses will be published separately and are not examined in this study. Our specific objectives were to: 1) compare levels of EBPs and their impact in autistic children to those in the two comparison groups; 2) evaluate, among children with ASD, the respective contributions of EBPs and autistic symptomatology to impaired functioning, controlling for background socio-demographic characteristics.

Methods

Participants

We recruited parents/guardians of children with Autism Spectrum Disorder (ASD), asthma, or neither condition (control group) for a longitudinal study of family costs of caring for children's chronic health conditions. Three Kaiser Permanente regions (Northwest, Hawaii, Northern California) and several health clinics in the OCHIN, Inc. community health center network were the recruitment sites. Using electronic health records (EHR) for initial eligibility screening and confirmatory screening with interested parent/guardians, we identified children between 3 and 17 years who met the inclusion and exclusion criteria. The EHR eligibility criteria were: a) At least one face-to-face encounter in the past 2 years, either as inpatient or outpatient; b) ASD (ICD-9 code 299.0) and asthma (ICD-9 codes 493.0, 493.1, 493.9) diagnoses made over one year of age to determine ASD and asthma status; c) for ASD: at least 2 occurrences of ASD diagnosis in EHR (separated by >30 days), *or* ASD diagnosis as not resolved/active in the problem list, *or* at least 1 ASD diagnosis by a specialty ASD clinic/provider; d) for asthma: at least 2 EHR diagnoses (separated by >30 days) of asthma in previous 2 years either encounter-based or medication-based *and* no ASD diagnosis; e) for the control group: not eligible for ASD or asthma groups; f) exclusion criteria were: child deceased, cancer diagnosis in last 3 years, the child was on the research center 'Do-not-contact' list. Fifty participants had both ASD and Asthma; comparisons of participants with ASD with or without Asthma showed that there were no differences between these two groups with respect to sex, age, emotional/behavioral problems, and autism severity (all *P*'s: NS). Thus, children with both ASD and asthma (N=50) were included in the ASD group.

Interested parents were further screened with the following criteria: a) parents had to confirm the presence of ASD and/or asthma, or their absence; b) for children in the ASD *and* asthma group: when ASD but not asthma was confirmed, the family was included in the

ASD group; when asthma but not ASD was confirmed, the family was excluded; c) child was excluded if respondent reported that the child had ever had cancer; d) other exclusion clauses were: the child did not live with the respondent over 50% of the time, respondent was a foster parent to the child, another sibling was already enrolled in the study.

Samples were extracted approximately every month over the 16-month recruitment period (November, 2017 through February, 2019). We oversampled racial and ethnic minority families. The asthma and control groups were matched to the age and gender distribution of the ASD group resulting in a preponderantly male study sample. Families were recruited through mailed letters, emails, and follow up phone calls. Respondents (parents/guardians) completed a series of 3 surveys, 4 months apart. Surveys were programmed in REDCap™ and could be completed on-line or over the phone with a trained interviewer. All recruitment and survey materials and phone participant contacts were provided in English and Spanish. The measures of interest for the present study (see below) were available only at baseline; accordingly, this cross-sectional study employed the first wave of data of the longitudinal survey.

Out of 6,533 potentially eligible families contacted, 1,707 consented to participate (26.1% response rate). After screening, 1,461 were eligible and enrolled in the study. Comparisons of participants and non-participants showed no significant differences with respect to gender (P=0.58) and age (P=0.066); significant differences (all P's<0.001) were found for race/ethnicity (White: 43.8% vs 37.2%, respectively), insurance type (publicly insured: 20.6% vs 26.3%, respectively), and clinical group with slightly higher enrollment rate in ASD (38.6%) as compared to the 2 other groups (asthma: 32.0%; controls: 29.4%). Further details about the study design and sample recruitment can be found elsewhere (Bulkley et al., submitted).

Of the 1,461 enrolled families, 1,267 caregivers (86.7%) completed the Strengths and Difficulties Questionnaire (SDQ) (472 ASD, 410 Asthma, 385 controls). There were no differences between SDQ completers and non-completers with respect to study site, and child's sex, race/ethnicity and age; however, SDQ completion rate was lower in families of children with ASD compared to the other two groups (ASD: 83.7%; asthma: 87.6%; Controls: 89.7%; P=.016).

In this study, we report on race and ethnicity since known disparities exist between Black, Hispanic and White autistic children in the U.S. with regard to prevalence, age at diagnosis, access to care, and comorbid intellectual disability that could influence our results. [2,15-16]

Instruments

Strengths and Difficulties Questionnaire & Impact Supplement (SDQ)[17]—The SDQ consists of 25 items scored 0, 1 or 2 ('Not true'; 'Somewhat true'; 'Certainly true') that ask about the child's behavior over the last six months. Four difficulty subscales (labeled: emotional, conduct, hyperactivity, peer problems) each comprising five items yield four subscale scores ranging from 0 to 10. The 4 subscale scores are summed up to generate a total SDQ score (range: 0-40); higher scores indicate more difficulties. The prosocial subscale (5 items; subscale score range: 0-10) measures positive attributes and was not used in this analysis. Exact SDQ items wording and item loading on each subscale is

available in Table S1 (Supplemental Digital Content). Of note, the five items included in the 'hyperactivity' subscale measure overactivity (2 items), inattention (2 items) and impulsivity (1 item) making that scale a robust measure of the ADHD construct and not solely one of hyperactivity. For this study, we computed a 15-item EBP score (range: 0-30) by summing up the emotional, hyperactivity and conduct subscale scores. We excluded the peer problem score from the total difficulty score since prior research had shown that the peer problems subscale overlapped with ASD symptoms,[9] and we needed measures of EBP uncontaminated by autism constructs. In addition, we used the single EBP measure in regression analyses since this 15-item composite achieved better reliability than its three component subscales, it provided a single measure of overall psychiatric disturbance well suited to our second study objective, and it provided for clearer interpretation.

The SDQ Impact Supplement measures distress and impairment in functioning resulting from behavioral problems. Parents first report if their child has any difficulty in behavior, emotions or getting along with others. If so, they report for how long have difficulties been present and whether they upset or distress their child using a four-point scale (Not at all=0, Only a little=0, A medium amount=1, A great deal=2). If some distress is endorsed, parents assess how much the difficulties interfere with child's everyday home life, friendships, classroom learning, and leisure activities. The Impact Score is the sum of these answers (range: 0-10). Parents who do not perceive a difficulty on the initial question receive an Impact Score of 0. In this study, we used the SDQ Impact score as a measure of impaired functioning.

Reliability and validity of the SDQ have been established in numerous studies, and in several languages and cultures. Population norms exist for various countries. In the US, the SDQ was included in the 2001 National Health Interview Survey Supplement with complete data obtained from a random sample of 9,878 US children ages 4 to 17. US population norms may assist in the interpretation of SDQ mean scores; for example, in the US normative sample combining all ages and sexes, a total SDQ value of 9 fell on the 73.4th centile, and a value of 18 on the 95.2th centile. The SDQ may be downloaded and used without charge for non-commercial purposes (sdqinfo.org/a0.html); US population norms data can be accessed from the same web site.

Social Responsiveness Scale-2nd edition (SRS)[18]—The SRS is a parent-completed measure of autistic symptoms and traits and of associated social impairment. It contains 65 questions assessing child's behavior over the last six months, each scored on a 4-point Likert scale ranging from 0 (*not true*) to 3 (*almost always true*), with 17 items being reverse-scored. Item scores combine into five scale scores, a total raw score and a total t-score. Reliability and validity of the SRS have been extensively examined and found to be excellent. In this study, we used total *T*-scores derived from a normative U.S. general population sample. T-scores over the cut-off of 60 have been suggested to indicate clinically significant ASD symptoms, and t-scores >=75 to predict a clinical diagnosis of ASD.

Statistical analyses

Comparisons between the three groups were performed with chi-square statistics for categorical variables, and ANOVAs for continuous variables. Internal consistency of computed scale scores was measured with Cronbach's alpha coefficient. Because EBP consistently vary by age and sex, between group comparisons of SDQ and Impact Scores were adjusted on both variables. Three-way between-subjects factorial ANOVA models were employed to test the main effects on Total SDQ, EBP and its three constituent subscales, and Impact Scores of group, sex, and age, and their interactions. Correlations between EBP, impact, and SRS scores were examined with Pearson correlation coefficients. Predictors of impairment within the ASD group were evaluated with stepwise multiple linear regression analysis. First, bivariate analyses (ANOVA, chi-square) tested for associations between Impact Score and socio-demographic characteristics. Variables with a weak association ($p<.15$) were retained for the stepwise regression. Next, the regression model was estimated with SRS, EBP and the selected socio-demographic variables entered as independent variables, and the Impact Score as dependent variable. A p-value of .05 was set to retain a predictor in the final model.

Previous studies have documented the failure of multiple regression indices such as beta weights to adequately partition variance of a criterion variable into its predictors when predictors are correlated[19]. To account for expected multicollinearity between SRS and EBP scores in our study, the interpretation of the regression model was therefore enhanced by 3 additional analyses[20,21]. First we computed the product measure (product of zero-order correlation and β weight) that sums to R^2 and provides non-overlapping partitions of the regression effect. Likewise, squared structure coefficients (correlation between a predictor and impairment score predicted from the regression equation) were calculated to estimate the variance in impairment attributable to a predictor adjusting on other predictors' effects. Second, we performed a commonality analysis to estimate how much impairment variance was uniquely explained by each predictor score (SRS and EBP) as opposed to being shared variance. Third, we used relative importance analysis and estimated both relative and dominance weights[19-21]. Relative weights (RW) add up to the R^2 value and partition the variance in a dependent variable (here, the SDQ impact score) into predictor effects (here, the independent variables SRS and EBP of the multiple regression model) that are freed from multicollinearity, permitting us to rank order their respective importance. We calculated 95% confidence intervals for RW using bootstrapping procedures with the RWA-Web software.[22] General dominance weights (GDW) also add up to R^2 and were calculated by averaging the additional variance contribution of a given predictor across all possible regression subset models. Both RWs and GDWs were rescaled to be presented as proportion of R^2 explained by each independent variable. The main results of the relative importance analysis are presented in the Results section; more detailed statistical results are made available in the Tables S3 and S4 of the Supplemental Digital Content.

Unless otherwise indicated (see above Multiple regression analysis), statistical significance was a priori set at 0.01 to account for the large sample size and the high number of comparisons. Effect sizes are reported with partial eta-square (η^2) statistics for three-way ANOVAs, R^2 in multiple linear regression models, and rescaled relative weights and general

dominance weights in the relative importance analysis. Analyses were conducted with SPSS version 26. Commonality analysis was performed with Nimon's SPSS script.[23] Relative weights analysis was performed with Tonidantel and LeBreton's RWA-Web tool;[22] general dominance weights were computed in Excel as per Tonidantel and LeBreton.[19]

This study was approved by the Kaiser Permanente Northwest IRB.

Results

Sample characteristics

Of the 1,267 participants, 79.4% were males; mean age was 9.16 (SD=3.9) with no difference across the 3 groups (Table 1). Compared to the other two groups, race/ethnicity distribution in the ASD group showed slightly more Hispanic and fewer Native Hawai'ian autistic children (*P*=.043; Table 1). Informants were mostly female (86%) and biological parents (95.7%). Statistical differences were found for *caregiver employment status*, *insurance type*, *monthly income*, *presence of another child with a serious health condition*, and *presence of another child with ASD*, the latter in keeping with known familial ASD recurrence risk. There was no statistically significant difference for informant age, gender, educational level, marital status, household size, or race/ethnicity (data not shown).

Behavioral problems and impact on functioning

Internal consistency of the 15-item EBP score was excellent (α=.87). Results of three-way (group x gender x age) between-subjects ANOVAs are provided in Table 2. In comparison to the asthma and control groups and to the published US population norms (Table 2, footnote a), the ASD group had significantly higher Total SDQ, subscale, EBP, and Impact scores. Of all comparisons, Impact showed the largest difference between groups (η^2=.263). For subscales, larger group differences were found for hyperactivity (η^2=.205) than for conduct (η^2=.081) and emotional (η^2=.064) scores. In post-hoc contrasts, all mean scores were significantly higher in ASD than in the asthma and control groups. Asthma scores were slightly higher than control scores but not significantly so. The few sex, age and interaction effects were of small magnitude. Reflecting general trends in developmental psychopathology, hyperactivity symptoms were more pronounced in males than in females, although this sex difference disappeared in the ASD group. Emotional problems increased and oppositional problems decreased with increasing age (Figure 1; and for individual items analyses, see Table S1 in the Supplemental Digital Content).

Predictors of impairment in children with ASD

In order to identify factors predictive of levels of impairment in autistic children, analyses were restricted to the ASD group; 446 had a completed SRS, and 6 were missing the Impact Score, leaving a sample of 440 for the regression analysis. Means, *SD*s and Pearson's correlations for the Impact, SRS and EBP scores are available in Table S2 (Supplemental Digital Content). The mean SRS score in this sample of children with diagnosed ASD was high (*X*=74.9), very close to the SRS value associated with a clinical diagnosis of ASD in other studies[18] providing further evidence of the validity of ASD diagnosis in our study. Both autistic symptomatology and non-autistic behavioral problems were highly

correlated with child's impairment as shown by the high (.64-.65) and significant (P<.01) correlations of SRS and EBP with Impact score. As expected, a high and significant correlation (.632; P<.01) was also found between the SRS and EBP scores indicating substantial multicollinearity.

Bivariate analyses showed no significant association between Impact and study site, child race (non-Hispanic white/other) and sex, parent marital and employment status (employed or not), household income ($0-5,000; $7,000; >=$10,000), presence of another child with ASD (yes/no) or another chronic health condition (yes/no), parental age, or number of people in the household (all P's>.15). Eight variables were associated (P<.15) with Impact: child age (3-5, 6-11, 12-17; P=.076), respondent's gender (P=.068) and race/ethnicity (non-Hispanic white/other; P=.124), number of children in the household (1, 2/3 or more; P=.136), insurance type (uninsured/private/public; P=.073), parent education level (high school or less/some college/college degree/graduate degree or more; P=.042), respondent self-evaluation of physical health (poor to fair/good to excellent; P <.001) and of mental health (poor to fair/good to excellent; P <.001). Stepwise multiple regression with forward selection was employed with Impact Score as dependent variable and EBP, SRS T-score and the eight socio-familial variables as independent variables. The resulting final model was highly significant (F(2,425)=117.2; P <.001) and explained 52.5% of the variance in Impact (Table 3). Of the candidate covariates, only child age and parental education were retained in the final model. The other 6 family-level variables, including self-reported parental mental health, did not contribute further to predicting child's impairment once child behavior variables were taken into account.

Inspection of traditional regression coefficients provides a first examination of which variables contribute to child's impaired functioning. Zero-order correlation coefficients and β weights showed significant positive predictions of SRS and EBP scores to impairment of comparable magnitude whereas child age and parental education contributions to the regression equation were respectively positive and negative, but small and barely significant. The product measure and squared structure coefficients provided a similar preliminary rank ordering of the predictors in the regression model: SRS and EBP accounted for nearly equal parts of the regression effect. Age and parental education were insignificant.

Because of the high multicollinearity between EBP and SRS (see above; and also Table S2 in the Supplemental Digital Content), other approaches were needed to compare the respective contributions to impairment of autism vs emotional/behavioral symptoms by partitioning the variance in the Impact score into unique, non overlapping contributing sources. Commonality analysis showed that a relatively small proportion of impairment variance was accounted for uniquely by both SRS (.113) and EBP (.076); shared variance between the two predictors was substantial. Both relative weights and dominance weights ranked the SRS and EBP in the same order of importance as defined by their overall contribution to R^2 that was at similar levels. Raw relative weights for SRS (0.271; 95% CI: .217-.324) and EBP (0.241; 95% CI: .186-.292) were significantly different from zero, but did not significantly differ from each other (mean difference: 0.03; 95% CI: −0.049-0.114; Table S3 in the Supplemental Digital Content). In sum, all analyses to evaluate the respective role of SRS and EBP in explaining Impairment variance showed

that both predictors shared substantial variance in Impact, each had a relatively small unique contribution, and that each accounted for about 50% of the impairment variance once multicollinearity was removed and other predictor effects taken into account. Further results of the relative importance analysis are presented in Tables S3 and S4 in the Supplemental Digital Content.

Discussion

In this large population-based sample, we observed a greatly increased prevalence of emotional and behavioral problems and resulting impairment in autistic children ages 3 to 17 compared to age- and sex-matched controls with or without a chronic medical condition. The results applied to all types of EBPs, and were not modified by age or sex. Moreover, child and parent/guardian race/ethnicity was not associated with impairment, and the study was well powered to detect any such association. Our in-depth analysis of linkage between impairment and symptom domains showed that, for children with ASD, impairment in functioning across settings and activities, as reported by parents, derived almost as much from EBPs as from autism symptoms per se.

The findings on the prevalence of individual behavioral problems are consistent with high levels of psychiatric comorbidity reported in previous surveys using either dimensional scores or diagnostic categories.[3-6] Several findings unique to our investigation are worth emphasizing. First, results held true when the ASD group was compared not only to a control group of healthy typically developing children but also to a group with another chronic medical condition: asthma. This comparison group allowed us to rule out general poor health as an explanation for the elevated rates of EBPs in children with ASD. Inclusion of children with co-occurring ASD and asthma in the ASD group enhanced the representativeness of our ASD sample but could have theoretically attenuated the ASD/Asthma comparisons; yet, the marked differences observed between the ASD and asthma groups alleviated this concern. Second, our sample spanned a large developmental period, and higher rates of EBPs were found at all ages and across sexes. Third, we found that hyperactivity problems most strongly differentiated the ASD and the two comparison groups, a result that concurs with high rates of ADHD symptoms and diagnoses reported in previous studies of both referred and non-referred autism samples.[3-7] Consistent with prior findings, hyperactivity scores among children with ASD were equally raised in males and in females. Fourth, both EBP and autism symptoms were associated with high levels of impaired functioning in home life, friendships, classroom learning and leisure activities when compared to each comparison group and to U.S. population norms (Table 2, footnote a), stressing the substantial health care needs of children with ASD and their families. Fifth, impairment levels were already high among young children with this early-onset disorder, pointing at the need to screen and detect early associated behavioral problems and to provide individual and family support around the time of diagnosis. Finally, self-reported parental mental health was much poorer among parents of children with ASD compared to parents of non-autistic children; furthermore, the statistical association of parental mental health with child's impairment lost significance once behavioral and autistic symptoms levels were taken into consideration, suggesting that both symptom domains are important to consider for optimization of care through caregiver well-being. Noticeably, parents of youth with

ASD experienced worse employment and income situations when compared to those from the two comparison groups; results from more in-depth analyses of family costs will be published separately.

We were able to partition impairment into its sources, and specifically, to compare the respective independent contributions to impairment of autistic versus EBP symptoms. The demonstration that both classes of symptoms contributed to impairment at the same level is a novel finding that has important implications. First, the co-occurrence of EBP in the developmental course of ASD has been associated with worse outcomes, suggesting it is an important target for intervention.[24,25] Second, our results demonstrating a linkage between poor mental health in caregivers of children with ASD and child's impairment concurs with those from several studies that have examined the reciprocal relationships between EBP and parental stress and/or mental health, documenting bidirectional effects that may contribute to the onset and maintenance of ASD children's EBPs over time.[26,27] Third, although there is no cure for autism, effective interventions exist that can decrease EBPs in a child with ASD, reduce parental stress and improve quality of life of families. Recent randomized clinical trials examining the efficacy of parent training, behavioral interventions, pharmacological treatments, and adaptations of cognitive-behavioral therapies to youth with ASD are showing beneficial effects.[2,28-29] Our study conveys hope that, starting at an early age, systematic detection and management of EBPs in the management of ASD could substantially improve autistic children's functioning and family quality of life.

Until recently, detection and diagnosis of psychiatric symptoms in autistic children remained problematic. Every symptom observed in a child with ASD was assumed to be due to ASD, thereby preventing formal psychiatric diagnosis and treatment, a phenomenon known as overshadowing.[7] Nosographical rules even precluded diagnosing co-occurring ADHD up to the latest DSM revision.[1] Our data confirm recent studies showing that the incidence of EBPs and psychiatric disorders is elevated among children with ASD.[3-7] Moreover, this study identifies EBPs in children with ASD as a substantial source of impairment separate from autistic symptomatology. Professional guidelines promote developmental screening to detect ASD[2] and screening for emotional and behavioral problems in the general pediatric population,[30] yet there is little integration of these two recommendations. Our findings suggest that screening for emotional and behavioral problems should be integrated with developmental screening and combined with access to effective treatment for EBPs. Recommendations for evidence-based treatment of EBP tailored to autistic children are becoming increasingly available.[29]

Strengths of the study include a large population-based sample recruited from diverse sites. The sample was ethnically and racially diverse, with a wide age range, and well powered to detect moderating effects of these factors. The inclusion of two control groups, one with a chronic medical condition, allowed us to establish that the high prevalence of EBPs in our sample was specific to autism and not simply due to poor general health. Outcome measures had good psychometric qualities and are widely regarded as valid instruments to measure autistic or general psychiatric symptomatology. Respondents were unaware of this study objectives making desirability bias unlikely. In addition, for the first time, inclusion of an *ad hoc* measure of impairment allowed us to separate out the contributions of autistic

and general behavioral disturbances to impairment in children's overall functioning. We used statistical techniques that circumvented classic interpretation problems of multiple regression analysis when predictors are correlated.

We acknowledge several study limitations. The design was cross-sectional, preventing causality inference from observed statistical associations. Although not uncommon in surveys, the overall response rate was low; participation bias may have occurred. Participants with ASD were not assessed directly. However, diagnoses were confirmed both by electronic health records and parental report, the validity of which is generally excellent for autism diagnosis[31], and the sample mean SRS score was consistent with published ASD clinical samples, making misclassification on ASD diagnosis very unlikely. Although the inclusion of asthma as a chronic health comparison group was an asset of our study design, alternative comparison groups of chronic health conditions involving the central nervous system (e.g. children with epilepsy, other neurodevelopmental disorders) could be more suitable and should be considered in future investigations. All outcome measures were completed by guardians with correlated error in measurement being a possibility; no school-based measure was available. We could not assess the moderating or mediating effects of children's language skills and intellectual levels. Regression models and relative importance analyses are dependent upon correct model specification. Impairment in a child with ASD can be modulated by familial, school or community characteristics that were not measured and unavailable to us. Replication in longitudinal studies is warranted.

Conclusion

Compared to other children, autistic youth had higher rates of emotional and behavioral problems and impairment across sexes and age groups. Non autistic behavioral problems contributed to impaired functioning as much as autistic symptoms. Systematic detection and management of EBPs could substantially improve autistic children's functioning, starting at an early age.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Funding/Support:

All phases of this study were supported by a NIMH grant RO1 MH106680 (Frances L. Lynch, PI)

Role of Funder/Sponsor:

The NIMH had no role in the design, conduct and interpretation of the study.

Abbreviations:

ASD	Autism Spectrum Disorder
SDQ	Strength and Difficulties Questionnaire
SRS	Social Responsiveness Scale

EBP	Emotional Behavioral Problem
M	male
F	female
RW	Relative Weights
GDW	General Dominance Weights

References

1. American Psychiatric Association. Diagnostic and statistical manual of mental disorders (5th ed.). Arlington, VA: American Psychiatric Publishing; 2013.
2. Hyman SL, Levy SE, Myers SM; Council on children with disabilities, Section on Developmental and Behavioral Pediatrics. Identification, Evaluation, and Management of Children With Autism Spectrum Disorder. Pediatrics. 2020 Jan;145(1):e20193447. [PubMed: 31843864]
3. Lai MC, Kassee C, Besney R, et al. Prevalence of co-occurring mental health diagnoses in the autism population: a systematic review and meta-analysis. Lancet Psychiatry. 2019;6(10):819–829. [PubMed: 31447415]
4. Gadow KD, Devincent CJ, Pomeroy J, et al. Comparison of DSM-IV symptoms in elementary school-age children with PDD versus clinic and community samples. Autism. 2005;9(4):392–415. [PubMed: 16155056]
5. Maskey M, Warnell F, Parr JR, et al. Emotional and behavioural problems in children with autism spectrum disorder. J Autism Dev Disord. 2013;43(4):851–9. [PubMed: 22895777]
6. Russell G, Rodgers LR, Ford T. The strengths and difficulties questionnaire as a predictor of parent-reported diagnosis of autism spectrum disorder and attention deficit hyperactivity disorder. PLoS One. 2013; 8(12): e80247. [PubMed: 24312466]
7. Rosen TE, Mazefsky CA, Vasa RA, et al. Co-occurring psychiatric conditions in autism spectrum disorder. Int Rev Psychiatry. 2018; 30(1):40–61. [PubMed: 29683351]
8. Davignon MN, Qian Y, Massolo M, et al. Psychiatric and Medical Conditions in Transition-Aged Individuals With ASD. Pediatrics. 2018;141(Suppl 4): S335–S345. [PubMed: 29610415]
9. Tyson KE, Cruess DG. Differentiating high-functioning autism and social phobia. J Autism Dev Disord. 2012;42(7):1477–90. [PubMed: 22038291]
10. Mosner MG, Kinard JL, Shah JS, et al. Rates of Co-occurring Psychiatric Disorders in Autism Spectrum Disorder Using the Mini International Neuropsychiatric Interview. J Autism Dev Disord. 2019; 49(9): 3819–32. [PubMed: 31175504]
11. Palmer M, Paris Perez J, Tarver J, et al. Development of the Observation Schedule for Children with Autism-Anxiety, Behaviour and Parenting (OSCA-ABP): A New Measure of Child and Parenting Behavior for Use with Young Autistic Children. J Autism Dev Disord. 2021;51(1):1–14. [PubMed: 32350790]
12. Kerns CM, Kendall PC, Wood JJ, et al. Anxiety disorders interview schedule – autism addendum: Reliability and validity in children with autism spectrum disorder. J Clin Child Adol Psychol. 2016; 46, 88–100.
13. Fombonne E, Green Snyder L, Daniels A, et al. Psychiatric and Medical Profiles of Autistic Adults in the SPARK Cohort. J Autism Dev Disord. 2020;50(10):3679–98. [PubMed: 32096123]
14. Plesa Skwerer D, Joseph RM, Eggleston B, et al. Prevalence and Correlates of Psychiatric Symptoms in Minimally Verbal Children and Adolescents With ASD. Front Psychiatry. 2019;10:43. [PubMed: 30833910]
15. Fombonne E, Zuckerman K. Clinical profiles of Black and White children referred for autism diagnosis. J Autism Dev Disord. 2021 Apr 19. doi: 10.1007/s10803-021-05019-3. Epub ahead of print. PMID: 33871736.

16. Zuckerman KE, Chavez AE, Wilson L, et al. Improving autism and developmental screening and referral in US primary care practices serving Latinos. Autism. 2021;25(1):288–299. [PubMed: 32921144]
17. Goodman R. The extended version of the Strengths and Difficulties Questionnaire as a guide to child psychiatric caseness and consequent burden. J Child Psychol Psychiatry. 1999; 40: 791–9. [PubMed: 10433412]
18. Constantino JN, Gruber CP. Social Responsiveness Scale. 2nd ed. Los Angeles, CA: Western Psychological Services; 2012.
19. Tonidandel S, LeBreton JM Relative importance analyses: A useful supplement to multiple regression analyses. J Bus Psychol. 2011; 26, 1–9. doi:10.1007/s10869-010-9204-3.
20. Nathans LL, Oswald FL, Nimon K. Interpreting Multiple Linear Regression: A Guidebook of Variable Importance, Practical Assessment, Research, and Evaluation. (2012). 17, Article 9. DOI: 10.7275/5fex-b874
21. Kraha A, Turner H, Nimon K, et al. Tools to support interpreting multiple regression in the face of multicollinearity. Front Psychol. 2012; 3:44. [PubMed: 22457655]
22. Tonidandel S, LeBreton JM. RWA Web: A Free, Comprehensive, Web-Based, and User-Friendly Tool for Relative Weight Analyses. J Bus Psychol. 2015; 30:207–216.
23. Nimon K. Regression commonality analysis: Demonstration of an SPSS solution. Multiple Linear Regression Viewpoints. 2010; 36(1), 10–17.
24. Visser JC, Rommelse NNJ, Lappenschaar M, et al. Variation in the Early Trajectories of Autism Symptoms Is Related to the Development of Language, Cognition, and Behavior Problems. J Am Acad Child Adolesc Psychiatry. 2017;56(8):659–668. [PubMed: 28735695]
25. McCauley JB, Elias R, Lord C. Trajectories of co-occurring psychopathology symptoms in autism from late childhood to adulthood. Dev Psychopathol. 2020;32(4):1287–1302. [PubMed: 32677592]
26. Rodriguez G, Hartley SL, Bolt D. Transactional Relations Between Parenting Stress and Child Autism Symptoms and Behavior Problems. J Autism Dev Disord. 2019;49(5):1887–1898. [PubMed: 30623270]
27. Yorke I, White P, Weston A, et al. The Association Between Emotional and Behavioral Problems in Children with Autism Spectrum Disorder and Psychological Distress in Their Parents: A Systematic Review and Meta-analysis. J Autism Dev Disord. 2018;48(10):3393–3415. [PubMed: 29777471]
28. Iadarola S, Levato L, Harrison B, et al. Teaching Parents Behavioral Strategies for Autism Spectrum Disorder (ASD): Effects on Stress, Strain, and Competence. J Autism Dev Disord. 2018;48(4):1031–1040. [PubMed: 28988339]
29. Ameis SH, Kassee C, Corbett-Dick P, et al. Systematic review and guide to management of core and psychiatric symptoms in youth with autism. Acta Psychiatr Scand. 2018;138(5):379–400. [PubMed: 29904907]
30. Weitzman, Wegner, the Section on Developmental and Behavioral Pediatrics, Committee on Psychosocial Aspects of Child and Family Health, Council on Early Childhood, and Society for Developmental and Behavioral Pediatrics. Promoting Optimal Development: Screening for Behavioral and Emotional Problems. Pediatrics. 2015;135(2):384–395. [PubMed: 25624375]
31. Fombonne E, Coppola L, Mastel S, et al. Validation of autism diagnosis and clinical data in the SPARK cohort. J Autism Dev Disord. 2021 Jul 30. doi: 10.1007/s10803-021-05218-y. Epub ahead of print. PMID: 34328611.

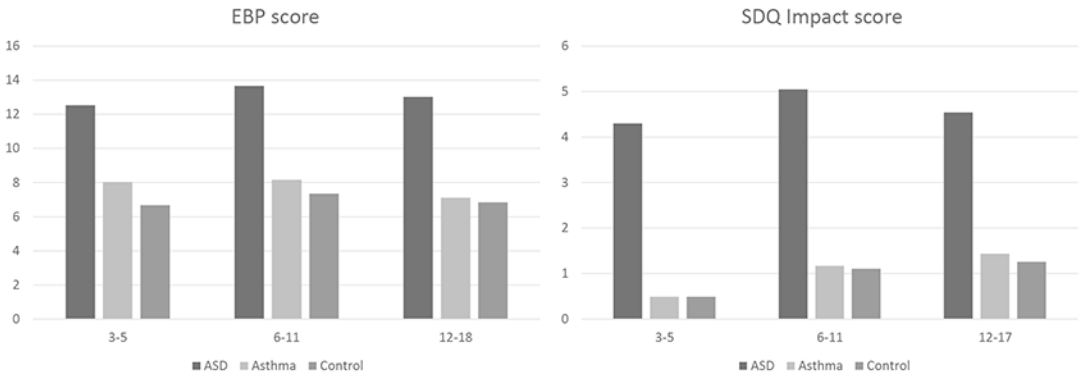


Figure 1: Emotional-Behavioral Problems (EBP) and impairment, by age and clinical group
Abbreviations: EBP: Emotional and Behavioral Problems; SDQ: Strengths and Difficulties Questionnaire; ASD: Autism Spectrum Disorder

Table 1.

Sample socio-demographic characteristics, by clinical group (N=1,267)

	All groups N=1,267	ASD N=472	Asthma N=410	Controls N=385	*P*
Age, N (%)					
• 3 to 5 years	282 (22.3)	104 (22.0)	77 (18.8)	101 (26.2)	.085
• 6 to 11 years	575 (45.4)	205 (43.4)	198 (48.3)	172 (44.7)	
• 12 to 18 years	410 (32.4)	163 (34.5)	135 (32.9)	112 (29.1)	
Gender male, N (%)	1,006 (79.4)	363 (76.9)	333 (81.2)	310 (80.5)	.23
Race/Ethnicity, N (%)					
White	553 (43.9)	215 (46.1)	162 (39.5)	176 (45.7)	.043
Hispanic	270 (21.4)	111 (23.8)	89 (21.7)	70 (18.2)	
Black or African American	44 (3.5)	15 (3.2)	19 (4.6)	10 (2.6)	
Asian	120 (9.5)	43 (9.2)	43 (10.5)	34 (8.8)	
Native Hawaiian Other Pacific Islander	109 (8.6)	26 (5.6)	45 (11.0)	38 (9.9)	
Other race/ethnicity or More than one	165 (13.1)	56 (12.2)	52 (12.7)	57 (14.8)	
Parent/Guardian female gender, N (%)	1,052 (86.0)	380 (85.0)	351(88.0)	321 (85.1)	.37
Parent age, X (SD)	40.1 (7.5)	40.3 (7.3)	39.8 (8.2)	40.2 (7.1)	.57
Parent/caregiver education, N (%)					
• High school or less	170 (14.0)	56 (12.6)	62 (15.7)	52 (13.8)	.67
• Some college	290 (23.9)	109 (24.6)	100 (25.3)	81 (21.5)	
• College degree	420 (34.6)	161 (36.3)	126 (31.9)	133 (35.4)	
• Graduate degree	334 (27.5)	117 (26.4)	107 (27.1)	110 (29.3)	
Parent/caregiver marital status, N (%)					

	All groups N=1,267	ASD N=472	Asthma N=410	Controls N=385	*P*
• Married/Living with partner	963 (79.0)	360 (80.9)	307 (77.1)	296 (78.7)	.12
• Single	124 (10.2)	32 (7.2)	53 (13.3)	39 (10.4)	
• Separated/Divorced	118 (9.7)	47 (10.6)	35 (8.8)	36 (9.6)	
• Widowed	14 (1.1)	6 (1.3)	3 (0.8)	5 (1.3)	
Employed, N (%)	961 (77.3)	342 (73.1)	320 (79.8)	299 (79.7)	.024
Monthly income ($), X (SD)	6,640 (5,462)	6,191 (4,264)	6,582 (6,200)	7,258 (5,863)	.024
Number of people in household, X (SD)	4.32 (1.4)	4.28 (1.3)	4.42 (1.5)	4.27 (1.4)	.20
Insurance type, N (%)					
• Uninsured	77 (6.2)	14 (3.0)	22 (5.5)	41 (10.8)	<.001
• Private	847 (67.7)	309 (65.7)	263 (65.3)	275 (72.8)	
• Public	327 (26.1)	147 (31.3)	118 (29.3)	62 (16.4)	
Another child with a serious health condition, N (%) [1]	335 (37.5)	137 (41.4)	140 (47.6)	58 (21.6)	<.001
Another child with ASD, N (%) [1]	53 (5.9)	45 (13.6)	4 (1.4)	4 (1.5)	<.001
Parent self-report on physical health, N (%)					
Poor/fair	223 (18.3)	97 (21.8)	74 (18.6)	52 (13.8)	
Good	451 (37.1)	159 (35.8)	152 (38.3)	140 (37.2)	.013
Very good	395 (32.5)	127 (28.6)	136 (34.3)	132 (35.1)	
Excellent	148 (12.2)	61 (13.7)	35 (8.8)	52 (13.8)	
Parent self-report on mental health, N (%)					
Poor/fair	152 (12.6)	71 (16.1)	40 (10.1)	41 (11.0)	

	All groups N=1,267	ASD N=472	Asthma N=410	Controls N=385	*P*
Good	377 (31.2)	139 (31.5)	138 (34.8)	100 (26.8)	
Very good	434 (35.9)	149 (33.8)	142 (35.9)	143 (38.3)	.017
Excellent	247 (20.4)	82 (18.6)	76 (19.2)	89 (23.9)	

1: Only includes households with at least two children

Abbr:. ASD: Autism Spectrum Disorder

Table 2:

Strength and Difficulties Questionnaire (SDQ) mean scores, by clinical group

	ASD (N=472)		Asthma (N=410)		Controls (N=385)		3-way ANOVA			
	mean	SD	mean	SD	Mean	SD	Group F, *P*, η^2	Sex *b* F, *P*, η^2	Age F, *P*, η^2	Interactions F, *P*, η^2
Total SDQ score	18.14 *a*	6.5	9.39 *a*	6.3	8.63 *a*	6.2	216.2, <.001, .257 ASD > Asthma, Controls	ns	ns	-
Emotional subscale	3.55	2.7	2.20	2.1	1.94	2.2	42.4, <.001, .064 ASD > Asthma, Controls	ns	22.2, <.001, .034 3-5 < 6-11 < 12-18	Sex x Age 4.7, .009, .007 M: 6-11 = 12-18
Conduct subscale	2.73	2.0	1.52	1.6	1.46	1.6	55.2, <.001, .081 ASD > Asthma, Controls	ns	7.7, <.001, .012 3-5 > 12-18	-
Hyperactivity subscale	6.91	2.5	4.08	2.7	3.62	2.7	161.1, <.001, .205 ASD > Asthma, Controls	12.8, <.001, .010 M>F	ns	Sex x Group 4.9, .007, .008 ASD: M=F
EBP score	13.19	5.3	7.79	5.2	7.02	5.2	136.0, <.001, .179 ASD > Asthma, Controls	ns	ns	-
Impact score	4.71 *a*	3.0	1.13 *a*	2.1	0.99 *a*	2.1	219.3, <.001, .263 ASD > Asthma, Controls	ns	6.8, =.001, .011 3-5 < 6-11, 12-18	-

Abbreviations.: SDQ: Strength and Difficulties Questionnaire; SRS: Social Responsiveness Scale; EBP: Emotional Behavioral Problem score (sum of 3 SDQ subscale scores: Emotional, Hyperactivity, Conduct); ASD: Autism Spectrum Disorder;

a: Using US population SDQ norms (N=9,878) for ages 4-17 (www.sdqinfo.org), the Total SDQ score had a mean of 7.1 (SD=5.7) and the Impact score of 0.4 (SD=1.3). The percentiles of Total SDQ score were: 68.3th for 8, 73.4th for 9, 77.9th for 10, 95.2th for 18. For the Impact score, the percentiles were: 87.8th for 0, 91.6th for 1, 97th for 4 and 98th for 5.

b: M= male; F= female

Table 3.

Predictors of impairment in 440 children with ASD

Predictor	B (SE)	β	*p*	r_s	r_s^2	Pearson's r	Product measure	U	C	Total (U+C)	RW	GDW
Constant	−5.652 (.627)											
SRS t-score	.102 (.010)	.437	<.001	.901	.812	.653	.285	.113	.313	.426	51.7	51.5
EBP score	.200 (.024)	.358	<.001	.880	.774	.637	.228	.076	.329	.406	45.9	46.0
Age 6-11	.516 (.203)	.086	.017	.141	.020	.099	.009	.007	.002	.010	1.7	1.7
Parental education: High school or less	−.718 (.300)	−.081	.011	−.043	.002	−0.031	.003	.006	−.005	.001	0.7	0.8

Abbr.: SRS: Social Responsiveness Scale; EBP: Emotional Behavioral Problem score (see text)

Explanatory note: In this multiple regression analysis, the SDQ impact score that measures impairment in child's functioning was regressed on 10 pre-selected variables (8 socio-familial variables, the Emotional Behavioral Problem score (EBP), the autism symptomatology score (SRS)). Only 4 variables contribute significantly to the final model. Child age and parental education have negligible effects whereas emotional/behavioral problems and autistic symptoms strongly and independently predict child's impairment and at about the same level (see text for details).

Model summary: multiple R=.724; R^2=.525; R^2_{adj}=.520;

Regression statistics: B (SE): regression weights (standard error); β: standardized regression weights; r_S: structure coefficient (r/R); r_S^2: squared structure coefficient; r: zero-order correlation between predictor and impairment variable; Product measure: predictor contribution to R^2 (product of r and β)

Commonality analysis: U: % of impairment variance explained uniquely by predictor; C: % of impairment variance explained by the predictor that is shared with one or more other predictors; Total: % of impairment variance (unique and shared) explained by the predictor; See Table S2 for detailed results on commonality coefficients and relative weights

Relative importance analysis: RW: Relative Weights; GDW: General Dominance Weights. Raw RW and GDW sum up to R^2 (see Table S2). In the Table, RW and GDW have been rescaled to 100% and values represent the proportion of R^2 explained by the predictor.

Neurotherapeutics (2015) 12:837–847
DOI 10.1007/s13311-015-0371-9

REVIEW

Endocannabinoid Signaling in Autism

Bhismadev Chakrabarti[1] · **Antonio Persico**[2,3] · **Natalia Battista**[4] · **Mauro Maccarrone**[2,5]

Published online: 28 July 2015

Abstract Autism spectrum disorder (ASD) is a complex behavioral condition with onset during early childhood and a lifelong course in the vast majority of cases. To date, no behavioral, genetic, brain imaging, or electrophysiological test can specifically validate a clinical diagnosis of ASD. However, these medical procedures are often implemented in order to screen for syndromic forms of the disorder (i.e., autism comorbid with known medical conditions). In the last 25 years a good deal of information has been accumulated on the main components of the "endocannabinoid (eCB) system", a rather complex ensemble of lipid signals ("endocannabinoids"), their target receptors, purported transporters, and metabolic enzymes. It has been clearly documented that eCB signaling plays a key role in many human health and disease conditions of the central nervous system, thus opening the avenue to the therapeutic exploitation of eCB-oriented drugs for the treatment of psychiatric, neurodegenerative, and neuroinflammatory disorders. Here we present a modern view of the eCB system, and alterations of its main components in human patients and animal models relevant to ASD. This review will thus provide a critical perspective necessary to explore the potential exploitation of distinct elements of eCB system as targets of innovative therapeutics against ASD.

Key Words Autism spectrum disorder · endocannabinoid system · fragile X syndrome · metabolic regulation · reward system

B. Chakrabarti, A. Persico and N. Battista are equal first authors.

✉ Mauro Maccarrone
m.maccarrone@unicampus.it

[1] Centre for Integrative Neuroscience and Neurodynamics, School of Psychology and Clinical Language Sciences, University of Reading, Reading, UK

[2] Center of Integrated Research and School of Medicine, Campus Bio-Medico University of Rome, Via Alvaro del Portillo 21, 00128 Rome, Italy

[3] Mafalda Luce Center for Pervasive Developmental Disorders, Milan, Italy

[4] Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Teramo, Italy

[5] European Center for Brain Research (CERC)/Santa Lucia Foundation, Rome, Italy

The Endocannabinoid System

Twenty-five years after the cloning and expression of a complementary DNA that encoded a G protein-coupled receptor, named type-1 cannabinoid (CB_1) receptor [1], there is a good deal of information on the main components of the so-called "endocannabinoid (eCB) system", as well as on its role in controlling cannabinergic signaling in human health and disease [2–4]. Anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are the most active eCBs as yet identified, although this family of bioactive lipids includes other arachidonic acid (AA) derivatives with cannabimimetic properties (i.e., noladin ether, virodhamine, *N*-arachidonoyldopamine, to name but a few). The classical dogma that eCBs are synthesized and released "on demand" upon (patho)physiological stimuli has been recently revisited on the basis of unexpected evidence for intracellular reservoirs and transporters of eCBs. These new entities have been shown to drive intracellular trafficking of eCBs, adding a new dimension to the regulation of their biological activity [5]. To date, several metabolic routes have

been described for AEA biosynthesis [6, 7], yet the most relevant pathway is believed to begin with the transfer of AA from the *sn*-1 position of 1,2-*sn*-di-arachidonoyl-phosphatidylcholine to phosphatidylethanolamine, generating the AEA precursor *N*-arachidonoyl-phosphatidylethanolamine. The latter compound is next cleaved by a specific *N*-acyl-phosphatidylethanolamine (NAPE)-specific phospholipase D, which has been characterized in detail [8]. However, the degradation of AEA to AA and ethanolamine is mainly due to 2 fatty acid amide hydrolases (FAAH and FAAH-2) [9, 10]. When FAAH and FAAH-2 are inhibited, *N*-acylethanolamine-hydrolyzing acid amidase cleaves AEA in an alternate route [11, 12]. The main enzymes responsible for AEA metabolism are reported in Fig. 1.

Much like AEA, the biological activity of 2-AG is controlled through cellular mechanisms that include: 1) synthesis through rapid hydrolysis of inositol phospholipids by a specific phospholipase C to generate diacylglycerol that is then converted into 2-AG by a *sn*-1-specific diacylglycerol lipase (DAGL) [13]; and 2) degradation to AA and glycerol by a monoacylglycerol lipase (MAGL) [14], as schematically represented in Fig. 1. AEA and 2-AG can be also oxidized by cyclooxygenase-2, different lipoxygenase isozymes, as well as by cytochrome P450, to generate, respectively, prostaglandin–ethanolamides [15] and prostaglandin-glyceryl esters [16], hydroxy-anandamides and hydroxyleicosatetraenoyl-glycerols [17], and epoxy-eicosatrienoyl-glycerols [18].

An open question concerning eCB metabolism remains the transport of these compounds across the plasma membrane [19, 20]. As yet, the most accepted mechanisms are: 1) passive diffusion, which can be favored by the formation of AEA–cholesterol complexes, possibly in preferred microdomains called "lipid rafts" [21, 22]; 2) facilitated transport through a purported eCB membrane transporter [23]; and 3) endocytosis assisted by caveolins (reviewed in [24]). Once taken up, intracellular AEA reaches distinct sites, where distinct metabolic and signaling pathways take place. Heat shock protein 70, and albumin and fatty acid binding proteins 5 and 7 have been shown to act as eCB intracellular transporters, able to ferry AEA (and likely also 2-AG) within the cytoplasm to the nucleus and other destinations, including storage compartments like adiposomes [5, 25].

eCBs act principally through type-1 and type-2 (CB_1 and CB_2) cannabinoid receptors. Interestingly, CB_1 but not CB_2 resides within lipid rafts, and their interaction with these specialized microdomains influences signal transduction thereof [26]. Additionally, eCBs are also able to interact with non-CB_1/non-CB_2 targets, such as 1) the transient receptor potential vanilloid type 1 channel, which is activated by both AEA and 2-AG [27, 28]; 2) peroxisome proliferator-activated receptor-α and peroxisome proliferator-activated receptor-γ [29]; and 3) the orphan G protein-coupled receptor GPR55 [30, 31]. By interacting with these receptors, eCBs trigger a multiplicity of signaling pathways that are involved in both physiological and pathological conditions [32]. On a final note, the existence of compounds structurally related to eCBs, and collectively known as "eCB-like" substances, should be recalled because of their "entourage effect". These compounds potentiate eCB activity at their receptors by increasing binding affinity or by inhibiting eCB hydrolysis [33–35]. A schematic representation of eCBs, their molecular targets, biosynthetic and hydrolizing enzymes, and extra- and intracellular transporters, is depicted in Fig. 1.

Autism Spectrum Disorder: Clinical Traits, Neuropsychological Deficits, and Neuroanatomical Underpinnings

Autism spectrum disorder (ASD) is a complex behavioral condition with onset during early childhood and a lifelong course in the vast majority of cases. It is characterized by deficits in communication and social interaction, as well as by stereotypic behaviors, restricted patterns of interest, and abnormal sensory issues [36]. The diagnosis is based on clinical observation, substantiated by standardized testing of the patient with the Autism Diagnostic Observation Schedule-Generic [37], later revised into the Autism Diagnostic Observation Schedule-2 [38], and/or by parental interview with the Autism Diagnostic Interview-Revised [39]. To date, no behavioral, genetic, brain imaging, or electrophysiological test can specifically validate a clinical diagnosis of ASD, although these medical procedures are regularly implemented in order to screen for syndromic forms of the disorder (i.e., autism due to known medical conditions). Two essential features distinguish ASD from most other behavioral disorders: 1) an impressive clinical and pathogenetic heterogeneity, which has led to the designaton, by the term "autisms", of a set of neurodevelopmental disorders with early onset in life, sharing autism as a common feature, but produced through distinct processes [40]; 2) the distribution of autistic features as a dimensional *continuum* in the general population, which fully justifies referring to the "autism spectrum" rather than to a categorical distinction between "affected" and "unaffected" [41, 42]. As many as 1 in 68 (1.6 %) 8-year-old children receive an ASD diagnosis [43], with a male:female ratio of 4:1. Siblings of children already diagnosed with ASD have a significantly higher incidence of the same disorder, reported at 18.7 % in a prospective follow-up study [44], and ranging from 15 % to 25 % depending on sex and clinical severity. The prospective follow-up of these siblings later diagnosed with ASD has led to the observation that some behavioral abnormalities can appear very early on (e.g.,, sensory issues [e.g., extreme responses to certain sounds/textures, fascination with lights/spinning objects] are already present at 7 months of age), others emerge at 12–14 months (e.g., disengagement

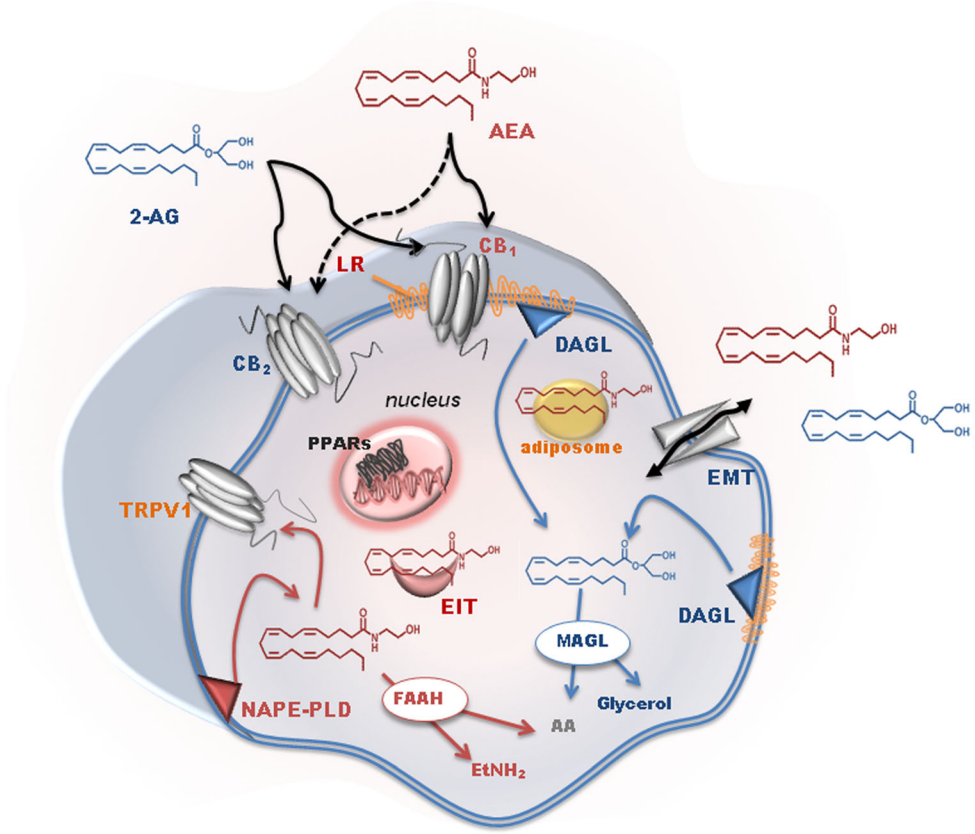


Fig. 1 Schematic representation of the main elements of the endocannabinoid (eCB) system. *N*-arachidonoylethanolamine (AEA) is mainly synthesized by the sequential activity of *N*-acyltransferase (not shown) and *N*-acylphosphatidyl-ethanolamine (NAPE)-specific phospholipase D (NAPE-PLD). The intracellular degradation of AEA is due to a fatty acid amide hydrolase (FAAH) that generates ethanolamine ($EtNH_2$) and arachidonic acid (AA). 2-Arachidonoylglycerol (2-AG) is released from membrane lipids through the activity of diacylglycerol lipase (DAGL), and can be hydrolyzed by a cytosolic monoacylglycerol lipase (MAGL), which releases glycerol and AA. Cyclooxygenase-2, lipoxygenase isozymes and cytochrome P450 were omitted for the sake of clarity. Extracellular eCBs can cross the plasma membrane through a purported eCB membrane transporter (EMT), and then they are trafficked within the cytoplasm through eCB intracellular transporters (EIT), which deliver them to their different targets or, alternatively, to storage organelles like adiposomes. Both AEA and 2-AG trigger several signal transduction pathways by acting at type-1 and type-2 cannabinoid receptors (CB_1 and CB_2, respectively), or at other non-CB_1/non-CB_2 targets, such as peroxisome proliferator-activated receptors (PPARs) in the nucleus. CB_1, but not CB_2, resides within specialized membrane microdomains enriched in cholesterol and sphingolipids, which are called lipid rafts (LR). AEA (and also 2-AG) can also translocate to the inner membrane leaflet, where it binds to transient receptor potential vanilloid type 1 (TRPV1) channels

of visual attention), while the bulk of more typical autistic abnormalities has an onset between 14 and 24 months [45–48]. Frequently, comorbid conditions include intellectual disability (65 %), seizures (30 %), and different forms of sleep problems [49–51]; less recognized, but equally impairing, are frequent psychiatric comorbidities, that include anxiety disorders, obsessive–compulsive disorders, and depression [52]. Altered neurodevelopment during early pregnancy represents the neuropathological cause of ASD [53, 54]. Postmortem studies have unveiled neuroanatomical and cytoarchitectonic abnormalities in the cerebellum, inferior olivary complex, deep cerebellar nuclei, hippocampus, amygdala, entorhinal cortex, fusiform gyrus, and anterior and posterior cingulate cortex, with thinner cortical minicolumns, excessive growth of the frontal lobes, and excessive dendritic spine density [55]. These abnormalities are suggestive of derangements occurring during the first/second trimester of pregnancy, namely reduced programmed cell death and/or increased cell proliferation, altered cell migration, abnormal cell differentiation with reduced neuronal body size, abnormal neurite sprouting, and pruning that result in atypical cell–cell wiring. In addition, neurodevelopmental mechanisms extending into late prenatal/postnatal life include reduced synapse formation and delayed myelination [40, 56, 57]. The latter result in abnormal neuronal wiring, which was previously believed to be characterized by long-range hypoconnectivity and local hyperconnectivity [58], but more recently has been shown to be a highly individualized mix of hyper- and hypoconnectivity specific to each single patient with ASD [59]. These abnormalities have been associated with deficits in multiple behavioral tasks that relate to social behavior, such as empathy, theory of mind, joint attention, and face and emotion

processing. Many of these observed behavioral features suggest a deficit in the social reward processing system in ASD, as we discuss in the following section. The neurocognitive phenotype in ASD stems from a complex and highly heterogeneous array of genetic and environmental causes, with patients ranging from "purely genetic" cases due to known ASD-causing chromosomal aberrations or mutations to "purely environmental" cases due to rare prenatal exposure to specific viral agents, drugs, and toxins [60–62]. In between these extremes, ASD for most cases fully qualifies for the definition of a "complex" disorder, whereby a host of rare and common genetic variants, often but not necessarily in conjunction with epigenetic factors [63], yield the neurodevelopmental abnormalities summarized above, resulting in autistic behaviors. Finally, neuroinflammation is also a frequent finding in postmortem brains of autistic individuals [64, 65]. It may represent a nonspecific consequence of insufficient neurite pruning and abnormal wiring of neural networks, resulting in elevated oxidative stress (possibly a common feature shared by several neurodevelopmental disorders) [66], but it could also stem from a broader immune dysfunction which, together with gastrointestinal disturbances and recurrent infections, collectively qualifies ASD as a systemic disorder [67–71].

Autism and Reward System

A deficit in theory of mind and empathy has commonly been suggested to underlie atypical social behavior in individuals with ASD [72]. A set of recent studies raises the possibility that some of these social behavioral deficits in ASD arise due to deficits in reward system functioning [73–75]. This hypothesis is supported by studies that report a lack of social motivation in children with autism [76, 77]. One rationale for the social motivation-based account of ASD relies on the following premise: if individuals with ASD do not find social stimuli rewarding, and hence do not attend to them as much as neurotypicals, then they are less likely to exhibit empathy toward them. An alternative formulation of the social motivation hypothesis suggests that the attention of individuals with and without ASD is drawn to social stimuli to a comparable extent, but individuals with ASD find social stimuli less rewarding, which leads to the observed deficits in empathy (Fig. 2).

Both of these possible accounts of the social motivation hypothesis are faced with a key question. Do people with ASD find social stimuli rewarding or do they have a domain-general dysfunction of the reward system? To test these possibilities, a number of studies have compared processing of social and nonsocial reward stimuli in people with and without ASD. An early study using a continuous performance task found no behavioral evidence for group differences in trials with monetary rewards *versus* nonrewards [78]. Similarly, comparable behavioral performance in a reward-processing task using a variant of the go–no-go task was found in children and adults with and without ASD [79, 80]. In contrast, using a similar task, DeMurie et al. [81] demonstrated that children with ASD were slower in responding to social rewards than monetary rewards, but this was not specific to ASD. Overall, the behavioral evidence summarized above appears equivocal about any circumscribed deficit in social reward processing in ASD. Yet, a main group effect in the majority of these studies points toward a domain-general deficit in the reward system. In contrast to the behavioral studies using button-press responses, eyegaze tracking, electroencephalography, and functional magnetic resonance imaging studies suggest clear differences in processing of social rewards in ASD compared with typically developed controls [82]. Eyegaze tracking studies typically involve measuring gaze fixation patterns in response to social and nonsocial stimuli. Children and adults with ASD are found to look less at social stimuli than at nonsocial stimuli [83–85]. Gaze fixation patterns have often been used as proxy metrics related to reward processing [86], thus supporting the hypothesis of atypical processing of social rewards in ASD. Using electroencephalography in children with ASD, a recent study reported lower magnitude of a component related to reward anticipation (stimulus preceding negativity) in response to social *versus* nonsocial stimuli [87]. Similarly, functional magnetic resonance imaging studies revealed lower activity in the ventral striatum in response to social stimuli (neutral faces) in individuals with ASD [88]. The latter finding is consistent with the observation that a reduced ventral striatal response to happy faces was associated with lower self-reported empathy in individuals with and without ASD [89].

In sum, there is substantial evidence across different techniques to suggest atypical reward processing in ASD. Irrespective of their domain specificity, such functional differences in the reward circuit in ASD have important consequences for the processing of social stimuli. Atypical response to social rewards from an early age can result in deficits in learning about the social world, which, in turn, can lead to social behavioral impairments in adulthood.

Alterations of the eCB System in Autism

The complexities that make autism hard to understand, from the diagnostic criteria and clinical heterogeneity to the genetic/environmental causes that provoke communication and behavioral problems to the innovative therapies to be applied in order to give patients the best quality of life, encourage scientists to look at predictive biomarkers and/or therapeutic targets for the pharmacological management of this disorder [90, 91]. It is now clear that eCB system is altered in several neurodegenerative diseases and, very interestingly, that

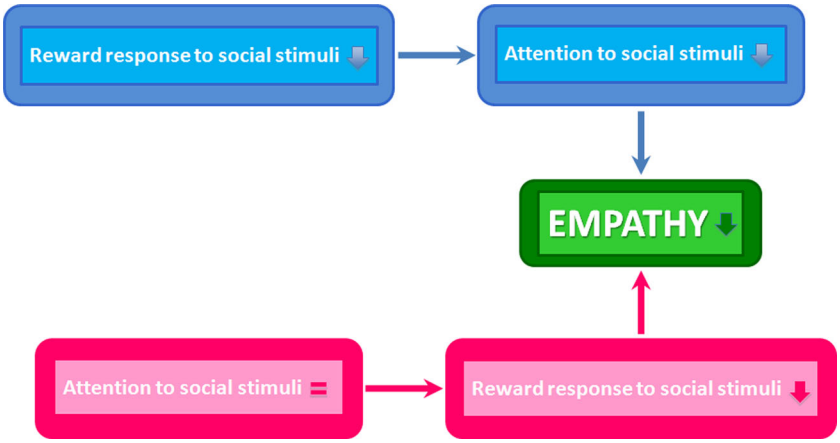


Fig. 2 Two possible routes through which atypical reward processing can lead to reduced empathy as seen in ASD. (Top panel) The first of these routes suggest that a lower reward response to social stimuli reduces the salience of social stimuli and hence how much attention they capture. The reduced empathy is therefore a product of this reduced attention to social stimuli. (Bottom panel) A second possible route suggests comparable salience for social and nonsocial stimuli in ASD, but lower value for social stimuli in ASD, leading to reduced empathy

distinct elements of the eCB system in peripheral blood mirror these perturbations, providing novel and noninvasive diagnostic tools for several neuroinflammatory diseases [92, 93]. In addition, the eCB system controls emotional responses [94], behavioral reactivity to context [95], and social interaction [96]. Thus, it can be hypothesized that alterations in this endogenous circuitry may contribute to the autistic phenotype. In this and the following sections, we critically discuss the evidence for this proposition from animal and human studies. Recent investigations have addressed the involvement of eCBs in autism, where, unfortunately, the role of these bioactive lipids remains poorly understood. Indeed, autism is uniquely human and there are only a few validated animal models (e.g., *fmr1* knockout mice, BTBR mice, and valproic acid-treated rats), that display autistic-like features. Fragile X syndrome (FXS) is an inherited disorder caused by mutations in *FMR1*, which is translated into the fragile X mental retardation 1 protein, which, in turn, plays a role in the development of synapses [97, 98]. Expansion mutations of *FMR1* produce autistic features in approximately 40 % of patients with FXS, and thus FXS provides a valuable model for identifying novel biomarkers/targets for autism and for dissecting the underlying neurochemical pathways [99]. In the first study addressing eCB system in FXS, it has been reported that the ablation *fmr1* gene causes a dysfunctional 2-AG metabolism, with increasing DAGL and MAGL activities in the striatum of *fmr1*$^{-/-}$ mutants, but unaltered striatal 2-AG levels [100]. According to a more recent study [101], stimulation of 2-AG signaling could be a useful treatment for mitigating FXS symptoms because it is able to normalize synaptic activity through type I metabotropic glutamate activation; additionally, genetic or pharmacological attenuation of CB_1-dependent signal transduction and blockade of the mammalian target of rapamycin pathway might provide alternative strategies to treat autistic patients [102].

A link between the eCB system and autism was put forward by Schultz [103], who proposed that acetaminophen, an antipyretic drug that is metabolized to a potent inhibitor of the purported eCB membrane transporter AM404, could trigger autism by activating CB receptors. In line with this, elevated levels of circulating AEA during pregnancy or in the first postnatal days might interefere with the neurodevelopment of offspring, and might increase the risk of delivering autistic children. Abnormalities in sociability and nociception tests, and alterations of distinct elements of eCB system have been reported in adolescent rats on valproic acid [104]. In particular, mRNA levels of the enzymes responsible for 2-AG metabolism (i.e., DAGL and MAGL), which is disrupted in the FXS model of autism [100], were altered in the cerebellum and hippocampus, whereas endogenous levels of 2-AG in the same regions remained at steady state [104]. Interestingly, the content of AEA, *N*-oleoylethanolamine (OEA), and *N*-palmitoylethanolamine (PEA), all of which are substrates of FAAH, were increased in the hippocampus following exposure to sociability tests, suggesting that a deficit in social play behaviors might be due to reduced AEA levels in critical brain areas [104]. Moreover, the same study documented a down-regulation of GPR55 and PPAR gene expression, supporting a role for these receptors in the cognitive mechanisms involved in autism [104]. Preliminary data also addressed CB_2 as a potential target for autism. Indeed, genomic studies have highlighted an upregulation of mRNA levels of the CB_2A, but not the CB_2B, isoform in the cerebellum of BTBR T+tF/J mice [105], which have an autism-like behavioral phenotype [106]. Also, an independent clinical study performed on young (3–9-year-old) children demonstrated that CB_2 is highly expressed, both at transcriptional and translational levels, in peripheral blood mononuclear cells of patients with autism, compared with matched healthy controls [107]. All the other elements of the eCB system remained unaltered, except for a

slight downregulation of NAPE–phospholipase D mRNA. According to a recent hypothesis on autism and inflammation [108], and in keeping with data on the key role of CB_2 in immune-related pathologies [109–111], it can be speculated that the increase in CB_2 expression may serve a compensatory role with respect to the inflammatory state associated with autism. Thus, the observed enhancement of CB_2 may be a negative feedback response aimed at counteracting the proinflammatory responses implicated in the pathogenesis of this neurobehavioral condition. In this context, it should be also recalled that AEA suppresses the release of proinflammatory cytokines from human lymphocytes through a CB_2-mediated mechanism [112]. The main alterations of the eCB system in human patients and animal models of autism are summarized in Tables 1 and 2, respectively. However, the paucity of the relevant human data and their largely correlational nature do not allow for a systematic comparison with the animal data.

Additionally, it is worth noting that plasma levels of polyunsaturated fatty acids (which are components of eCBs) are lower in patients with autism, and that 2 derivatives of docosahexaenoic acid and eicosapentaenoic acid (also components of eCBs) are able to activate both CB_1 and CB_2 receptors [113]. These data further suggest that a dysregulation of eCB signaling might be driven by diet, resulting in an imbalance of pro- and anti-inflammatory metabolites, and thus favoring the development of autism [114].

Suggested Roles of the eCB System in Autism

As discussed in the previous sections, there is phenotypic evidence across multiple levels that suggests a role for atypical reward system functioning in ASD. It is therefore vital to investigate in detail the eCB system in autism and related endophenotypes, also in view of its key role in modulating mesolimbic dopaminergic neurotransmission. The majority of studies on the eCB system in autism-related endophenotypes in humans have tested the role of the *CNR1*, which is strongly expressed in striatal structures implicated in processing rewards [115]. The previous section presented an overview of largely animal studies that point to a role for eCB signaling in autism-relevant phenotypes. Further clues from both human and animal studies are discussed in the following section, and are summarized in Fig. 3.

Table 1 Molecular markers of the endocannabinod (eCB) system in autism and related phenotypes in humans

Biological sample	Model	eCB alterations	Reference
Postmortem brain	Human	↓CB_1	[121]
PBMCs	Human	↓NAPE–PLD mRNA = FAAH, CB_1 mRNA ↑CB_2 mRNA and protein	[107]
Saliva	Human	CB_1 SNP	[116, 119]

PBMCs=peripheral blood mononuclear cells; CB^1 =type-1 cannabinoid receptor; NAPE–PLD=*N*-acylphosphatidyl-ethanolamine-specific phospholipase D; FAAH=fatty acid hydrolase; CB2=type-2 cannabinoid receptor; SNP=single nucleotide polymorphism

The first clue comes from a human neuroimaging study that measured striatal response to social rewards (happy faces *vs* neutral faces). This investigation found that common single nucleotide polymorphisms in *CNR1* are associated with activity in the ventral striatal cluster in response to happy (but not to disgusted) faces [116], and later on it was replicated in an independent cohort [117]. In view of the central role of the ventral striatum in reward processing, it is reasonable to infer that variation in *CNR1* was linked to differences in sensitivity to social rewards such as happy faces. Another study in an independent sample used eyegaze tracking to show that the same *CNR1* polymorphisms were associated with greater gaze duration to happy faces, but again not to disgust faces [118]. A parallel population genetic study found a nominal association of the same *CNR1* genetic variations with trait empathy [119]. Individuals with ASD score low in trait empathy, and, consistent with this, a gene expression study in postmortem brains of individuals with autism had earlier reported a reduced expression of CB_1 [120, 121]. In sum, multiple indirect lines of evidence suggest a role for *CNR1* genetic variations in underlying social reward responsivity, a putative endophenotype for autism. These findings in human patients parallel observations in animal models that show a strong role for the eCB system in social play behavior, which is a proxy measure for social reward responsivity [122, 123].

A second clue for the role of eCB system in autism comes from observations in early neural development [124]. Autism is neurodevelopmental in nature, and atypical development of neural connectivity has been suggested to underlie its key phenotypic features [125]. A set of genes involved in neurodevelopmental processes that mediate the formation, stabilization, and pruning of synapses has been consistently associated with autism-related phenotypes in animal models [126–128]. Neuroligins (NLGN) represent a significant part of this set, and indeed several genes of NLGN family have been associated with autism [129]. In a mouse model, an autism-associated mutation in NLGN3 was found to be associated with deficits in social behavior and disrupted tonic eCB signaling [130, 131]. Evidence from this study and several others (reviewed in [124]) provides a potential causal bridge between atypical neural development and potential dysfunction of the eCB system in autism.

A third clue comes from the role of the eCB system in influencing circadian rhythm in animal models [132–134]. Autism has been associated with atypical sleep patterns and circadian rhythms [135]. Polymorphisms in *ASMT* (involved in melatonin synthesis), paralleled by reduced levels of circulating melatonin, have been reported in autism [136].

Table 2 Molecular markers of the eCB system in autism-related animal models

Biological sample	Model	eCBs alterations	Reference
Striatum	*fmr1*$^{-/-}$ mouse	=2-AG levels ↑DAGL and MAGL activity	[100]
Cerebellum	Rat valproic acid	= AEA, OEA, PEA and 2-AG levels ↓DAGLα mRNA	[104]
Frontal cortex		= AEA, OEA, PEA and 2-AG levels ↓PPAR-α and ↓GPR55 mRNA	
Hippocampus		↑AEA, ↑OEA, ↑PEA content* ↓PPAR-γ and ↓GPR55 mRNA ↓MAGL mRNA ↑MAGL activity	
Cerebellum	BTBR T+tF/J mouse	↑CB_2A isoform mRNA	[105]

2-AG=2-arachidonoylglycerol; DAGL=diacylglycerol; MAGL=monacylglycerol lipase; AEA=anandamide; OEA=*N*-oleoylethanolamine; PEA=*N*-palmitoylethanolamine; PPAR=peroxisome proliferator-activated receptor

*After sociability tests

Interestingly, the eCB system also plays a role in regulating circadian rhythms [134], thus making it a putative target to examine using animal models of autism-related phenotypes.

The fourth clue comes from comorbidities commonly observed in autism. First of these is anxiety, which is highly comorbid with autism (42–56 %). The eCB system, and cannabidiol in particular, is known to mediate anxiety and related phenotypes [137, 138]. Anecdotal reports of cannabis use in autism suggest a reduction in anxiety-related symptoms. A potential role for the eCB system in ASD can thus also be mediated through its influence on the anxiety-related component of the disease. The second commonly occuring comorbidity in autism relevant to the current review is epilepsy (up to 30 %). The eCB system is studied intensively as targets for potential antiepileptic drugs [139]. It is therefore possible that a potential future drug acting on the eCB system is better able to ameliorate epilepsy-related comorbidities in ASD.

Conclusions

Accumulated evidence suggests that the eCB system constitutes a relatively less investigated piece of a puzzle that brings together 4 phenotypic features known to be atypical in autism: 1) social reward responsivity; 2) neural development; 3) circadian rhythm; and 4) anxiety-related symptoms. Therefore, the potential therapeutic exploitation of distinct elements of this system (e.g., receptor targets, biosynthetic and hydrolytic enzymes, and transmembrane/intracellular transporters)

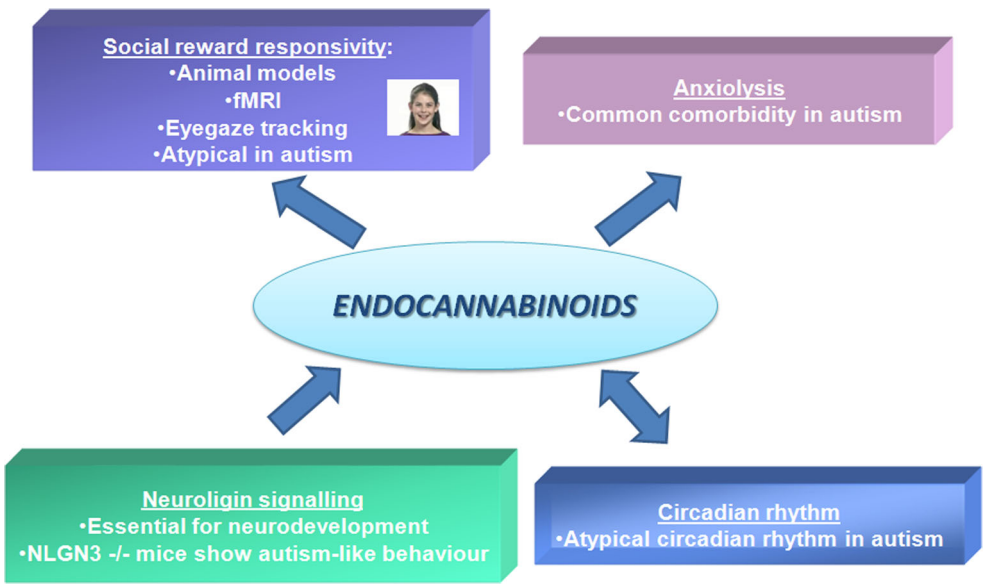


Fig. 3 Four clues pointing to the role of the endocannabinoid (eCB) system in autism. The eCB system constitutes a relatively less investigated piece of a puzzle that brings together 4 phenotypic features known to be atypical in autism: 1) social reward responsivity; 2) neural development; 3) circadian rhythm; and 4) anxiety-related symptoms. fMRI = functional magnetic resonance imaging

seems immense. As supported by the evidence presented in the previous sections in humans and animal models, any potential therapeutic approach is unlikely to involve a simple choice between activation *versus* inhibition of the eCB system to target specific features related to autism. Any such approach will need to be precisely tuned to the developmental timeline and to the specific pathogenetic underpinnings of autism in the single patient. Our understanding of eCB signaling in autism is still in its infancy compared with other disorders of the central nervous system or of peripheral tissues, where eCB-based therapies have already reached preclinical and clinical phases [4]. However, research in this field is rapidly evolving, and novel drugs able to hit specifically a distinct element of the eCB system are developed at a surprising speed [4]. Among them, those that target metabolic enzymes of eCBs, and, at the same time, key enzymes of oxidative pathways like cyclooxygenases seem to hold promise as next-generation therapeutics against human disorders with an inflammatory component [140], and therefore they will possibly result in also being beneficial for ASD. A second medium-term target could focus on the antiepileptic drugs that are being developed, focusing on the eCB system. These drugs could potentially ameliorate the epilepsy-related symptoms that commonly co-occur with ASD. On a final note, it seems of major interest that preliminary data, showing consistency between changes in distinct eCB system elements (i.e., CB_2) in animal models of ASD and in peripherabl blood mononuclear cells from young patients with ASD [106, 107], support a role for these elements in the (early) diagnosis of the disease. Future work should test expression profiles for key players of the eCB system in prospective samples to test the potential of these as diagnostic biomarkers. In this context, it should be recalled that easily accessible biomarkers of neurological disorders are highly searched for, and some of them have been already identified to hold promise in human neurodegenerative/neuroinflammatory diseases [141].

Acknowledgments This investigation was partly supported by Ministero dell'Istruzione, dell'Università e della Ricerca (PRIN 2010-2011 grant) to MM. BC was supported by Medical Research Council UK (grant: G1100359/1).

Required Author Forms Disclosure forms provided by the authors are available with the online version of this article.

References

1. Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 1990;346:561-564.
2. Pacher P, Kunos G. Modulating the endocannabinoid system in human health and disease-successes and failures. FEBS J 2013;280:1918-1943.
3. Maccarrone M, Guzman M, Mackie K, Doherty P, Harkany T. Programming and reprogramming neural cells by (endo-)cannabinoids: from physiological rules to emerging therapies. Nature Rev Neurosci 2014;15:786-801.
4. Maccarrone M, Bab I, Bíró T, et al. Endocannabinoid signaling at the periphery: 50 years after THC. Trends Pharmacol Sci 2015;36:277-296.
5. Maccarrone M, Dainese E, Oddi S. Intracellular trafficking of anandamide: new concepts for signaling. Trends Biochem Sci 2010;35:601-608.
6. Ueda N, Tsuboi K, Uyama T. Metabolism of endocannabinoids and related N-acylethanolamines: canonical and alternative pathways. FEBS J 2013;280:1874-1894.
7. Fezza F, Bari M, Florio R, Talamonti E, Feole M, Maccarrone M. Endocannabinoids, related compounds and their metabolic routes. Molecules 2014;19:17078-17106.
8. Okamoto Y, Morishita J, Tsuboi K, Tonai T, Ueda N. Molecular characterization of a phospholipase D generating anandamide and its congeners. J Biol Chem 2004;279:5298-5305.
9. Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature 1996;384:83-87.
10. Wei BQ, Mikkelsen TS, McKinney MK, Lander ES, Cravatt BF. A second fatty acid amide hydrolase with variable distribution among placental mammals. J Biol Chem 2006;281:36569-36578.
11. Tsuboi K, Sun YX, Okamoto Y, Araki N, Tonai T, Ueda N. Molecular characterization of *N*-acylethanolamine-hydrolyzing acid amidase, a novel member of the choloylglycine hydrolase family with structural and functional similarity to acid ceramidase. J Biol Chem 2005;280:11082-11092.
12. Ueda N, Tsuboi K, Uyama T. *N*-acylethanolamine metabolism with special reference to *N*-acylethanolamine-hydrolyzing acid amidase (NAAA). Prog Lipid Res 2010;49:299-315.
13. Bisogno T, Howell F, Williams G, et al. Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. J Cell Biol 2003;163:463-468.
14. Dinh TP, Freund TF, Piomelli D. A role for monoglyceride lipase in 2-arachidonoylglycerol inactivation. Chem Phys Lipids 2002;121:149-158.
15. Kozak KR, Crews BC, Morrow JD, et al. Metabolism of the endocannabinoids, 2-arachidonylglycerol and anandamide, into prostaglandin, thromboxane, and prostacyclin glycerol esters and ethanolamides. J Biol Chem 2002;277:44877-44885.
16. Kozak KR, Crews BC, Ray JL, Tai HH, Morrow JD, Marnett LJ. Metabolism of prostaglandin glycerol esters and prostaglandin ethanolamides in vitro and in vivo. J Biol Chem 2001;276:36993-36998.
17. Van der Stelt M, van Kuik JA, Bari M, et al. Oxygenated metabolites of anandamide and 2-arachidonoylglycerol: conformational analysis and interaction with cannabinoid receptors, membrane transporter, and fatty acid amide hydrolase. J Med Chem 2002;45:3709-3720.
18. Chen JK, Chen J, Imig JD, et al. Identification of novel endogenous cytochrome p450 arachidonate metabolites with high affinity for cannabinoid receptors. J Biol Chem 2008;283:24514-24524.
19. Fowler CJ. Anandamide uptake explained? Trends Pharmacol Sci 2012;33:181-185.
20. Fowler CJ. Transport of endocannabinoids across the plasma membrane and within the cell. FEBS J 2013;280:1895-1904.
21. Ehehalt R, Füllekrug J, Pohl J, Ring A, Herrmann T, Stremmel W. Translocation of long chain fatty acids across the plasma membrane – lipid rafts and fatty acid transport proteins. Mol Cell Biochem 2006;284:135-140.

22. Di Pasquale E, Chahinian H, Sanchez P, Fantini J. The insertion and transport of anandamide in synthetic lipid membranes are both cholesterol-dependent. PLoS One 2009;4:e4989.
23. Chicca A, Marazzi J, Nicolussi S, Gertsch J. Evidence for bidirectional endocannabinoid transport across cell membranes. J Biol Chem 2012;287:34660-34682.
24. Dainese E, Oddi S, Bari M, Maccarrone M. Modulation of the endocannabinoid system by lipid rafts. Curr Med Chem 2007;14:2702-2715.
25. Oddi S, Fezza F, Pasquariello N, et al. Evidence for the intracellular accumulation of anandamide in adiposomes. Cell Mol Life Sci 2008;65:840-850.
26. Maccarrone M, Bernardi G, Finazzi Agrò A, Centonze D. Cannabinoid receptor signalling in neurodegenerative diseases: a potential role for membrane fluidity disturbance. Br J Pharmacol 2011;163:1379-1390.
27. Di Marzo V, De Petrocellis L. Endocannabinoids as regulators of transient receptor potential (TRP) channels: a further opportunity to develop new endocannabinoid-based therapeutic drugs. Curr Med Chem 2010;17:1430-1449.
28. Zygmunt PM, Ermund A, Movahed P, et al. Monoacylglycerols activate TRPV1—a link between phospholipase C and TRPV1. PLoS One 2013;8:e81618.
29. Pistis M, Melis M. From surface to nuclear receptors: the endocannabinoid family extends its assets. Curr Med Chem 2010;17:1450-1467.
30. Moriconi A, Cerbara I, Maccarrone M, Topai A. GPR55: current knowledge and future perspectives of a purported "type-3" cannabinoid receptor. Curr Med Chem 2010;17:1411-1429.
31. Ross RA. L-α-lysophosphatidylinositol meets GPR55: a deadly relationship. Trends Pharmacol Sci 2011;32:265-269.
32. Di Marzo V, Stella N, Zimmer A. Endocannabinoid signalling and the deteriorating brain. Nat Rev Neurosci 2015;16:30-42.
33. Ben-Shabat S, Fride E, Sheskin T, et al. An entourage effect: inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. Eur J Pharmacol 1998;353:23-31.
34. Costa B, Comelli F, Bettoni I, Colleoni M, Giagnoni G. The endogenous fatty acid amide, palmitoylethanolamide, has anti-allodynic and anti-hyperalgesic effects in a murine model of neuropathic pain: involvement of CB(1), TRPV1 and PPARgamma receptors and neurotrophic factors. Pain 2008;139:541-550.
35. Ho WS, Barrett DA, Randall MD. "Entourage" effects of N-palmitoylethanolamine and *N*-oleoylethanolamine on vasorelaxation to anandamide occur through TRPV1 receptors. Br J Pharmacol 2008;155:837-846.
36. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 5th ed. American Psychiatric Association, Arlington, VA, 2013.
37. Lord C, Rutter M, DiLavore PC, Risi S. ADOS, Autism Diagnostic Observation Schedule. Western Psychological Services, Los Angeles, CA, 2002.
38. Gotham K, Risi S, Pickles A, Lord C. The Autism Diagnostic Observation Schedule: revised algorithms for improved diagnostic validity. J Autism Dev Disord 2007;37:613-627.
39. Rutter M, Le Couter A, Lord C. ADI-R, Autism Diagnostic Interview-Revised. Western Psychological Services, Los Angeles, CA, 2003.
40. Persico AM. Autisms. In: Neural circuit development and function in the healthy and diseased brain: comprehensive developmental neuroscience, vol. 3 (Rakic P. and Rubenstein J, eds). Elsevier, New York, 2013, pp. 651-694.
41. Piven J, Palmer P, Jacobi D, Childress D, Arndt S. Broader autism phenotype: evidence from a family history study of multiple-incidence autism families. Am J Psychiatry 1997;154:185-190.
42. Baron-Cohen S, Wheelwright S, Skinner R, Martin J, Clubley E. The autism-spectrum quotient (AQ): evidence from Asperger syndrome/high-functioning autism, males and females, scientists and mathematicians. J Autism Dev Disord 2001;31:5-17.
43. Developmental Disabilities Monitoring Network Surveillance Year 2010 Principal Investigators; Centers for Disease Control and Prevention (CDC). Prevalence of autism spectrum disorder among children aged 8 years – autism and developmental disabilities monitoring network, 11 sites, United States, 2010. MMWR Surveill Summ 2014;63:1-21.
44. Ozonoff S, Young GS, Carter A, et al. Recurrence risk for autism spectrum disorders: a Baby Siblings Research Consortium study. Pediatrics 2011;128:e488-e495.
45. Elsabbagh M, Fernandes J, Jane Webb S, et al. Disengagement of visual attention in infancy is associated with emerging autism in toddlerhood. Biol Psychiatry 2013;74:189-194.
46. Chawarska K, Shic F, Macari S, et al. 18-month predictors of later outcomes in younger siblings of children with autism spectrum disorder: a baby siblings research consortium study. J Am Acad Child Adolesc Psychiatry 2014;53:1317-1327.
47. Gangi DN, Ibañez LV, Messinger DS. Joint attention initiation with and without positive affect: risk group differences and associations with ASD symptoms. J Autism Dev Disord 2014;44:1414-1424.
48. Gliga T, Jones EJ, Bedford R, Charman T, Johnson MH. From early markers to neuro-developmental mechanisms of autism. Dev Rev 2014;34:189-207.
49. Tuchman R, Rapin I. Epilepsy in autism. Lancet Neurol 2002;1:352-358.
50. Fombonne E. Epidemiology of autistic disorder and other pervasive developmental disorders. J Clin Psychiatry 2005;66:3-8.
51. Souders MC, Mason TB, Valladares O, et al. Sleep behaviors and sleep quality in children with autism spectrum disorders. Sleep 2009;32:1566-1578.
52. Tarazi F, Sahli Z, Pleskow J, Mousa S. Asperger's syndrome: diagnosis, comorbidity and therapy. Expert Rev Neurother 2015;15:281-293.
53. Di Cicco-Bloom E, Lord C, Zwaigenbaum L, et al. The developmental neurobiology of autism spectrum disorder. J Neurosci 2006;26:6897-6906.
54. Amaral DG, Schumann CM, Nordahl CW. Neuroanatomy of autism. Trends Neurosci 2008; 31:137-145.
55. Blatt GJ. The neuropathology of autism. Scientifica 2012;2012:703675.
56. Bauman ML, Kemper TL. Neuroanatomic observations of the brain in autism: a review and future directions. Int J Dev Neurosci 2005;23:183-187.
57. Rice D, Barone S Jr. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. Environ Health Perspect 2000;108(Suppl. 3):511-533.
58. Geschwind DH, Levitt P. Autism spectrum disorders: developmental disconnection syndromes. Curr Opin Neurobiol 2007;17:103-111.
59. Hahamy A, Behrmann M, Malach R. The idiosyncratic brain: distortion of spontaneous connectivity patterns in autism spectrum disorder. Nat Neurosci 2015;18:302-309.
60. Geschwind DH. Genetics of autism spectrum disorders. Trends Cogn Sci 2011;15:409-416.
61. Persico AM, Napolioni V. Autism genetics. Behav Brain Res 2013;251:95-112.
62. Persico AM, Merelli S. Environmental factors and autism spectrum disorder. Curr Dev Disord Rep 2014;1:8-19.
63. Tordjman S, Somogyi E, Coulon N, et al. Gene×environment interactions in autism spectrum disorders: role of epigenetic mechanisms. Front Psychiatry 2014;5:53.

64. Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann Neurol 2005;57:67-81.
65. Garbett KA, Ebert PJ, Mitchell A, et al. Immune transcriptome alterations in the temporal cortex of subjects with autism. Neurobiol Dis 2008;30:303-311.
66. Lintas C, Sacco R, Persico AM. Genome-wide expression studies in Autism spectrum disorder, Rett syndrome, and Down syndrome. Neurobiol Dis 2012;45:57-68.
67. Sacco R, Curatolo P, Manzi B, et al. Principal pathogenetic components and biological endophenotypes in autism spectrum disorders. Autism Res 2010;3:237-252.
68. Fox E, Amaral D, Van de Water J. Maternal and fetal antibrain antibodies in development and disease. Dev Neurobiol 2012;72: 1327-1334.
69. Onore C, Careaga M, Ashwood P. The role of immune dysfunction in the pathophysiology of autism. Brain Behav Immun 2012;26:383-392.
70. McElhanon BO, McCracken C, Karpen S, Sharp WG. Gastrointestinal symptoms in autism spectrum disorder: a meta-analysis. Pediatrics 2014;133:872-883.
71. Piras I, Haapanen L, Napolioni V, Sacco R, Van de Water J, Persico A. Anti-brain antibodies are associated with more severe cognitive and behavioural profiles in Italian children with Autism Spectrum Disorder. Brain Behav Immun 2014;38:91-99.
72. Chakrabarti B, Baron-Cohen S. Empathizing: neurocognitive developmental mechanisms and individual differences. Prog Brain Res 2006;156:403-417.
73. Chevallier C, Kohls G, Troiani V, Brodkin ES, Schultz RT. The social motivation theory of autism. Trends Cogn Sci 2012;16:231-239.
74. Sims TB, Van Reekum CM, Johnstone T, Chakrabarti B. How reward modulates mimicry: EMG evidence of greater facial mimicry of more rewarding happy faces. Psychophysiology 2012;49: 998-1004.
75. Sims TB, Neufeld J, Johnstone T, Chakrabarti B. Autistic traits modulate frontostriatal connectivity during processing of rewarding faces. Soc Cogn Affect Neurosci 2014;9:2010201-2010206.
76. Dawson G, Carver L, Meltzoff AN, Panagiotides H, McPartland J, Webb SJ.Neural correlates of face and object recognition in young children with autism spectrum disorder, developmental delay, and typical development. Child Dev 2002;73:700-717.
77. Pierce K, Conant D, Hazin R, Stoner R, Desmond J. Preference for geometric patterns early in life as a risk factor for autism. Arch Gen Psychiatry 2011;68:101-109.
78. Schmitz N, Rubia K, van Amelsvoort T, Daly E, Smith A, Murphy DG. Neural correlates of reward in autism. Br J Psychiatry 2008;192:19-24.
79. Dichter GS, Richey JA, Rittenberg AM, Sabatino A, Bodfish JW. Reward circuitry function in autism during face anticipation and outcomes. J Autism Dev Disord 2012;42:147-160.
80. Kohls G, Schulte-Rüther M, Nehrkorn B, Reward system dysfunction in autism spectrum disorders. Soc Cogn Affect Neurosci 2013;8:565-572.
81. Demurie E, Roeyers H, Baeyens D, Sonuga-Barke E. Common alterations in sensitivity to type but not amount of reward in ADHD and autism spectrum disorders. J Child Psychol Psychiatry 2011;52:1164-1173.
82. Dawson G, Bernier R, Ring RH. Social attention: a possible early indicator of efficacy in autism clinical trials. J Neurodev Disord 2012;4:11.
83. Fletcher-Watson S, Leekam SR, Benson V, Frank MC, Findlay JM. Eye-movements reveal attention to social information in autism spectrum disorder. Neuropsychologia 2009;47:248-257.
84. Klin A, Jones W, Schultz R, Volkmar F, Cohen D. Visual fixation patterns during viewing of naturalistic social situations as predictors of social competence in individuals with autism. Arch Gen Psychiatry 2002;59:809-816.
85. Sasson NJ, Dichter GS, Bodfish JW. Affective responses by adults with autism are reduced to social images but elevated to images related to circumscribed interests. PLoS One 2012;7:e42457.
86. Krajbich I, Armel C, Rangel A. Visual fixations and the computation and comparison of value in simple choice. Nat Neurosci 2010;13:1292-1298.
87. Stavropoulos KK, Carver LJ. Effect of familiarity on reward anticipation in children with and without autism spectrum disorders. PLoS One 2014;9:e106667.
88. Richey JA, Rittenberg A, Hughes L, et al. Common and distinct neural features of social and non-social reward processing in autism and social anxiety disorder. Soc Cogn Affect Neurosci 2014;9:367-377.
89. Chakrabarti B, Bullmore E, Baron-Cohen S. Empathizing with basic emotions: common and discrete neural substrates. Soc Neurosci 2006;1:364-384.
90. Ruggeri B, Sarkans U, Schumann G, Persico AM. Biomarkers in autism spectrum disorder: the old and the new. Psychopharmacology 2014;231:1201-1216.
91. Vorstman JA, Spooren W, Persico AM, et al. Using genetic findings in autism for the development of new pharmaceutical compounds. Psychopharmacology 2014;231:1063-1078.
92. Battista N, Bari M, Tarditi A, et al. Severe deficiency of the fatty acid amide hydrolase (FAAH) activity segregates with the Huntington's disease mutation in peripheral lymphocytes. Neurobiol Dis 2007;27:108-116.
93. Centonze D, Battistini L, Maccarrone M. The endocannabinoid system in peripheral lymphocytes as a mirror of neuroinflammatory diseases. Curr Pharm Des 2008;14:2370-2382.
94. Marco EM, Scattoni ML, Rapino C, et al. Emotional, endocrine and brain anandamide response to social challenge in infant male rats. Psychoneuroendocrinology 2013;38:2152-2162.
95. Sciolino NR, Bortolato M, Eisenstein SA, et al. Social isolation and chronic handling alter endocannabinoid signaling and behavioral reactivity to context in adult rats. Neuroscience 2010;168: 371-386.
96. Marco EM, Rapino C, Caprioli A, Borsini F, Maccarrone M, Laviola G. Social encounter with a novel partner in adolescent rats: activation of the central endocannabinoid system. Behav Brain Res 2011;220:140-145.
97. Bagni C, Tassone F, Neri G, Hagerman R. Fragile X syndrome: causes, diagnosis, mechanisms, and therapeutics. J Clin Invest 2012;122:4314-4322.
98. Ludwig AL, Espinal GM, Pretto DI, et al. CNS expression of murine fragile X protein (FMRP) as a function of CGG-repeat size. Hum Mol Genet 2014;23:3228-3238.
99. Gürkan CK, Hagerman RJ. Targeted treatments in autism and Fragile X syndrome. Res Autism Spectr Disord 2012;6:1311-1320.
100. Maccarrone M, Rossi S, Bari M, et al. Abnormal mGlu 5 receptor/endocannabinoid coupling in mice lacking FMRP and BC1 RNA. Neuropsychopharmacology. 2010;35:1500-1509.
101. Jung KM, Sepers M, Henstridge CM, et al. Uncoupling of the endocannabinoid signalling complex in a mouse model of fragile X syndrome. Nat Commun 2012;3:1080.
102. Busquets-Garcia A, Gomis-González M, Guegan T, et al. Targeting the endocannabinoid system in the treatment of fragile X syndrome. Nat Med 2013;19:603-607.
103. Schultz ST. Can autism be triggered by acetaminophen activation of the endocannabinoid system? Acta Neurobiol Exp (Wars) 2010;70:227-231.

104. Kerr DM, Downey L, Conboy M, Finn DP, Roche M. Alterations in the endocannabinoid system in the rat valproic acid model of autism. Behav Brain Res 2013;249:124-132.
105. Liu QR, Pan CH, Hishimoto A, et al. Species differences in cannabinoid receptor 2 (*CNR2* gene): identification of novel human and rodent CB2 isoforms, differential tissue expression and regulation by cannabinoid receptor ligands. Genes Brain Behav 2009;8:519-530.
106. Onaivi ES, Benno R, Halpern T, et al. Consequences of cannabinoid and monoaminergic system disruption in a mouse model of autism spectrum disorders. Curr Neuropharmacol 2011;9:209-214.
107. Siniscalco D, Sapone A, Giordano C, et al. Cannabinoid receptor type 2, but not type 1, is up-regulated in peripheral blood mononuclear cells of children affected by autistic disorders. J Autism Dev Disord 2013;43:2686-2695.
108. Depino AM. Peripheral and central inflammation in autism spectrum disorders. Mol Cell Neurosci 2013;53:69-76.
109. Leleu-Chavain N, Desreumaux P, Chavatte P, Millet R. Therapeutical potential of CB_2 receptors in immune-related diseases. Curr Mol Pharmacol 2013;6:183-203.
110. Rom S, Persidsky Y. Cannabinoid receptor 2: potential role in immunomodulation and neuroinflammation. J Neuroimmune Pharmacol 2013;8:608-620.
111. Chiurchiù V, Battistini L, Maccarrone M. Endocannabinoid signaling in innate and adaptive immunity. Immunol 2015;144:352-364.
112. Cencioni MT, Chiurchiù V, Catanzaro G, et al. Anandamide suppresses proliferation and cytokine release from primary human T-lymphocytes mainly via CB2 receptors. PLoS One 2010;5:e8688.
113. Brown I, Cascio MG, Rotondo D, Pertwee RG, Heys SD, Wahle KW. Cannabinoids and omega-3/6 endocannabinoids as cell death and anticancer modulators. Prog Lipid Res 2013;52:80-109.
114. Das U. Autism as a disorder of deficiency of brain-derived neurotrophic factor and altered metabolism of polyunsaturated fatty acids. Nutrition 2013;29:1175-1185.
115. Van der Stelt M, Di Marzo V. The endocannabinoid system in the basal ganglia and in the mesolimbic reward system: implications for neurological and psychiatric disorders. Eur J Pharmacol 2003;480:133-150.
116. Chakrabarti B, Kent L, Suckling J, Bullmore E, Baron-Cohen S. Variations in the human cannabinoid receptor (CNR1) gene modulate striatal responses to happy faces. Eur J Neurosci 2006;23:1944-1948.
117. Domschke K, Dannlowski U, Ohrmann P, et al. Cannabinoid receptor 1 (CNR1) gene: impact on antidepressant treatment response and emotion processing in major depression. Eur Neuropsychopharmacol 2008;18:751-759.
118. Chakrabarti B, Baron-Cohen S. Variation in the human cannabinoid receptor CNR1 gene modulates gaze duration for happy faces. Mol Autism 2011;2:10.
119. Chakrabarti B, Dudbridge F, Kent L, et al. Genes related to sex steroids, neural growth, and social–emotional behavior are associated with autistic traits, empathy, and Asperger syndrome. Autism Res 2009;2:157-177.
120. Baron-Cohen S, Wheelwright S. The empathy quotient: an investigation of adults with Asperger syndrome or high functioning autism, and normal sex differences. J Autism Dev Disord 2004;34:163-175.
121. Purcell AE, Jeon OH, Zimmerman AW, Blue ME, Pevsner J. Postmortem brain abnormalities of the glutamate neurotransmitter system in autism. Neurol 2001;57:1618-1628.
122. Trezza V, Vanderschuren LJ. Bidirectional cannabinoid modulation of social behavior in adolescent rats. Psychopharmacol 2008;197:217-227.
123. Trezza V, Baarendse PJ, Vanderschuren LJ. The pleasures of play: pharmacological insights into social reward mechanisms. Trends Pharmacol Sci 2010;31:463-469.
124. Maccarrone M, Guzmán M, Mackie K, Doherty P, Harkany T. Programming of neural cells by (endo)cannabinoids: from physiological rules to emerging therapies. Nat Rev Neurosci 2014;15:786-801.
125. Belmonte MK, Allen G, Beckel-Mitchener A, Boulanger LM, Carper RA, Webb SJ. Autism and abnormal development of brain connectivity. J Neurosci 2004;24:9228-9231.
126. Persico AM, Bourgeron T. Searching for ways out of the autism maze: genetic, epigenetic and environmental clues. Trends Neurosci 2006;29:349-358.
127. Spooren W, Lindemann L, Ghosh A, Santarelli L. Synapse dysfunction in autism: a molecular medicine approach to drug discovery in neurodevelopmental disorders. Trends Pharmacol Sci 2012;33:669-684.
128. Tsai NP, Wilkerson JR, Guo W, et al. Multiple autism-linked genes mediate synapse elimination via proteasomal degradation of a synaptic scaffold PSD-95. Cell 2012;151:1581-1594.
129. Bourgeron T. A synaptic trek to autism. Current Opin Neurobiol 2009;19:231-234.
130. Jaramillo TC, Liu S, Pettersen A, Birnbaum SG, Powell CM. Autism-related neuroligin-3 mutation alters social behavior and spatial learning. Autism Res 2014;7:264-272.
131. Földy C, Malenka RC, Südhof TC. Autism-associated neuroligin-3 mutations commonly disrupt tonic endocannabinoid signaling. Neuron 2013;78:498-509.
132. Cota D, Steiner MA, Marsicano G, et al. Requirement of cannabinoid receptor type 1 for the basal modulation of hypothalamic-pituitary-adrenal axis function. Endocrinol 2007;148:1574-1581.
133. Atkinson HC, Leggett JD, Wood SA, Castrique ES, Kershaw YM, Lightman SL. Regulation of the hypothalamic-pituitary-adrenal axis circadian rhythm by endocannabinoids is sexually diergic. Endocrinol 2010;15:3720-3727.
134. Vaughn LK, Denning G, Stuhr KL, de Wit H, Hill MN, Hillard CJ. Endocannabinoid signalling: has it got rhythm? Br J Pharmacol 2010;160:530-543.
135. Glickman G. Circadian rhythms and sleep in children with autism. Neurosci Biobehav Rev 2010;34:755-768.
136. Melke J, Goubran Botros H, Chaste P. Abnormal melatonin synthesis in autism spectrum disorders. Mol Psychiat 2008;13:90-98.
137. Campos AC, Guimarães FS.Involvement of 5HT1A receptors in the anxiolytic-like effects of cannabidiol injected into the dorsolateral periaqueductal gray of rats. Psychopharmacology 2008;199:223-230.
138. Rubino T, Guidali C, Vigano D, et al. CB1 receptor stimulation in specific brain areas differently modulate anxiety-related behaviour. Neuropharmacol 2008;54:151-160.
139. Katona I, Freund TF. Endocannabinoid signaling as a synaptic circuit breaker in neurological disease. Nat Med 2008;14:923-930.
140. Sasso O, Migliore M, Habrant D, et al. Multitarget fatty acid amide hydrolase/cyclooxygenase blockade suppresses intestinal inflammation and protects against nonsteroidal anti-inflammatory drug dependent gastrointestinal damage. FASEB J 2015;29:2616-2627.
141. Arosio B, D'Addario C, Gussago C, et al. Peripheral blood mononuclear cells (PBMCs) as a laboratory to study dementia in the elderly. BioMed Res Int 2014;2014:169203.

Neuropsychopharmacology (2007) 32, 1384–1390

www.neuropsychopharmacology.org

Endocannabinoids in Chronic Migraine: CSF Findings Suggest a System Failure

Paola Sarchielli*[,1], Luigi Alberto Pini[2], Francesca Coppola[1], Cristiana Rossi[1], Antonio Baldi[2,3], Maria Luisa Mancini[1] and Paolo Calabresi[2,3]

[1]Neurologic Clinic, Department of Medical and Surgical Specialties and Public Health, University of Perugia, Perugia, Italy; [2]Department of Biomedical Sciences, Headache Study Center, Institute of Toxicology and Clinical Pharmacology, University of Modena and Reggio Emilia, Modena, Italy; [3]IRCCS, Fondazione Santa Lucia, Rome, Italy

Based on experimental evidence of the antinociceptive action of endocannabinoids and their role in the modulation of trigeminovascular system activation, we hypothesized that the endocannabinoid system may be dysfunctional in chronic migraine (CM). We examined whether the concentrations of *N*-arachidonoylethanolamide (anandamide, AEA), palmitoylethanolamide (PEA), and 2-arachidonoylglycerol (2-AG) in the CSF of patients with CM and with probable CM and probable analgesic-overuse headache (PCM + PAOH) are altered compared with control subjects. The above endocannabinoids were measured by high-performance liquid chromatography (HPLC), and quantified by isotope dilution gas-chromatography/mass-spectrometry. Calcitonin gene-related peptide (CGRP) levels were also determined by RIA method and the end products of nitric oxide (NO), the nitrites, by HPLC. CSF concentrations of AEA were significantly lower and those of PEA slightly but significantly higher both in patients with CM and PCM + PAOH than in nonmigraineur controls ($p<0.01$ and $p<0.02$, respectively). A negative correlation was found between AEA and CGRP levels in CM and PCM + PAOH patients ($r=0.59$, $p<0.01$ and $r=-0.65$, $p<0.007$; respectively). A similar trend was observed between this endocannabinoid and nitrite levels. Reduced levels of AEA in the CSF of CM and PCM + PAOH patients may reflect an impairment of the endocannabinoid system in these patients, which may contribute to chronic head pain and seem to be related to increased CGRP and NO production. These findings support the potential role of the cannabinoid (CB)1 receptor as a possible therapeutic target in CM.
Neuropsychopharmacology (2007) **32,** 1384–1390. doi:10.1038/sj.npp.1301246; published online 22 November 2006

Keywords: endocannabinoids; calcitonin gene-related peptide; nitrites; chronic migraine; analgesic overuse headache; cerebrospinal fluid

INTRODUCTION

Cannabinoid (CB) receptors and their endogenous ligands constitute a novel modulatory system that is involved in specific brain functions, such as control of movement, memory, neuroendocrine regulation, and also nociception (Howlett *et al*, 2004).

Several endocannabinoids (endogenous cannabis-like substances), which are small molecules derived from arachidonic acid, have been detected so far: *N*-arachidonoylethanolamide (anandamide, AEA), 2-arachidonoylglycerol (2-AG) and its ether, the 2-arachidonyl glyceryl ether (noladin ether, NE), palmitoylethanolamide (PEA), *N*-oleoylethanolamide (OEA), virodhamine (VA), and *N*-arachidonoyl-dopamine (NADA). AEA and PEA are the best characterized (Drysdale and Platt, 2003).

Two CB receptor types have been identified in mammalian tissues, the CB(1) and CB(2) receptors, which mediate several pharmacological effects of AEA and are primarily localized to the nervous system and immune system, respectively. By being coupled to G-proteins, they can be seen as AEA 'metabotropic' receptors (Di Marzo *et al*, 2002a, b; Grotenhermen, 2004).

To date, the only reasonably well-characterized, non-cannabinoid site of action for AEA is the transient receptor potential vanilloid type 1 (TRPV1), a nonselective cation channel gated also by capsaicin, protons, and heat (Szallasi, 2002; Ross, 2003).

Endocannabinoids are released upon demand from lipid precursors in a receptor-dependent manner and serve as retrograde signaling messengers in GABAergic and glutamatergic synapses, as well as modulators of postsynaptic transmission, interacting with other neurotransmitters, including dopamine (Fride, 2002; Gubellini *et al*, 2002; Freund *et al*, 2003; Breivogel *et al*, 2004; Melis *et al*, 2004). Endocannabinoids are transported into cells by a specific uptake system and degraded by two well-characterized

*Correspondence: Dr P Sarchielli, Neurologic Clinic, Department of Medical and Surgical Specialties and Public Health, University of Perugia, Santa Maria della Misericordia Hospital, Sant'Andrea delle Fratte, 06156 Perugia, Italy, Tel: +39 075 578 3609, Fax: +39 075 578 3609, E-mails: neuro.pg@tiscalinet.it; headache@unipg.it
Received 13 June 2006; revised 7 August 2006; accepted 14 August 2006
Online publication: 26 September 2006 at http://www.acnp.org/citations/Npp092606060397/default.pdf

enzymes, fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (Szallasi, 2002; Grotenhermen, 2004).

A clinical endocannabinoid deficiency (CECD) has been hypothesized to underlie the pathophysiology of migraine, fibromyalgia, irritable bowel syndrome (IBS), and other functional conditions alleviated by clinical cannabis but no clear evidence to support this deficiency has been reported until now in this regard (Russo, 2004).

Migraine, in particular, has numerous relationships to endocannabinoid function. AEA potentiates 5-HT_{1A} and inhibits 5-HT_{2A} receptors supporting a potential therapeutic efficacy in acute antimigraine treatment with triptans, which are specific agonists of 5-$HT_{1B/1D}$ receptors but also exert their agonistic effect on 5-HT_{1A} receptors, and in antimigraine preventive treatment, with particular regard to serotonin antagonists (Kimura *et al*, 1998; Cheer *et al*, 1999). CBs also demonstrate antinociceptive and anti-inflammatory effects (Calignano *et al*, 1998, 2001; Hohmann, 2002; Cravatt and Lichtman, 2004; Di Marzo *et al*, 2002a, b; Pertwee, 2001; Mbvundula *et al*, 2004). AEA is also tonically active in the periaqueductal gray matter, which is believed to be a putative migraine generator (Vaughan *et al*, 2000).

Recent experimental findings suggest that AEA, the endogenous ligand of CB(1) and CB(2) receptors, is tonically released to play a modulatory role in the trigeminovascular system. AEA seems to act both presynaptically, to prevent CGRP release from trigeminal sensory fibers, and postsynaptically to inhibit the CGRP-induced nitric oxide (NO) release in the smooth muscle of dural arteries. AEA is tonically released to play some form of modulatory role in the trigeminovascular system (Akerman *et al*, 2004a). All these suggestions prompted us to test the hypothesis that the endogenous CB system may be dysfunctional in chronic migraine (CM), as in other chronic pain entities such as fibromyalgia and IBS. We therefore examined whether the concentrations of AEA, PEA, and 2-AG in the CSF of CM patients and probable CM and probable analgesic-overuse headache (PCM + PAOH) patients are altered compared with control subjects and if an association exists between their concentrations and those of CGRP and nitrites, the end products of NO.

PATIENTS AND METHODS

Patients and Controls

The study protocol was approved by the Ethics Committee of the Azienda of Umbria and all patients gave their written consent to the study.

Fifteen consecutive patients affected by CM and 15 patients diagnosed with PCM + PAOH according to the criteria of the International Classification of Headache Disorders, ICHD-II (Headache Classification Subcommittee of the International Headache Society, 2004), and attending the Headache Center of the Neurologic Clinic of the University of Perugia were admitted to the study.

The analgesic abused drugs in the PCM + PAOH group included nonsteroidal anti-inflammatory drugs, simple or combination analgesics, but not triptans. Combination analgesics included butalbital + propyphenazone + caffeine and indomethacin + prochlorperazine + caffeine. Among the patients with PCM + PAOH, eight (53.3%) were predominantly overusing one class of symptomatic medication, four (26.7%) were overusing combinations of two or three classes of symptomatic medication, whereas the remaining three (20.0%) patients were overusing combination analgesics.

None of the PCM + PAOH patients had opiate abuse or signs of a withdrawal syndrome at the time of assessment.

The monthly drug intake averaged 71.4 ± 12.6 (mean $\pm$ SD) tablets or suppositories. None of the patients used drugs containing codeine and/or took preventive medication for at least 1 month from inclusion into the study but they were allowed to take medication for the acute relief of pain.

All patients were admitted to the Neurologic Clinic of the University of Perugia to undergo lumbar puncture.

During their stay in the Hospital, each patient completed a questionnaire consisting of six questions derived from five of seven criteria of DSM-IV substance dependence (American Psychiatric Association, 1994) according to Fuh *et al* (2005), and the Hospital Anxiety and Depression Scale (HADS), a self-completed questionnaire specifically developed for use in the hospital outpatient setting that yields two subscales: anxiety and depression. According to this scale, clinically significant anxiety and depression were defined as anxiety scores of HADS $\geqslant 8$ and depression scores of HADS $\geqslant 8$, respectively.

Control CSF specimens were also obtained from 20 age-matched subjects who underwent lumbar puncture for diagnostic purposes. In all these subjects, CSF and blood tests excluded CNS or systemic diseases. All control subjects were drug-free for at least 2 months and none of them were taking any medication at the time of CSF sampling or had a personal or family history of migraine or suffered from tension-type headache. None of the above controls developed postlumbar puncture headache. All control subjects were assessed with the same questionnaires administered to patient groups. The details of patients and control subjects are reported in Table 1.

Routine CSF determinations both in patients and controls included total cell count, total protein, measurement of the concentration of albumin and IgG in CSF and serum, determination of oligoclonal bands by isoelectrophocusing, and extensive virological and microbiological testing. Samples were stored at $-80^{\circ}C$ until analysis.

Methods

CSF determination of endocannabinoids. This was performed according to the method of Giuffrida and Piomelli (1998) adapted to CSF by Leweke *et al* (1999) and Giuffrida *et al* (2004), which involves separation of endocannabinoids by high-performance liquid chromatography (HPLC) and then quantification by gas chromatography/mass spectrometry (GS-MS).

Standards for [$^2H_4$]AEA, [$^2H_4$]oleoylethanolamide, and [$^2H_4$]PEA were synthesized by the reaction of fatty acyl chlorides with unlabeled or [$^2H_4$]ethanolamine, provided by Cambridge Isotope Laboratories (Andover, MA). [$^2H_8$]-2-AG was custom-synthesized by Deva Biotech (Hatboro, PA). Fatty acyl chlorides in dichloromethane (10 mg/ml) were mixed with one equivalent of ethanolamine, and allowed to react for 15 min at 0–4°C. Reactions were stopped by adding

Table 1 Details of Patients and Control Subjects

	CM patients	PCM+PAOH patients	Controls
Number	15	15	20
Males	$n=4$	$n=2$	$n=7$
Females	$n=11$	$n=13$	$n=13$
Age (years)	37.4±4.9	40.1±5.4	36.3±7.4
Duration of chronic pain (years)	11.2±4.9	12.8±3.9	
No. of days/month	24.5±5.1	23.8±4.7	
VAS[a]	81.2±18.7	79.6±14.6	
Hospital anxiety and depression scores	14.2±7.4	15.9±8.1*	7.4±3.4
Anxiety score	7.1±4.1	7.9±3.9**	3.4±1.9
Depression score	6.2±3.9	8.0±4.2***	4.1±2.2

[a]All patients were asked to make one assessment of pain by means of a visual analogue scale (VAS) (0–100 score) during the 3 months of diary recording. Average values during the monitoring period are presented.
Statistical significance : *$p<0.03$; **$p<0.05$; ***$p<0.01$.

water. After vigorous mixing, the upper aqueous phases were discarded to remove unreacted ethanolamine. The organic phases were washed twice with water, concentrated to dryness under a stream of N_2, and the reaction products were reconstituted in methanol. Identity and chemical purity (>98%) of the synthesized acylethanolamides (AEs) and [$^2H_4$]AEs were determined by GC/MS.

These standards were added to three aliquots of CSF (1.2 nmol in 15 ml) to improve recovery and allow for quantification. After acetone precipitation of plasma proteins in CSF samples, the supernatants were collected and subjected to lipid extraction with methanol/chloroform. Enough of each solvent was added to reach a final ratio buffer/methanol/chloroform of 1:1:2 (v/v/v). The chloroform phases were recovered, evaporated to dryness under N_2, and reconstituted in chloroform (150 µl).

HPLC fractionations were performed on a Hewlett-Packard 1090 Liquid Chromatograph, equipped with a normal-phase Resolve Silica column (3.9 mm × 15 cm, 5 µm; Waters Associates, Milford, MA), eluted with a gradient of isopropyl alcohol (B) in *n*-hexane (A) (100% A initial; 90% A, 10% B for 1 min; 60% A, 40% B for 7 min, 50% A, 50% B for 12 min) at a flow rate of 1.7 ml/min. Under these conditions, all AEs were eluted from the HPLC column between 4.7 and 5.3 min. The AE-containing fractions were collected in glass reaction vessels, dried under N_2 and converted to trimethylethers by treatment with *Bis*(trimethylsilyl)trifluoroacetamide (BSTFA) for 30 min at room temperature. The trimethylsilylether (TMS) derivatives produced in this reaction were dried under N_2, reconstituted in *n*-hexane and injected in the splitless mode into a Hewlett-Packard 5890 GC equipped with an HP-5MS capillary column (30 m; internal diameter, 0.25 mm) and interfaced with a Hewlett-Packard 5972 MS.

Details on isotope dilution and GC/MS methods are reported elsewhere (Giuffrida and Piomelli, 1998).

The concentrations of analytes in the CSF samples were expressed as pmol/ml and calculated in three separate determinations.

Lowest limits of detection were 0.16 pmol/sample for AEA, 0.10 pmol/sample for PEA and 0.20 pmol/sample for 2-AG. Accuracy and precision of the assays were obtained by measuring the recovery of known amounts of the three endocannabinoids in the presence of 0.5 nmol of isotope-labeled standards in five independent determinations. Accuracy, expressed as the ratio between the actual and nominal values (%) was 98, 96, and 95%, respectively, for AEA, PEA, and 2-AG. Precision, expressed as percent coefficient of variation (CV), by dividing the standard deviation by the sample mean and multiplying the resulting value by 100, was 3.9, 4.2, and 5.6%.

Accuracy expressed as CV (%) was 3.9, 4.5, and 5.9% for AEA, PEA, and 2-AG, respectively.

CSF determination of CGRP. Five ml of CSF were collected according to the study protocol in polypropylene tubes containing EDTA (1 mg/ml) and kallikrein (500 IU/ml).

CGRP immunoreactivity was eluted with 60% acetonitrile in 0.1% trifluoroacetic acid in SEP-C18 columns activated with 0.1% trifluoroacetic acid and 60% acetonitrile in 0.1% trifluoroacetic acid. Eluates were associated with a centrifuge concentrator (Supervap PL-CC-180). Residues were dissolved in buffer and determined with RIA kits (Peninsula Laboratories, Belmont, CA). Neuropeptide data were expressed as pmol/l. Standards for the above substances were dissolved in 0.1 mol/l phosphate buffer, pH 7.5, containing 0.1% bovine albumin, 0.01% sodium azide and 500 IU/ml kallikrein. CGRP human antiserum was obtained from rabbit and was specific for the C-terminal end for the neuropeptide. CGRP antiserum showed a crossreactivity with rat and chicken CGRP (100%) but not with other neuropeptides. The intra- and interassay variabilities were 3 and 6%, respectively. The detection limit of the assay was <1 pmol/l. Specificity data on CGRP were provided by Peninsula Laboratories.

CSF determination of nitrite. The oxidation products of NO, nitrite and nitrate, were determined as total NO_2^- after enzymatic reduction of nitrate to nitrite by fluorimetric HPLC analysis with precolumn hydralazine derivatization, as reported in one previous paper of our group (Gallai *et al*, 2003). Detection was achieved by fluorescence evaluation of the nitrite derivative at 360 nm emission and 228 nm excitation; quantification was performed by external standard calibration. The detection limit of the method was 0.3–0.5 pmol of NO_2^- in the column. The recovery of nitrite was calculated to range between 96 and 99%. The reproducibility, including sample preparation and analysis, was specified with a CV of 6%. The day-to-day variability of identical samples was 0.9% (CV). Data were expressed as µmol/l.

Statistical Analysis

Data from the patient and control groups were expressed as mean±SEM and compared using ANOVA, and Fisher's least significant difference (LSD) was also used to compare the main effect means in ANOVA. The correlation between variables was determined using Spearman's rank correlation test. All statistical tests were two-sided. *P*-values less than 0.05 were considered significant.

RESULTS

Table 1 shows the details of the patient and control groups and for the patient groups also displays data relative to pain parameters and HADS.

No differences in pain parameters were found between the two patients groups, whereas patients with PCM + PAOH had slightly significantly higher HADS scores. Nine patients in the CM patient group, 10 in the PCM + PAOH, and none of controls showed depression scale scores indicative of clinically relevant depression.

According to the modified DSM-IV substance dependence criteria, 12 patients (80%) with PCM + PAOH were classified as having substance dependence, whereas none of the patients with CM were classified as having substance dependence. The prevalence of DSM-IV dependence was highest among overusers of multiple classes or combination medication (100%) than simple analgesics (62.5%).

CSF concentrations of AEA was significantly lower both in patients with CM and in patients with PCM + PAOH than in nonmigraine controls ($p<0.01$). Conversely, significantly higher levels of PEA were found compared to control subjects ($p<0.02$) without significant difference between the two patient groups (Table 2). Levels of 2-AG were below detection limits in both patient and control groups.

No significant correlation emerged between CSF levels of endogenous CBs and age in both CM and PCM + PAOH patients and controls. This can be explained by the limited variance in the age of patients and control groups.

We also did not find any difference between CSF levels of the two CBs between CM patients classified as having medication dependence and those without when medication dependence was assumed as a categorical variable in ANOVA.

Besides the lack of any significant correlation between AEA and HADS scores, in particular depression scores, in all patient and control groups, significantly lower levels of AEA emerged in patients with a clinically significant depression (CM: 0.19 ± 0.054 and PCM + PAOH: 0.20 ± 0.063 compared with those without (CM: 0.23 ± 0.069 and PCM + PAOH: 0.24 ± 0.058) ($p<0.02$ and $p<0.001$). Patients without significant depression had in any case values significantly lower than those of control groups ($p<0.0005$ and $p<0.0003$, respectively). Conversely, there were no significant differences in CSF AEA levels between patients with and without concomitant depression in CM and PCM + PAOH groups.

As shown in previous papers by our group (Gallai *et al*, 2003), significantly higher levels of CGRP and nitrites were found in patients with CM, and this was also evident in the CSF of PCM + PAOH patients with values of nitrites significantly greater than those found in CM patients (Table 2).

In both CM and PCM + PAOH patient groups, CSF values of AEA, 2-AG, and PEA did not appear correlated with the duration of CM and the average intensity of pain during the monitoring period of 3 months, as measured by the visual analogue scale.

A negative correlation was found between AEA and CGRP levels in CM and PCM + PAOH patients ($r=-0.59$, $p<0.018$; and $r=-0.65$, $p<0.007$; respectively) (Figure 1a and b) and also emerged for PEA levels *vs* CGRP in both patient groups ($r=-0.45$, $p<0.002$; and $r=-0.57$, $p<0.003$) (plots not shown).

Table 2 Levels of AEA, PEA, 2-AG, CGRP, and Nitrites (Mean ± SE) in Controls and CM and PCM+PAOH Patient Groups

	Controls	CM	PCM+PAOH
AEA (pmol/ml)	0.39 ± 0.09	$0.21\pm0.060^{*}$	$0.22\pm0.05^{*}$
PEA (pmol/ml)	4.91 ± 0.47	$6.21\pm0.59^{\S}$	$6.23\pm0.52^{\S\S}$
CGRP (pmol/l)	29.37 ± 4.67	$44.16\pm4.63^{\wedge}$	$43.96\pm5.22^{\wedge\wedge}$
Nitrites (μmol/l)	13.66 ± 2.89	$24.51\pm4.44^{\#}$	$31.42\pm6.93^{\#\#\circ}$

Statistical significance *vs* controls: AEA = $^{*}p<0.0001$; PEA = $^{\S}p<0.0002$; $^{\S\S}p<0.0004$; CGRP = $^{\wedge}p<0.0005$; $^{\wedge\wedge}p<0.0001$; Nitrites = $^{\#}p<0.0003$; $^{\#\#}p<0.001$.
Statistical significance *vs* CM: Nitrites = $^{\circ}p<0.02$.

A similar trend toward a negative correlation was observed between the two endogenous CBs, AEA and PEA, and nitrite levels in the CSF of both CM and PCM + PAOH patient groups ($r=-0.63$, $p<0.01$; $r=-0.77$, $p<0.006$; Figure 2a and b, respectively; PEA plots not shown).

DISCUSSION

Migraine has been suggested to have an underlying CECD (Russo, 2004).

Current anecdotal references continue to refer to the putative efficacy of AEA for migraine, but biochemical studies providing a scientific basis for such treatment are lacking (Russo, 1998). In the present study, reduced AEA levels in the CSF of CM patients support the hypothesis of the failure of this endogenous CB system in CM, which seems to be related to increased CGRP and NO production in this pathological condition. This finding might be due to a failure of the inhibitory role of the endocannabinoid AEA on the trigeminovascular system activation via CB(1) receptors localized on fibers in the spinal trigeminal tract and spinal trigeminal nucleus caudalis. This inhibitory effect has been demonstrated in experimental settings where AEA was able to inhibit dural blood vessel dilation due either to electrical stimulation or to CGRP, capsaicin, or NO application. This effect was reversed by the CB(1) receptor antagonist AM251 (Akerman *et al*, 2004a).

The reduction in CSF levels of AEA does not seem specific for CM because it was also evident in the CSF of patients with PCM + PAOH, but appears to be related to chronic head pain *per se*.

In contrast to the reduction of AEA, significantly higher levels of PEA were found in both patient groups compared to nonmigraineur controls. It can hypothesized that increased PEA levels represent a compensatory response to reduced levels of AEA, the latter due probably to an acceleration of its metabolism in CM patients and PCM + PAOH. Experimental findings support in fact the role of PEA and PEA derivatives in the inhibition of FAAH-catalyzed hydrolysis of AEA, which can result in a potentiation of AEA action (Vandevoorde *et al*, 2003). Based on these findings, increased levels of PEA can be interpreted as a physiological mechanism counteracting accelerated AEA catabolism in these pathological chronic pain conditions.

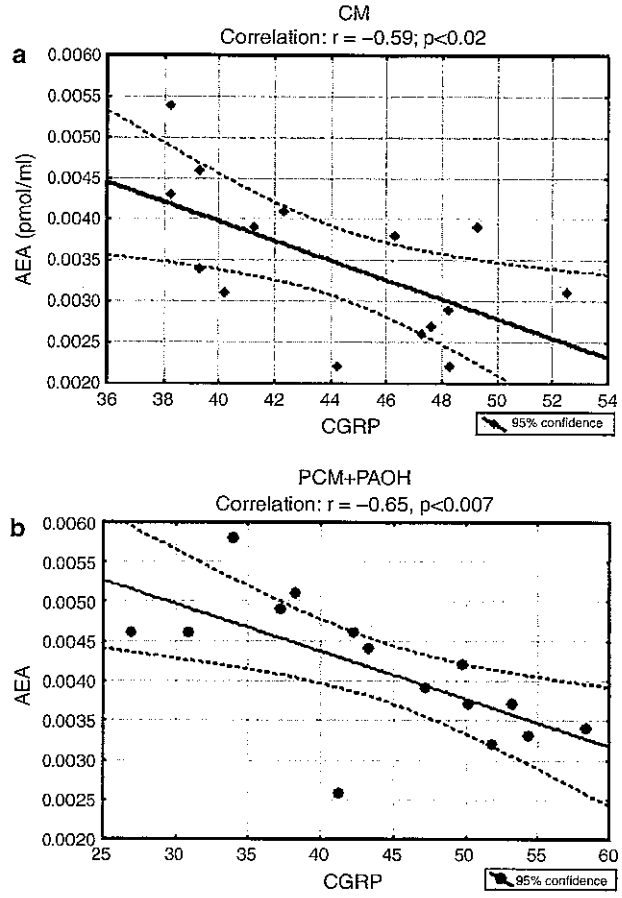


Figure 1 (a and b) Plot of the levels of AEA *vs* CGRP, expressed as pmol/ml and pmol/l, in patients with CM (a) and in patients with PCM + PAOH (b).

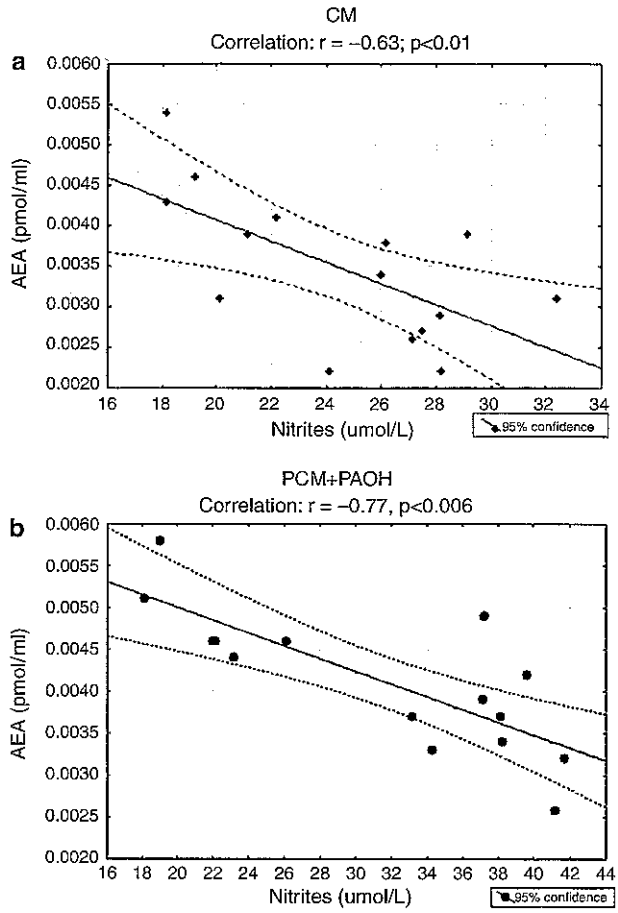


Figure 2 (a and b) Plot of the levels of AEA *vs* nitrite, expressed as pmol/ml and μmol/l, in patients with CM (a) and in patients with PCM + PAOH (b).

Based on our findings, it can be hypothesized that the failure of the inhibitory role of AEA can contribute to maintaining central sensitization in chronic head pain, and represents a further mechanism which intervenes in increasing the release of the sensory neuropeptide CGRP and NO production, together with nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) release via glutamatergic transmission (Sarchielli *et al*, 2001, 2002).

It has been demonstrated that endocannabinoids, in particular AEA, positively influence serotonin levels in the brain of experimental animals, supporting its putative role in mood regulation, which pointed to FAAH as a previously uncharacterized target for antidepressant drugs (Gobbi *et al*, 2005).

As migraine has been related to a serotonergic dysfunction, which is more accentuated in chronic forms, including those with analgesic overuse (Srikiatkhachorn *et al*, 1998; Sarchielli *et al*, 1999), it is plausible that lower levels of AEA can reflect lower levels of this monoamine in both CM and PCM + PAOH patient groups.

The chemical theory of depression postulates, at the basis of its debilitating and often chronic symptoms, a perturbation of monoamine transmission and depletion of these neurotransmitters. This is strongly supported by the effectiveness of monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs), and more recently of serotonin reuptake inhibitors (SSRIs) and serotonin/norepinephrine reuptake inhibitors (SNRIs), which elevate levels of monoamines, by preventing their metabolism and blocking their reuptake, the latter two acting more selectively on the serotonin system (Schechter *et al*, 2005). The majority of these drugs are included among preventive treatment of migraine based on the assumption of a serotonin system derangement in migraine, particularly in the chronic forms.

Although we did not measure serotonin levels in the CSF of subjects of all groups and could not relate these levels to AEA, we found that patients with scores indicative of clinically relevant depression in CM and PCM + PAOH groups showed lower levels of AEA than those without. Patients without clinically significant depression had, however, CSF values of AEA significantly reduced than those of controls in both patient groups.

These findings thus support not only chronic pain as being characterized by a deficiency of this endocannabinoid but also the contribution of depression comorbidity to AEA reduction in the CSF of both CM and PCM + PAOH patients.

Whether this reduction is also peculiar for other chronic pain conditions should be clarified by future research

investigating the levels of endogenous CBs in other, similar conditions, such as fibromyalgia or IBS.

The findings of the present study offer some speculations on the pathophysiologic aspects of chronic pain in general, and chronic head pain in particular. Chronic head pain, like other chronic pain syndromes, is characterized by altered neuronal excitability in the pain matrix (van der Stelt and Di Marzo, 2003). In these conditions, neuroplastic changes and reorganization of neuronal pathways take place at several levels in the spinal cord, in thalamic nuclei, and in cortical and subcortical (limbic) areas integrating pain threshold, intensity, and affective components. The endogenous CB system plays a central role in the extinction of aversive memories, including that related to pain, by facilitating their selective inhibitory effects on GABAergic networks at different levels in the pain matrix (Marsicano *et al*, 2002). Reduction in endocannabinoids can therefore result in a failure of discharge activity of interneurons, controlling neurons conveying nociceptive information and counteracting long-term changes in the pain matrix following nociceptor activation (Walker and Huang, 2002). This may explain not only the transition of acute pain signaling to chronic pain states, including chronic head pain, but also their maintenance.

Moreover, it cannot be excluded that changes in CSF levels of AEA and PEA can reflect migraine, since both CM and PCM + PAOH share the same biological substrate. To clarify the role for endocannabinoids in chronic head pain would require a further primary chronic headache study.

It could be wise to study endocannabinoids in the CSF of patients with chronic tension-type headache. This could discriminate whether endocannabinoid system changes are a response unique in some way to migraine or can be extended to other chronic head pain conditions. This issue will be the aim of future research of our group.

It has been recently suggested that the endocannabinoid system might be a component of the brain reward circuitry and thus play a role not only in CB tolerance/dependence but also in dependence/withdrawal to other drugs of abuse (Di Marzo *et al*, 2000; Gonzalez *et al*, 2003; Centonze *et al*, 2004). Changes in endocannabinoid ligands and their receptors have been shown in different brain regions, with particular attention to those areas related to reinforcement processes, during dependence on powerful addictive drugs. There is also a growing body of evidence supporting the potential intervention of the endocannabinoid system in the motivational and dopamine-releasing effects of several drugs of abuse and dependence (Parolaro *et al*, 2005; Calabresi and Cupini, 2005).

It remains to be established if similar mechanisms are activated in the case of analgesic overuse involved in migraine chronicity as suggested by our findings.

In our study we did not find any significant differences in CSF AEA levels between the two patient groups with and without medication overuse, supporting the involvement of AEA reduction in chronic head pain independently of its relation to drug abuse and dependence.

Other aspects related to this dysfunction and potential failure of CB(1) mediated signaling also need to be clarified.

Some experimental evidence allows, in fact, to hypothesize other targets for AEA or analogs other than CB(1). In an *in vitro* setting, AEA has been demonstrated to activate TRPV1, the nonselective cation channel that belongs to the large family of TRP ion channels, activated by the pungent ingredient of hot chilli peppers, capsaicin (Akerman *et al*, 2004b). They are expressed in some nociceptor efferent neurons, where they act as a molecular sensor of noxious heat and low pH. The activation of TRPV1 receptors in trigeminovascular system activation via AEA promotes CGRP release and causes vasodilation independent of any action at the CB(1) receptor, and therefore moderates an opposite effect of that attributed to endocannabinoids through metabotropic receptors.

Whether and how the above mechanisms play a pivotal role in chronic pain and CM, in particular, remains to be established in the search for new therapeutic strategies in migraine. In this regard, other endogenous substances have been discovered, which need to be investigated in future research: the endovanilloids, which are defined as endogenous ligands of the TRPV1 protein. Other than AEA, they include some of its congeners, such as unsaturated *N*-acyldopamines and lipoxygenase products of arachidonic acid (Van der Stelt and Di Marzo, 2004). Research in their regard is warranted.

ACKNOWLEDGEMENTS

We express our gratitude to John A Toomey for editing the English and Marisa M Morson for technical assistance.

CONFLICT OF INTEREST

We have no financial or nonfinancial conflict of interests to declare.

REFERENCES

Akerman S, Kaube H, Goadsby PJ (2004a). Anandamide is able to inhibit trigeminal neurons using an *in vivo* model of trigeminovascular-mediated nociception. *J Pharmacol Exp Ther* **309**: 56–63.

Akerman S, Kaube H, Goadsby PJ (2004b). Anandamide acts as a vasodilator of dural blood vessels *in vivo* by activating TRPV1 receptors. *Br J Pharmacol* **142**: 1354–1360.

American Psychiatric Association (1994). *Diagnostic and Statistical Manual of Mental Disorders*, 4th edn. American Psychiatric Association: Washington, DC.

Breivogel CS, Walker JM, Huang SM, Roy MB, Childers SR (2004). Cannabinoid signaling in rat cerebellar granule cells: G-protein activation, inhibition of glutamate release and endogenous cannabinoids. *Neuropharmacology* **47**: 81–91.

Calabresi P, Cupini LM (2005). Medication-overuse headache: similarities with drug addiction. *Trends Pharmacol Sci* **26**: 62–68.

Calignano A, La Rana G, Giuffrida A, Piomelli D (1998). Control of pain initiation by endogenous cannabinoids. *Nature* **394**: 277–281.

Calignano A, La Rana G, Piomelli D (2001). Antinociceptive activity of the endogenous fatty acid amide, palmitylethanolamide. *Eur J Pharmacol* **419**: 191–198.

Centonze D, Battista N, Rossi S, Mercuri NB, Finazzi-Agro A, Bernardi G *et al* (2004). A critical interaction between dopamine D2 receptors and endocannabinoids mediates the effects of cocaine on striatal gabaergic transmission. *Neuropsychopharmacology* **29**: 1488–1497.

Cheer JF, Cadogan AK, Marsden CA, Fone KC, Kendall DA (1999). Modification of 5-HT2 receptor mediated behaviour in the rat by

oleamide and the role of cannabinoid receptors. *Neuropharmacology* 38: 533–541.
Cravatt BF, Lichtman AH (2004). The endogenous cannabinoid system and its role in nociceptive behavior. *J Neurobiol* 61: 149–160.
Di Marzo V, Berrendero F, Bisogno T, Gonzalez S, Cavaliere P, Romero J *et al* (2000). Enhancement of anandamide formation in the limbic forebrain and reduction of endocannabinoid contents in the striatum of delta9-tetrahydrocannabinol-tolerant rats. *J Neurochem* 74: 1627–1635.
Di Marzo V, Blumberg PM, Szallasi A (2002a). Endovanilloid signaling in pain. *Curr Opin Neurobiol* 12: 372–379.
Di Marzo V, De Petrocellis L, Fezza F, Ligresti A, Bisogno T (2002b). Anandamide receptors. *Prostaglandins Leukot Essent Fatty Acids* 66: 377–391.
Drysdale AJ, Platt B (2003). Cannabinoids: mechanisms and therapeutic applications in the CNS. *Curr Med Chem* 10: 2719–2732.
Freund TF, Katona I, Piomelli D (2003). Role of endogenous cannabinoids in synaptic signaling. *Physiol Rev* 83: 1017–1066.
Fride E (2002). Endocannabinoids in the central nervous system-an overview. *Prostaglandins Leukot Essent Fatty Acids* 66: 221–233.
Fuh J-L, Wang S-J, Lu S-R, Juang K-D (2005). Does medication overuse headache represent a behavior of dependence? *Pain* 119: 49–55. (E-pub November 17, 2005).
Gallai V, Alberti A, Gallai B, Coppola F, Floridi A, Sarchielli P (2003). Glutamate and nitric oxide pathway in chronic daily headache: evidence from cerebrospinal fluid. *Cephalalgia* 23: 166–174.
Giuffrida A, Leweke FM, Gerth CW, Schreiber D, Koethe D, Faulhaber J *et al* (2004). Cerebrospinal anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. *Neuropsychopharmacology* 29: 2108–2114.
Giuffrida A, Piomelli D (1998). Isotope dilution GC/MS determination of anandamide and other fatty acylethanolamides in rat blood plasma. *FEBS Lett* 422: 373–376.
Gobbi G, Bambico FR, Mangieri R, Bortolato M, Campolongo P, Solinas M *et al* (2005). Antidepressant-like activity and modulation of brain monoaminergic transmission by blockade of anandamide hydrolysis. *Proc Natl Acad Sci USA* 102: 18620–18625. (E-pub December 13, 2005. Erratum in: *Proc Natl Acad Sci USA* 2006; 103: 2465).
Gonzalez S, Schmid PC, Fernandez-Ruiz J, Krebsbach R, Schmid HH, Ramos JA (2003). Region-dependent changes in endocannabinoid transmission in the brain of morphine-dependent rats. *Addict Biol* 8: 159–166.
Grotenhermen F (2004). Pharmacology of cannabinoids. *Neuro Endocrinol Lett* 25: 14–23.
Gubellini P, Picconi B, Bari M, Battista N, Calabresi P, Centonze D *et al* (2002). Experimental parkinsonism alters endocannabinoid degradation: implications for striatal glutamatergic transmission. *J Neurosi* 22: 6900–6907.
Headache Classification Subcommittee of the International Headache Society (2004). The International Classification of Headache Disorders, 2nd edn. *Cephalalgia* 24(Suppl 1): 1–160.
Hohmann AG (2002). Spinal and peripheral mechanisms of cannabinoid antinociception: behavioral, neurophysiological and neuroanatomical perspectives. *Chem Phys Lipids* 121: 173–190.
Howlett AC, Breivogel CS, Childers SR, Deadwyler SA, Hampson RE, Porrino LJ (2004). Cannabinoid physiology and pharmacology: 30 years of progress. *Neuropharmacology* 47(Suppl 1): 345–358.
Kimura T, Ohta T, Watanabe K, Yoshimura H, Yamamoto I (1998). Anandamide, an endogenous cannabinoid receptor ligand, also interacts with 5-hydroxytryptamine (5-HT) receptor. *Biol Pharm Bull* 21: 224–226.
Leweke FM, Giuffrida A, Wurster U, Emrich HM, Piomelli D (1999). Elevated endogenous cannabinoids in schizophrenia. *Neuroreport* 10: 1665–1669.
Marsicano G, Wotjak CT, Azad SC, Bisogno T, Rammes G, Cascio MG *et al* (2002). The endogenous cannabinoid system controls extinction of aversive memories. *Nature* 418: 530–534.
Mbvundula EC, Rainsford KD, Bunning RA (2004). Cannabinoids in pain and inflammation. *Inflammopharmacology* 12: 99–114.
Melis M, Pistis M, Perra S, Muntoni AL, Pillolla G, Gessa GL (2004). Endocannabinoids mediate presynaptic inhibition of glutamatergic transmission in rat ventral tegmental area dopamine neurons through activation of CB1 receptors. *J Neurosci* 24: 53–62.
Parolaro D, Vigano D, Rubino T (2005). Endocannabinoids and drug dependence. *Curr Drug Targets CNS Neurol Disord* 4: 643–655.
Pertwee RG (2001). Cannabinoid receptors and pain. *Prog Neurobiol* 63: 569–611.
Ross RA (2003). Anandamide and vanilloid TRPV1 receptors. *Br J Pharmacol* 140: 790–801.
Russo E (1998). Cannabis for migraine treatment: the once and future prescription? An historical and scientific review. *Pain* 76: 3–8.
Russo EB (2004). Clinical endocannabinoid deficiency (CECD): can this concept explain therapeutic benefits of cannabis in migraine, fibromyalgia, irritable bowel syndrome and other treatment-resistant conditions? *Neuro Endocrinol Lett* 25: 31–39.
Sarchielli P, Alberti A, Floridi A, Gallai V (2001). Levels of nerve growth factor in cerebrospinal fluid of chronic daily headache patients. *Neurology* 57: 132–134.
Sarchielli P, Alberti A, Gallai B, Coppola F, Baldi A, Gallai V (2002). Brain-derived neurotrophic factor in cerebrospinal fluid of patients with chronic daily headache: relationship with nerve growth factor and glutamate levels. *J Headache Pain* 3: 129–135.
Sarchielli P, Alberti A, Russo S, Codini M, Panico R, Floridi A *et al* (1999). Nitric oxide pathway, Ca2+, and serotonin content in platelets from patients suffering from chronic daily headache. *Cephalalgia* 19: 810–816.
Schechter LE, Ring RH, Beyer CE, Hughes ZA, Khawaja X, Malberg JE *et al* (2005). Innovative approaches for the development of antidepressant drugs: current and future strategies. *NeuroRx* 2: 590–611.
Srikiatkhachorn A, Maneesri S, Govitrapong P, Kasantikul V (1998). Derangement of serotonin system in migrainous patients with analgesic abuse headache: clues from platelets. *Headache* 38: 43–49.
Szallasi A (2002). Vanilloid (capsaicin) receptors in health and disease. *Am J Clin Pathol* 118: 110–121.
van der Stelt M, Di Marzo V (2003). The endocannabinoid system in the basal ganglia and in the mesolimbic reward system: implications for neurological and psychiatric disorders. *Eur J Pharmacol* 480: 133–150.
van Der Stelt M, Di Marzo V (2004). Endovanilloids. Putative endogenous ligands of transient receptor potential vanilloid 1 channels. *Eur J Biochem* 271: 1827–1834.
Vandevoorde S, Jonsson KO, Fowler CJ, Lambert DM (2003). Modifications of the ethanolamine head in *N*-palmitoylethanolamine: synthesis and evaluation of new agents interfering with the metabolism of anandamide. *J Med Chem* 46: 1440–1448.
Vaughan CW, Connor M, Bagley EE, Christie MJ (2000). Actions of cannabinoids on membrane properties and synaptic transmission in rat periaqueductal gray neurons *in vitro*. *Mol Pharmacol* 57: 288–295.
Walker JM, Huang SM (2002). Cannabinoid analgesia. *Pharmacol Ther* 95: 127–135.

Trends in Psychiatry and Psychotherapy

Original Article

Evaluation of the efficacy and safety of cannabidiol-rich cannabis extract in children with autism spectrum disorder: randomized, double-blind, and placebo-controlled clinical trial

Estácio Amaro da **Silva Junior**,[1] Wandersonia Moreira Brito **Medeiros**,[1] João Paulo Mendes dos **Santos**,[2] João Marçal Medeiros de **Sousa**,[2] Filipe Barbosa da **Costa**,[2] Katiúscia Moreira **Pontes**,[2] Thaís Cavalcanti **Borges**,[2] Carlos Espínola **Neto Segundo**,[2] Ana Hermínia **Andrade e Silva**,[3] Eliane Lima Guerra **Nunes**,[4] Nelson Torro **Alves**,[5] Marine Diniz da **Rosa**,[6] Katy Lísias Gondim Dias de **Albuquerque**[4,7]

Abstract

Objective: Autism spectrum disorder (ASD) is characterized by persistent deficits in social communication and social interaction and by restricted and repetitive patterns of behavior. Some studies have shown that substances derived from *Cannabis sativa* improve the quality of life of children with ASD without causing serious adverse effects, thus providing an alternative therapeutic option. The objective of this study was to evaluate the efficacy and safety of a cannabis extract rich in cannabidiol (CBD) in children with ASD.
Methods: In this randomized, double-blind, placebo-controlled clinical trial, 60 children, aged from 5 to 11 years, were selected and divided into two groups: the treatment group, which received the CBD-rich cannabis extract, and the control group, which received the placebo. They both used their respective products for a period of 12 weeks. Statistical analysis was done by two-factor mixed analysis of variance (two-way ANOVA).
Results: Significant results were found for social interaction ($F_{1,116}$ = 14.13, p = 0.0002), anxiety ($F_{1,116}$ = 5.99, p = 0.016), psychomotor agitation ($F_{1,116}$ = 9.22, p = 0.003), number of meals a day ($F_{1,116}$ = 4.11, p = 0.04), and concentration ($F_{1,48}$ = 6.75, p = 0.01), the last of which was only significant in mild ASD cases. Regarding safety, it was found that only three children in the treatment group (9.7%) had adverse effects, namely dizziness, insomnia, colic, and weight gain.
Conclusion: CBD-rich cannabis extract was found to improve one of the diagnostic criteria for ASD (social interaction), as well as features that often co-exist with ASD, and to have few serious adverse effects.
Keywords: Autism spectrum disorder, child behavior, clinical trial, cannabis, cannabidiol.

Introduction

According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by persistent deficits in social communication and social interaction, in multiple contexts, and by the presence of restricted and repetitive patterns of behavior, interests, or activities. The DSM-5 also adopts ASD severity level as a specifying criterion, which varies according to

[1] Programa de Pós-Graduação em Neurociência Cognitiva e Comportamento, Departamento de Psicologia, Universidade Federal da Paraíba (UFPB), João Pessoa, PB, Brazil. [2] UFPB, João Pessoa, PB, Brazil. [3] Departamento de Estatística, UFPB, João Pessoa, PB, Brazil. [4] Sociedade Brasileira de Estudos da Cannabis Sativa (SBEC), São Paulo, SP, Brazil. [5] Departamento de Psicologia, UFPB, João Pessoa, PB, Brazil. [6] Departamento de Fonoaudiologia, UFPB, João Pessoa, PB, Brazil. [7] Departamento de Fisiologia e Patologia, UFPB, João Pessoa, PB, Brazil.

Submitted Sep 14 2021, accepted for publication May 19 2022.

Suggested citation: Silva Junior EA, Medeiros WMB, Santos JPM, Sousa JMM, Costa FB, Pontes KM, et al. Evaluation of the efficacy and safety of cannabidiol-rich cannabis extract in children with autism spectrum disorder: randomized, double-blind, and placebo-controlled clinical trial. Trends Psychiatry Psychother. 2024;46:e20210396. http://doi.org/10.47626/2237-6089-2021-0396

the need for support: mild (needs support), moderate (needs substantial support), or severe (needs very substantial support).[1]

There are no treatments proven to target the core features of ASD. Existing treatments are solely symptomatic, mainly aiming to reduce aggressiveness and psychomotor agitation symptoms and usually using psychotropic medications to effect these behavioral changes. In order to help patients affected by this disorder, there is great interest in discovering new therapeutic options to overcome the ineffectiveness of some of the conventional psychotropic drugs used to treat ASD, or even to enable their suspension, reducing the adverse effects associated with these drugs.[2]

Endocannabinoids are substances that are part of the endocannabinoid system (ECS) that are key modulators of socioemotional responses, cognition, seizure susceptibility, nociception, and neuronal plasticity and all of these responses are altered in autism.[3,4] Phytocannabinoids, mainly cannabidiol (CBD) and tetrahydrocannabinol (THC), present in several subspecies of the Cannabis genus, have been widely studied as a potential therapeutic alternative for treatment of symptoms associated with ASD, because they activate cannabinoid receptors present in the central nervous system, alleviating some symptoms associated with autism.[5]

Even though cannabis is becoming a topic of great interest among scientists, it is still difficult to find clinical trials with humans due to the restrictions and legal issues involving the plant.[6] Studies using cannabis for treatment of difficult-to-control epilepsy have found that, in addition to seizure reduction, there were also improvements in behavior and social interaction in children who had ASD as a comorbidity.[7]

In light of the above, the purpose of this research is to evaluate the efficacy of CBD-rich cannabis extract in children with ASD, monitoring aspects from the DSM-5 diagnostic criteria (social interaction, speech, and stereotypes) and other aspects that often coexist with ASD (aggressiveness, psychomotor agitation, impaired concentration, eating disorders, sleep disorders, and anxiety), as well as to assess the tolerability and safety of the therapeutic adjuvant.

Method

This study was a randomized, double-blind, placebo-controlled, 12-week clinical trial following the CONSORT recommendations. Initially, G Power software was used to calculate the sample size based on studies by Handen et al.[8] with children with ASD who took donepezil. An alpha of 0.05 and power of 0.80 were used. The calculation showed that 62 subjects would be needed (31 per group).

This study included children aged from 5 to 11 years who lived in the state of Paraíba, Brazil, or in neighboring states (Pernambuco and Rio Grande do Norte), who had a medical diagnosis of ASD, regardless of whether they had mild, moderate, or severe levels of ASD impairment, and whose caregivers signed the informed consent form. This age group was selected because children aged from 5 to 11 years exhibit greater similarity in brain development, making the group more homogeneous for analysis of the results. In addition, children older than 5 years would be more likely to have developed verbal language and be better able to respond during neuropsychological testing. For these reasons, we chose this age group to participate in this study. Children who had comorbidities such as diabetes mellitus, hypertension, autoimmune diseases, or refractory epilepsy or who had used a cannabis product in the last two months before starting the study were excluded.

To recruit the sample, the study was publicized widely through autism support institutions, with informative lectures and posts on WhatsApp and social media (Facebook and Instagram). Thereafter, those interested in the study registered on a website created exclusively for this clinical trial and filled out the sociodemographic questionnaire and the Childhood Autism Rating Scale (CARS) screening instrument.[9] The cut-off score is 15 points and CARS results were also used to assess severity. After applying the eligibility criteria, the researchers contacted caregivers to provide further explanations about the research.

After recruitment, the 64 selected children were randomized and stratified by severity. The randomization was conducted using the True Random Number Service, available at www.random.org. Any researchers who had direct contact with the patients were blinded to the treatment provided, except for one pharmacy student, who was responsible for delivering the vials to the researching physician weekly. Finally, baseline evaluations were scheduled with all children participating in this study and performed by the same child and adolescent psychiatrist responsible for the trial, to whom the products for the trial were also delivered. Caregivers were instructed to always start with a dose of three drops every 12 hours, preferably during a fasting period and with an interval of at least 1 hour before or after use of psychotropic medication, especially antipsychotics, according to the protocol suggested by the product supplier, since it was a plant extract. The starting dose of CBD-rich cannabis extract

used in this study was six drops daily, increased by two drops daily twice a week, if necessary, up to a maximum dose of 70 drops daily.

This clinical study was conducted in the outpatient sector, on the campus of the Hospital Universitário Lauro Wanderley, Universidade Federal da Paraíba (UFPB), which is located in João Pessoa, Paraíba. This research was approved by the institutional ethics review board at the UFPB Centro de Ciências da Saúde, under CAAE number 89392518.4.0000.5188. It is registered on the Brazilian Registry of Clinical Trials (ReBEC) under number 10743.

The product used was CBD-rich cannabis extract at a concentration of 0.5% (5 mg/mL), in the ratio of 9CBD:1THC, supplied by the Associação Brasileira de Apoio Cannabis Esperança (ABRACE). The extract used throughout the clinical trial was from the same batch, in order to ensure the same phytochemical and pharmacobotanical characteristics during production of the extract. The CBD-rich cannabis extract and the placebo product without it had the same consistency, color, odor, and other organoleptic characteristics, making it impossible for patients or the multidisciplinary team accompanying them to differentiate between the two.

To evaluate the effectiveness of the treatment, we used a semi-structured interview prepared by the authors containing questions related to ASD symptoms and the Autism Treatment Evaluation Checklist (ATEC),[10] which were administered to and answered by caregivers before and after the clinical trial. The number of daily meals was reported by the child's caregiver during the psychiatric consultation after they had answered the semi-structured interview questionnaire. In order to assess safety, before starting the study, all children underwent a laboratory evaluation, including kidney and liver function tests, as well as complete blood count and fasting glucose levels.

All analyses were performed using R version 4.0.2, which is free software available at https://www.r-project.org/. A 5% significance level was adopted for all analyses. Statistical analysis was performed using the mixed variance test for two factors (two-way ANOVA). In cases in which the null hypothesis was rejected, Tukey's multiple comparisons post-hoc test was applied to identify which groups had significant differences. As there is no non-parametric technique available in the literature regarding mixed analysis of variance for two factors, a simple non-parametric analysis was used for each factor individually, in order to support the results obtained by the parametric technique. The treatment and control groups and before and after data were compared using the Wilcoxon test for independent and dependent samples respectively.

Results

Sociodemographic analysis of participants' parents

A range of different sociodemographic variables regarding the parents were evaluated, including: age; education; whether they had another child, and whether they had other children on the autism spectrum; whether father and/or mother went to work away from the home; whether one of the parents had had to stop working because the child was diagnosed with ASD; and parents' marital status (Table 1).

Sociodemographic analysis of the children

In general, there were no significant differences between the treatment and control groups for the sociodemographic variables evaluated in the children with ASD who participated in this study. However, it is important to observe whether the child was receiving any professional healthcare intervention for ASD (public, private, or mixed) and the professional(s) responsible for this care (occupational therapist, physical therapist, speech therapist, psychologist, psychopedagogue, or others); whether the child was using psychotropic drugs before, during, or after the clinical trial; whether or not the child had food selectivity and whether this eating pattern was modified; the severity of ASD (mild [needs support], moderate [needs substantial support], or severe [needs very substantial support], according to the DSM-5 classification); whether there were adverse effects associated with the use of the product (CBD-rich extract or placebo); the number of drops of the product being taken at the end of the trial, since the increase was gradual and as directed by the researcher; whether the caregiver was in doubt, did not notice, or could not see improvement with the test product at the final consultation, before the researcher or the participants were aware whether the child had been allocated to the treatment or the control group. Finally, because the coronavirus (coronavirus disease 2019 [COVID-19]) pandemic broke out during the clinical trial, the children were isolated at home or could not receive professional attention, causing changes to their routine, which in itself is a disorganizing factor for those with ASD, so the researchers included the participants' parents' reports of the effects of isolation on their children's symptoms in the final evaluation (Table 2).

Analysis of the semi-structured interview, ATEC, and CARS

Important variables associated with ASD were assessed using a semi-structured questionnaire. The symptoms evaluated were aggressiveness,

psychomotor agitation, concentration, meals (number of meals/day), sleep (number of hours of sleep/day), social interaction with peers, verbal language (speech), anxiety; and repetitive and stereotyped movements (stereotypies). Mean scores were also calculated for the ATEC scale (and its subdivisions: ATEC L, related to language; ATEC S, related to socialization; ATEC P, related to sensory and cognitive perception; ATEC SC, related to health, physical aspects, and behavior; and ATEC T, which is the total sum of the scale) and the CARS scale (Table 3).

It can be observed from the results of the semi-structured interview that children who received the CBD-rich cannabis extract showed a significant improvement in psychomotor agitation, started to accept more meals a day, had much improved social interaction, and were less anxious, when compared to children in the control group, suggesting improvement in some symptoms associated with the ASD condition. On the other hand, regarding the "concentration" variable, it was observed that only children with mild ASD who received the CBD-rich cannabis extract showed significant improvement in this variable (Table 4). For this reason, it could be suggested that there is a difference between different levels of ASD severity for the "concentration" variable only.

Four children dropped out of the study during recruitment and laboratory testing prior to the start of the clinical trial. Three of these had been recruited for the control group and one for the treatment group,

Table 1 - Sociodemographic data on the parents of children with autism spectrum disorder (ASD)

Variable (parents)	Treatment group (n = 31)	Control group (n = 29)	Total (n = 60)
Mother's age (at conception)	29.00 (29.00) ± 6.09	30.00 (30.00) ± 6.85	29.46 (30.00) ± 6.42
Father's age (at conception)	34.05 (34.00) ± 6.69	32.10 (31.00) ± 5.67	33.10 (33.00) ± 6.21
Mother's education			
Incomplete elementary	1 (3.57)	2 (7.69)	3 (5.56)
Complete elementary	0 (0.00)	1 (3.85)	1 (1.85)
Incomplete secondary	2 (7.14)	0 (0.00)	2 (3.70)
Complete secondary	9 (32.14)	7 (26.92)	16 (29.63)
Incomplete higher	4 (14.29)	6 (23.08)	10 (18.52)
Complete higher	6 (21.43)	4 (15.38)	10 (18.52)
Postgraduate	6 (21.43)	6 (23.08)	12 (22.22)
Father's education			
Incomplete elementary	0 (0.00)	1 (5.56)	1 (2.63)
Complete elementary	1 (5.00)	1 (5.56)	2 (5.26)
Incomplete secondary	3 (15.00)	6 (33.33)	9 (23.68)
Complete secondary	2 (10.00)	2 (11.11)	4 (10.53)
Incomplete higher	8 (40.00)	4 (22.22)	12 (31.58)
Complete higher	6 (30.00)	4 (22.22)	10 (26.32)
Other children			
No	11 (35.48)	10 (34.48)	21 (35.00)
Yes	14 (45.16)	14 (48.28)	28 (46.67)
Yes and with ASD	6 (19.35)	5 (17.24)	11 (18.33)
Working parents			
No	1 (3.23)	2 (6.90)	3 (5.00)
Yes	20 (64.52)	14 (48.28)	34 (56.67)
One of the parents had to stop	10 (32.26)	13 (44.83)	23 (38.33)
Father or mother's marital status			
Single	8 (25.81)	6 (20.69)	14 (23.33)
Married	15 (48.39)	17 (58.62)	32 (53.33)
Divorced	8 (25.81)	5 (17.24)	13 (21.67)
Other	0 (0.00)	1 (3.45)	1 (1.67)

Qualitative variables: n (%); quantitative variables: average (median) ± standard deviation.

Table 2 - Sociodemographic data and information about the children participating in the research

Variable (children)	Treatment group (n = 31)	Control group (n = 29)	Total (n = 60)
Gender			
Male	25 (80.65)	27 (93.10)	52 (86.67)
Female	6 (19.35)	2 (6.90)	8 (13.33)
Age	7.64 (7.00) ± 1.76	7.72 (7.00) ± 1.75	7.68 (7.00) ± 1.74
Child's education			
Does not attend	2 (6.45)	2 (6.90)	4 (6.67)
Beneath the expected grading	4 (12.90)	6 (20.69)	10 (16.67)
Within the expected grading	25 (80.65)	21 (72.41)	46 (76.67)
Treatment type			
Does not receive treatment	6 (19.35)	7 (24.14)	13 (21.67)
Public	14 (45.16)	9 (31.03)	23 (38.33)
Private	7 (22.58)	11 (37.93)	18 (30.00)
Mixed	4 (12.90)	2 (6.90)	6 (10.00)
Occupational therapy	16 (51.61)	17 (58.62)	33 (55.00)
Physiotherapy	1 (3.23)	1 (3.45)	2 (3.33)
Speech therapy	19 (61.29)	20 (68.97)	39 (65.00)
Psychology	15 (48.39)	17 (58.62)	32 (53.33)
Psychopedagogy	11 (35.48)	12 (41.38)	23 (38.33)
Other treatments	6 (19.35)	2 (6.90)	8 (13.33)
Psychotropics			
Does not use	17 (54.84)	10 (34.48)	27 (45.00)
Used and continued	12 (38.71)	16 (55.17)	28 (46.67)
Used and stopped	1 (3.23)	2 (6.90)	3 (5.00)
Did not use and started	1 (3.23)	1 (3.45)	2 (3.33)
Selective eating			
No	16 (51.61)	17 (58.62)	33 (55.00)
Yes and continued	8 (25.81)	7 (24.14)	15 (25.00)
Yes and stopped	7 (22.58)	5 (17.24)	12 (20.00)
Severity			
Mild	13 (41.94)	13 (44.83)	26 (43.33)
Moderate	16 (51.61)	13 (44.83)	29 (48.33)
Severe	2 (6.45)	3 (10.34)	5 (8.33)
Adverse side effects	4 (12.90)	5 (17.24)	9 (15.00)
Subjective improvement			
No	7 (22.58)	12 (41.38)	19 (31.67)
Unsure	3 (9.68)	7 (24.14)	10 (16.67)
Yes	21 (67.74)	10 (34.48)	31 (51.67)
Number of drops being taken	47.42 (52.00) ± 15.22	40.96 (44.00) ± 18.86	44.30(50.00) ± 17.23
Isolation interfered?			
No	19 (61.29)	19 (65.52)	38 (63.33)
Only initially	4 (12.90)	1 (3.45)	5 (8.33)
Yes	8 (25.81)	9 (31.03)	17 (28.33)

Qualitative variables: n (%); quantitative variables: average (median) ± standard deviation.

Table 3 - Assessment of different variables in children with autism spectrum disorder (ASD) in the control group and the treatment group

Variable	Treatment group (n = 31)	Control group (n = 29)	p-value
Aggressiveness	0.81 (0.00) ± 1.05	1.39 (1.00) ± 1.36	0.2149
Psychomotor agitation	1.64 (2.00) ± 1.28	2.65 (3.00) ± 1.14	0.00295*
Concentration	1.71 (2.00) ± 1.07	2.96 (3.00) ± 0.86	0.269
Meals	1.32 (0.00) ± 1.90	0.38 (0.00) ± 0.82	0.045†
Sleep	0.77 (0.00) ± 1.61	0.28 (0.00) ± 0.59	0.0711
Social interaction	1.68 (2.00) ± 1.01	2.83 (3.00) ± 1.10	0.000268‡
Speech	1.32 (1.00) ± 1.42	1.72 (1.00) ± 1.55	0.3918
Anxiety	1.84 (2.00) ± 1.39	2.90 (3.00) ± 1.23	0.0159*
Stereotypy	1.45 (1.00) ± 1.06	2.07 (2.00) ± 1.03	0.3853
ATEC L	12.16 (12.00) ± 7.49	13.14 (13.00) ± 8.18	0.254
ATEC S	13.64 (15.00) ± 6.31	17.83 (18.00) ± 9.83	0.113
ATEC P	13.68 (13.00) ± 7.77	16.86 (18.00) ± 8.53	0.212
ATEC SC	25.35 (25.00) ± 10.79	27.17 (25.00) ± 11.03	0.119
ATEC T	64.84 (63.00) ± 26.82	75.00 (78.00) ± 32.89	0.098
CARS	33.47 (31.00) ± 8.48	37.83 (39.00) ± 9.02	0.188

CARS = Childhood Autism Rating Scale.
Results are expressed as average (median) ± standard deviation.
All p-values were calculated for the treatment after versus the control after groups using the two-factor mixed analysis of variance (two-way ANOVA) test followed by Tukey and Wilcoxon.
The Autism Treatment Evaluation Checklist (ATEC) subscales are as follows: ATEC L, related to language; ATEC S, related to socialization; ATEC P, related to sensory and cognitive perception; and ATEC SC, related to health, physical aspect, and behavior, while ATEC T, is the total sum of the scale.
* $p < 0.01$; † $p < 0.05$; ‡ $p < 0.001$.

Table 4 - Mixed analysis of variance for two factors (R software version 4.0.2)

Variable	df	Sum Sq	Mean Sq	F value	Pr(>F)
Social interaction	1	17.63	17.633	14.133	0.000268*
Residuals	116	144.73	1.248		
Psychomotor agitation	1	14.70	14.700	9.225	0.00295†
Residuals	116	184.84	1.593		
Anxiety	1	10.21	10.208	5.989	0.0159‡
Residuals	116	197.73	1.705		
Number of meals per day	1	9.63	9.633	4.109	0.045‡
Residuals	116	271.99	2.345		
Concentration (mild group)	1	5.56	5.558	6.747	0.0124‡
Residuals	48	39.54	0.824		

All p-values were calculated for the treatment after versus the control after groups using the two-factor mixed analysis of variance (Two-way ANOVA) test followed by Tukey and Wilcoxon.
* $p < 0.001$; † $p < 0.01$; ‡ $p < 0.05$.

thus resulting in a final sample of 60 children (31 in the treatment group and 29 in the control group). The reason for dropping out was that they did not live in the city where the clinical trial was being run and had difficulties traveling there. Furthermore, we also analyzed some important hematological parameters, including complete blood test, glycemia, aspartate aminotransferase (ALT), alanine aminotransferase (AST), urea and creatinine, and it was observed that all these parameters were within normal limits in all children.

The Supplementary Material (online-only) presents additional two-way mixed ANOVA statistical analyses.

Discussion

Even though the CBD-rich extract was used at a low concentration (2.5 mg/mL), using three drops twice a day, and the basic dose was determined by the ABRACE protocol, improvements were observed in social interaction, psychomotor agitation, number of meals, anxiety, and concentration, and the adverse effects experienced by a few of the children were mild and transient. Results showing improved concentration were only observed in the mild group.

The sample comprised 86.7% male children and was not selected by gender, but it is known that ASD is more

common in boys than in girls.[5,11] Females, as a rule, are more rarely affected (four boys to each girl for autism and 10 boys to each girl for Asperger's Syndrome). This pattern led to the hypothesis of a "female protective effect," a purported biological aspect by which females require a greater etiological "burden" to manifest autism.[12,13] Enrollment for possible participation in the clinical trial was open to the general population, but there were significantly fewer female children (296 children enrolled, 45 of whom were female, thus corresponding to only 15.2%, and eight girls [13.3%] and 52 boys [86.7%] were selected for the clinical trial).

Concerning the challenges of the COVID-19 pandemic for people with ASD, given the importance of routine in their lives, families with children with ASD face enormous challenges to mitigate the impact of the condition, as they often fail to carry out preventive measures. Sudden changes, such as social isolation, can cause emotional and behavioral changes, such as anxiety, agitation, and agressiveness.[14] It is not clear whether COVID-19 had any impact on the study, but differences were observed in medical consultations. For this reason, at the end of the study, parents were asked whether social isolation, which led to temporary discontinuation of multidisciplinary treatments and consequent changes in routine, had interfered with the children's functioning and 71.7% reported that there was no interference with any significant impairment.

The neuropsychological assessment is crucial to complement a diagnosis of ASD and to monitor the child's progress while undergoing medication-based interventions. It was also important that neuropsychological tests, such as executive functions, Theory of Mind, empathy, and attention[15] were performed to evaluate the main psychological functions related to ASD in several areas, such as severity of autism and verbal language.

Efficacy of CBD-rich cannabis extract

One of the core symptoms of ASD, and one of its DSM-5 diagnostic criteria, is persistent impairment in social interaction. In the present study, the result revealing the most robust improvement ($p < 0.001$) was social interaction. The reduction in psychomotor agitation is of great relevance. Parents of ASD children often report several food restriction problems and inadequate diet.[16,17] These issues were observed in our sample, in which the caregivers said that many participants had fewer daily meals than desired and had difficulty eating due to sensory and food selectivity issues. An improvement was observed in eating habits in the treatment group, seen in the parameter "meals." Anxiety accompanies many children with ASD and can lead to behavioral changes, given that they often cannot express what they feel, and also leads to psychological suffering.[18-20]

Another major complaint of parents of ASD children relates to concentration, so it was very important to analyze this according to the degree of severity of ASD, because mild children possibly have less cognitive impairment, since they require less support, which could suggest that improvement in the ability to concentrate might only occur at this severity level, as was found in this study, but we cannot confirm such a hypothesis on the basis of this clinical trial.

A study with 400 individuals in New Zealand evaluating the prescription of CBD in clinical practice also assessed neurological symptoms, which included Parkinson's disease, multiple sclerosis, epilepsy, ASD with challenging behavior, amyotrophic lateral sclerosis, multiple system atrophy, chronic pain, various neuropathies, and tremors. Mental health symptoms include anxiety disorders, depressive disorders, post-traumatic stress disorder, stress disorder, and insomnia.[21] However, to date, there have been no randomized, double-blind, placebo-controlled clinical trials with samples composed only of children with ASD, regardless of stratification by severity.

Although retrospective, one research study with a sample of 60 individuals with an average age of 11 years did show improvements in behavioral outbursts (61%) and anxiety (39%).[22] A prospective study with cannabis use, which also included adults (53 participants aged 4 to 22 years) and employed biweekly evaluations using structured interviews, resulted in 67.6% improvement in self-injury and bouts of anger, 68.4% improvement in hyperactivity, 71.4% improvement in sleep disturbances, and 47.1% improvement in anxiety, with mild to moderate adverse effects, such as drowsiness and decreased appetite.[23]

In another prospective study, 188 children were observed for 6 months, with all subjects receiving cannabis. From the results of structured questionnaires filled out by their parents, it was found that 30.1% of the subjects presented significant improvement, 53.7% moderate improvement, 6.4% slight improvement, and 8.6% presented no improvement, with agitation (6.6%) and drowsiness (3.2%) as adverse effects.[24]

One observational study looked at efficacy and tolerability over 6 to 9 months, including analysis of comorbidities using monthly structured questionnaires, and found that 93% improved 30% or more in at least one symptom category, 47% improved 30% or more in four or more symptom categories, 13% improved 30% or more in two symptom categories; and 33% improved 30% or more in one symptom category. The symptom categories were as follows: 1) ADHD; 2) behavioral

disorders; 3) motor deficits; 4) autonomy deficits; 5) communication and social interaction deficits; 6) cognitive deficits; 7) sleep problems; 8) seizures.[25]

Individuals with ASD who used the CBD-rich cannabis extract showed improvement in the following symptoms: self-injury and bouts of anger, hyperactivity, sleep problems, anxiety, restlessness, psychomotor agitation, irritability, aggressiveness, sensory sensitivity, cognition, attention, social interaction, language, perseveration, and depression. Regarding the benefits of the intervention with cannabis, the restlessness symptom showed the greatest improvement (91%) in relation to the other symptoms studied.[26]

Therefore, our results show what the scientific literature demonstrates about its efficacy in hyperactivity, restlessness, and psychomotor agitation; in anxiety; in cognition, attention and concentration; in facilitating learning; in nutrition; and in social interaction in children on the autism spectrum.

Safety of CBD-rich cannabis extract

Currently, CBD is approved by the Food and Drug Administration (FDA) for treatment of Dravet and Lennox-Gastaut syndromes, which are related to seizures.[27] Randomized clinical trials have shown that when CBD is added to an anticonvulsant, the frequency of seizures decreases.[28,29] As the scientific literature shows good response to epilepsy, we included children who presented epilepsy as a comorbidity as an exclusion criterion, in order to specifically analyze the characteristics of cannabis for ASD.[30,31]

Numerous pre-clinical studies[32,33] and neuroimaging studies[34] have demonstrated the anxiolytic effects of CBD. A published case series in psychiatric patients found CBD was beneficial for anxiety and sleep.[35] According to the results described, anxiety is identified as a relevant characteristic associated with ASD, corroborating the scientific literature that presents overall improvement in anxiety in some studies.[32-35]

Researchers advise medical professionals, who encounter young patients using CBD, to discuss its quality and possible adverse effects and drug interactions, which were carefully analyzed in this study. If any subject had exhibited poorly understood symptoms such as fever, diarrhea, vomiting, or drowsiness, the adverse effects of CBD oil would not have gone unnoticed.

In this trial, it was found that only three children in the treatment group (9.7%) had adverse effects, which were dizziness and insomnia in one child, colic in one, and weight gain in another. In some studies, the following symptoms of adverse effects were observed: sleep disorders, restlessness, and nervousness, as well as moderate irritability, diarrhea, increased appetite, conjunctival hyperemia, behavioral problems, decreased cognition, fatigue, and aggression/agitation.[24,25]

These agents need to be evaluated over time for the long-term effects of these drugs on development, which remains an open question.

One of the limitations of the study was the coronavirus pandemic, which started during the clinical trial. Since routine is crucial in the lives of children with ASD, their families were faced with enormous challenges to mitigate the impact of the condition. As the children were divided into six groups of 10 for consultation and product initiation, there was a difference regarding the start of use: those who started before the pandemic (COVID-19) and those who started during the pandemic, since the clinical trial started in January 2020 and a countrywide quarantine was implemented in March of the same year in Brazil. For the same reason, laboratory tests could not be performed after the end of the clinical trial.

New randomized, double-blind, placebo-controlled clinical trials using CBD-rich cannabis extract at higher concentrations and even using isolated CBD (phytochemical) for similar analyses would be an important contribution.

Conclusion

The CBD-rich cannabis extract was found to be safe at the doses used in this study (ranging from six to 70 drops/day), given that only three of the 31 children who received the extract reported very mild side effects, such as dizziness, insomnia, colic, and weight gain. Titration could reach a maximum of 100 drops per day, as directed by the product supplier.

Based on the results obtained, it can be concluded that CBD-rich cannabis extract showed significant improvement in social interaction, anxiety, and psychomotor agitation when compared to children who received the placebo and that CBD-rich cannabis extract did not interfere with the children's sleep quality.

Another important result in this study was an increased number of meals per day among children who received the CBD-rich cannabis extract when compared to the children who received the placebo. This result may be related to the decreased anxiety levels of these children observed after administration of the extract.

Therefore, it is observed that CBD-rich cannabis extract is effective and can be used safely, at least in the short term, to relieve some important symptoms related to ASD in children, such as social interaction, psychomotor agitation, and anxiety.

Acknowledgements

The authors are grateful to all the families who allowed their children to participate in this study and to ABRACE for donating the product for the trial.

Disclosure

No conflicts of interest concerning the publication of this article.

References

1. Associação Americana de Psiquiatria. DSM-5: Manual diagnóstico e estatístico de transtornos mentais, 5ª edição. Porto Alegre: Artmed Editora; 2014.
2. Gomes FA. Comorbidades clínicas em psiquiatria. São Paulo: Atheneu; 2012.
3. Marco EM, Laviola G. The endocannabinoid system in the regulation of emotions throughout lifespan: a discussion on therapeutic perspectives. J Psychopharmacol. 2012;26:150-63.
4. Trezza V, Damsteegt R, Manduca A, Petrosino S, Van Kerkhof LWM, Pasterkamp RJ, et al. Endocannabinoids in amygdala and nucleus accumbens mediate social play reward in adolescent rats. J Neurosci. 2012;32:14899-908.
5. Chakrabarti B, Persico A, Battista N, Maccarrone M. Endocannabinoid signaling in autism. Neurotherapeutics. 2015;12:837-47.
6. Anderson CL, Evans VF, DeMarse TB, Febo M, Johnson CR, Carney PR. Cannabidiol for the treatment of drug-resistant epilepsy in children: current state of research. J Pediatr Neurol. 2017;15:143-50.
7. Gu B. Cannabidiol provides viable treatment opportunity for multiple neurological pathologies of autism spectrum disorder. Glob Drugs Therap. 2017;2:1-4. DOI: 10.15761/GDT.1000134.
8. Handen BL, Johnson CR, McAuliffe-Bellin S, Murray PJ, Hardan AY. Safety and efficacy of donepezil in children and adolescents with autism: neuropsychological measures. J Child Adolesc Psychopharmacol. 2011;21:43-50.
9. Schopler E, Reichler RJ, Renner BR. CARS: The childhood autism rating scale. Los Angeles: Western Psychological Services; 1988.
10. Rimland B, Edelson S. Autism Treatment Evaluation Checklist (ATEC). San Diego: Autism Research Institute; 1999.
11. de la Torre-Ubieta L, Won H, Stein JL, Geschwind DH. Advancing the understanding of autism disease mechanisms through genetics. Nat Med. 2016;22:345-61.
12. Happé F, Frith U. Annual Research Review: Looking back to look forward – changes in the concept of autism and implications for future research. J Child Psychol Psychiatry. 2020;61:218-32.
13. Baron-Cohen S, Tsompanidis A, Auyeung B, Nørgaard-Pedersen B, Hougaard DM, Abdallah M, et al. Foetal oestrogens and autism. Mol Psychiatry. 2019;1-9.
14. Rocha AB, Santoro RA, Crenzel G, Mendonça ASA, Cabral RL, Abranches C. Autism and the new challenges imposed by the COVID-19 pandemic. Rev Pediatr SOPERJ. 2020;6.
15. Jones CRG, Simonoff E, Baird G, Pickles A, Marsden AJS, Tregay J, et al. The association between theory of mind, executive function, and the symptoms of autism spectrum disorder. Autism Res. 2018;11:95-109.
16. Lázaro CP, Pondé MP. Narrativa de mães de crianças com transtorno do espectro do autismo: Foco no comportamento alimentar. Trends Psychiatry Psychother. 2017;39:180-7.
17. Cermak AS, Curtin C, Bandini LG. Seletividade alimentar e sensibilidade sensorial em crianças com transtornos do espectro do autismo. J Am Diet Assoc. 2010;110:238-46.
18. Melas PA, Scherma M, Fratta W, Cifani C, Fadda P. Cannabidiol as a potential treatment for anxiety and mood disorders: molecular targets and epigenetic insights from preclinical research. Int J Mol Sci. 2021;22:1863.
19. Petrie GN, Nastase AS, Aukema RJ, Hill MN. Endocannabinoids, cannabinoids and the regulation of anxiety. Neuropharmacology. 2021;195:108626.
20. Spinella TC, Stewart SH, Naugler J, Yakovenko I, Barrett SP. Evaluating cannabidiol (CBD) expectancy effects on acute stress and anxiety in healthy adults: a randomized crossover study. Psychopharmacology (Berl). 2021;238:1965-77.
21. Gulbransen G, Xu W, Arroll B. Cannabidiol prescription in clinical practice: an audit on the first 400 patients in New Zealand. BJGP Open. 2020;4:bjgpopen20X101010.
22. Aran A, Cassuto H, Lubotzky A, Wattad N, Hazan E. Brief Report: Cannabidiol-rich cannabis in children with autism spectrum disorder and severe behavioral problems – a retrospective feasibility study. J Autism Dev Disord. 2019;49:1284-8.
23. Barchel D, Stolar O, De-Haan T, Ziv-Baran T, Saban N, Fuchs DO, et al. Oral cannabidiol use in children with autism spectrum disorder to treat related symptoms and co-morbidities. Front Pharmacol. 2019;9:1521.
24. Bar-Lev Schleider L, Mechoulam R, Saban N, Meiri G, Novack V. Real life Experience of medical cannabis treatment in autism: analysis of safety and efficacy. Sci Rep. 2019;9:200.
25. Fleury-Teixeira P, Caixeta FV, Ramires da Silva LC, Brasil-Neto JP, Malcher-Lopes R. Effects of CBD-enriched cannabis sativa extract on autism spectrum disorder symptoms: an observational study of 18 participants undergoing compassionate use. Front Neurol. 2019;10:1145.
26. Food and Drug Administration. FDA approves first drug comprised of an active ingredient derived from marijuana to treat rare and severe forms of epilepsy. www.fda.gov/news-events/press-announcements/fda-approves-first-drug-comprised-active-ingredient-derived-marijuana-treat-rare-severe-forms. Accessed 2020 Nov 19.
27. Devinsky O, Patel AD, Cross JH, Villanueva V, Wirrell EC, Privitera M, et al. Effect of cannabidiol on drop seizures in the Lennox-Gastaut syndrome. N Engl J Med. 2018;378:1888-97.
28. Devinsky O, Cross JH, Laux L, Marsh E, Miller I, Nabbout R, et al. Trial of cannabidiol for drug-resistant seizures in the Dravet syndrome. N Engl J Med. 2017;376:2011-20.
29. Morano A, Fanella M, Albini M, Cifelli P, Palma E, Giallonardo AT, et al. Cannabinoids in the treatment of epilepsy: current status and future prospects. Neuropsychiatr Dis Treat. 2020;16:381.
30. Russo EB. Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. Br J Pharmacol. 2011;163:1344-64.
31. Bergamaschi MM, Queiroz RHC, Chagas MHN, de Oliveira DCG, De Martinis BS, Kapczinski F, et al. Cannabidiol reduces the anxiety induced by simulated public speaking in treatment-naïve social phobia patients. Neuropsychopharmacology. 2011;36:1219-26.
32. Zuardi AW, Cosme RA, Graeff FG, Guimarães FS. Effects of ipsapirone and cannabidiol on human experimental anxiety. J Psychopharmacol. 1993;7 Suppl 1:82-8.
33. Crippa JAS, Derenusson GN, Ferrari TB, Wichert-Ana L, Duran FLS, Martin-Santos R, et al. Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: a preliminary report. J Psychopharmacol. 2011;25:121-30.
34. Shannon S, Lewis N, Lee H, Hughes S. Cannabidiol in anxiety and sleep: a large case series. Perm J. 2019;23.
35. Wolff D, Reijneveld SA. Use of cannabidiol oil in children. Ned Tijdschr Geneeskd. 2019;163.

Correspondence:
Estácio Amaro da Silva Junior
Universidade Federal da Paraíba
Cidade Universitária, s/nº, Bairro Castelo Branco III
58051-085 - João Pessoa, PB - Brazil
E-mail: estacioamaro@yahoo.com.br

Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors

Article *in* Biochemical Pharmacology · July 1995

Source: PubMed

CITATIONS
2,404

READS
990

10 authors, including:

Raphael Mechoulam
Hebrew University of Jerusalem
503 PUBLICATIONS **60,244** CITATIONS
SEE PROFILE

Lumir Hanus
School of Pharmacy, Ein Kerem Campus, Hebrew University of Jerusal…
196 PUBLICATIONS **21,312** CITATIONS
SEE PROFILE

Ellen Kaminski
Heidelberg University
120 PUBLICATIONS **8,191** CITATIONS
SEE PROFILE

Asher Gopher
Sheba Medical Center
33 PUBLICATIONS **5,675** CITATIONS
SEE PROFILE

Pergamon

Biochemical Pharmacology, Vol. 50, No. 1, pp. 83–90, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(95)00109-3

IDENTIFICATION OF AN ENDOGENOUS 2-MONOGLYCERIDE, PRESENT IN CANINE GUT, THAT BINDS TO CANNABINOID RECEPTORS

RAPHAEL MECHOULAM,*† SHIMON BEN-SHABAT,* LUMIR HANUŠ,* MOSHE LIGUMSKY,* NORBERT E. KAMINSKI,‡ ANTHONY R. SCHATZ,§ ASHER GOPHER,‖ SHLOMO ALMOG,‖ BILLY R. MARTIN,§ DAVID R. COMPTON,§ ROGER G. PERTWEE,¶ GRAEME GRIFFIN,¶ MICHAEL BAYEWITCH,** JACOB BARG** and ZVI VOGEL**

*Faculty of Medicine, The Hebrew University, Jerusalem 91120, Israel; ‡Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824-1317, U.S.A.; ‖Institute of Clinical Pharmacology and Toxicology, Chaim Sheba Medical Center, Tel Hashomer, Israel; §Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298, U.S.A.; ¶Department of Biomedical Sciences, Marischal College, University of Aberdeen, Aberdeen AB9 1AS, U.K.; and **Department of Neurobiology, The Weizmann Institute of Science, Rehovot 76100, Israel

(*Received* 18 *September* 1994; *accepted* 9 *January* 1995)

Abstract—In this study, we report the isolation from canine intestines of 2-arachidonyl glycerol (2-Ara-Gl). Its structure was determined by mass spectrometry and by direct comparison with a synthetic sample. 2-Ara-Gl bound to membranes from cells transiently transfected with expression plasmids carrying DNA of either CB_1 or CB_2—the two cannabinoid receptors identified thus far—with K_i values of 472 ± 55 and 1400 ± 172 nM, respectively. In the presence of forskolin, 2-Ara-Gl inhibited adenylate cyclase in isolated mouse spleen cells, at the potency level of Δ^9-tetrahydrocannabinol (Δ^9-THC). Upon intravenous administration to mice, 2-Ara-Gl caused the typical tetrad of effects produced by THC: antinociception, immobility, reduction of spontaneous activity, and lowering of the rectal temperature. 2-Ara-Gl also shares the ability of Δ^9-THC to inhibit electrically evoked contractions of mouse isolated vasa deferentia; however, it was less potent than Δ^9-THC.

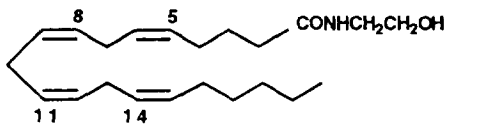
Key words: 2-arachidonyl glycerol; anandamide; tetrahydrocannabinol; arachidonylethanolamide; immune system; transfection; mouse behavior; adenylate cyclase inhibition

A cannabinoid receptor, CB_1††, was originally identified and cloned from rat brain [1, 2]. This receptor is negatively coupled to adenylate cyclase via a GTP-binding protein. Examination of other tissues known to be modulated by cannabinoid compounds subsequently led to the tentative identification of CB_1 receptors in spleen [3, 4] and testis [5]. More recently, a second cannabinoid receptor, termed CB_2, was identified in rat spleen and in the human promyelocytic leukemic line HL60 [6]. Unlike CB_1, which is abundantly expressed in a variety of brain regions, CB_2 mRNA transcripts have not been detected in rat brain, suggesting either the absence or very low receptor expression of CB_2 in this tissue. The CB_2 receptor was therefore defined by Munro *et al.* [6] as a peripheral receptor for cannabinoids. Little is known about the tissue specificity and exact localization of this receptor in the various peripheral tissues and whether it mediates cannabinoid signaling in the peripheral nervous system.

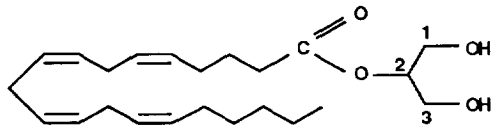
With the discovery of CB_1, and later of CB_2, research was initiated on the identification and function of their endogenous ligand(s). Using a CB_1 based binding strategy for screening potential endogenous ligands, we isolated ethanolamides of unsaturated fatty acids from porcine brain preparations [7–10]. Arachidonylethanolamide (anandamide) (Fig. 1), the ligand most widely investigated, binds to both CB_1 [7, 11, 12] and CB_2 [6], inhibits the production of cAMP [11, 12], and parallels the actions of the active constituent of cannabis, Δ^9-THC, in many *in vivo* and *in vitro* studies [13–15]. Thus far, anandamide has been identified only in brain tissue preparations. In the present studies, we describe the isolation of a second type of cannabinoid receptor ligand, 2-Ara-Gl, an ester isolated from canine gut (Fig. 1). This is the first putative endogenous cannabinoid receptor ligand isolated from a peripheral tissue. Included in this report is also the structure elucidation of 2-Ara-

† Corresponding author: Prof. R. Mechoulam, Faculty of Medicine, The Hebrew University, Ein Kerem Campus, Jerusalem 91120, Israel. Tel. 972-2-758634; FAX 972-2-410740 or 972-2-784010.

†† Abbreviations: 2-Ara-Gl, 2-arachidonyl glycerol; BSTFA, *N,O*-bis(trimethylsilyl) trifluoroacetamide; cAMP, adenosine 3′,5′-cyclic phosphate; CB_1, cannabinoid receptor originally found in rat brain (see text); CB_2, cannabinoid receptor not expressed in brain; expressed in macrophages in the marginal zone of spleen (see text); CI, chemical ionization; EBSS, Earle's balanced salt solution; EI, electron impact; IBMX, 1-methyl-3-isobutylxanthine; NBCS, newborn calf serum; RPMI, Roswell Park Memorial Institute medium; THC, tetrahydrocannabinol; and TMS, trimethyl silyl.



anandamide



2-arachidonyl glycerol (2-Ara-Gl)

Fig. 1. Structures of anandamide and 2-arachidonyl glycerol (2-Ara-Gl).

Gl, as well as an examination of the cannabimimetic activity of 2-Ara-Gl using a diverse group of established biological endpoints in tissues previously shown to exhibit sensitivity to the effects of cannabinoids. Included are the binding characteristics of 2-Ara-Gl in CB_1 and CB_2 transfected COS cells and the effects of this putative ligand on adenylate cyclase activity in mouse spleen cells, electrically evoked contractions of mouse isolated vasa deferentia, and behavioral effects in mice.

MATERIALS AND METHODS

Drugs. Anandamide was synthesized as previously described [7]. Δ^9-THC was prepared from cannabidiol as previously described [16]. 2-Ara-Gl was purchased from Deva Biotech, Inc. (Hatboro, PA). It was stored at −20° and protected from light. Forskolin, IBMX, 2-palmityl glycerol and 1-linoleyl glycerol were purchased from the Sigma Chemical Co. (St. Louis, MO). Emulphor (EL-620), a polyoxyethylated vegetable oil, was obtained from the GAF Corp. (Linden, NJ).

Materials. TLC plates (RP-18, $F_{254}S$) were purchased from Merck (Darmstadt, Germany). Silica gel was from ICN (Eschwege, Germany), Catalog No. 4530.

GC-MS. GC-MS analyses were carried out with a Finnigan SSQ 70 mass spectrometer coupled to a Varian 3400 gas chromatograph. Chromatographic separations were performed on a cross-linked methyl silicone (HP-1) capillary column (length, 25 m; internal diameter, 0.2 mm; film thickness, 0.33 μm); column temperature was programmed to increase from 70° to 300° at a rate of 20°/min following a 13-min holding time at 300°. Helium was used as the carrier gas at a head pressure of 10 psi. Injection temperature was 25° in the splitless mode. Mass spectra were obtained in both the EI and the CI (methane as the reagent gas) mode with electron energy of 70 eV. Ion source and transfer-line temperatures were 150° and 275°, respectively. The quadrupole was scanned in the m/z range 50–550 at 1 scan/sec.

Silylation of the endogenous compounds and standards was made by adding BSTFA to the dry sample. After 30 min of incubation at room temperature, the silylated material was injected into the GC-MS.

NMR. NMR spectra were recorded in $CDCl_3$ on a Varian VXR-300 S instrument at 300 MHz for ^1H. Due to the small amounts of material available, the spectra were scanned for 3–5 hr. The chemical shifts are described in parts per million.

Isolation of fatty acid esters of glycerol. Canine small intestines (350 g wet weight) were extracted with methanol (700 mL). Addition of acetone (1000 mL) to the extract precipitated inactive materials. The supernatant was evaporated and the residue was partitioned between chloroform and water. The chloroform soluble material (7.4 g) was chromatographed on silica gel with a solvent mixture consisting of chloroform : petroleum ether : methanol starting with a ratio of 40:6:0.02 followed by a gradual increase in the polarity of the solvent system. The active fractions were found mostly in the portion eluted with the above mixture with a solvent ratio of 40:6:1. Activity was measured by inhibition of the specific binding of the labeled cannabinoid [^{3}H]-HU-243 on synaptosomal membranes from rat brains in a centrifugal assay as previously described [7, 8, 17]. The active fractions were collected and separated again on TLC plates. The TLC plates were first predeveloped (without the extract) with chloroform : methanol (1:1). The elution was performed with methanol. The materials present in the bands were initially assayed for binding to the brain cannabinoid receptor in the centrifugation-based ligand binding assay. Only one fraction showed reproducible activity. It had an R_f on TLC of 0.68. It was investigated further by NMR and GC-MS. Pure compounds were tested for binding in transfected cells as described below.

Binding to transfected cells. COS-7 cells were transfected with plasmids containing the CB_1 or CB_2 receptor genes as previously described [2, 6]. Crude membranes were prepared, spun (20 min, at 15,000 g) and resuspended in 50 mM Tris–HCl, pH 7.4, containing 5 mM $MgCl_2$ and 2.5 mM EDTA, and binding of [^{3}H]HU-243 was carried out as previously described [11]. For determination of K_i values, data were analyzed by the Inplot4 computer program (GraphPad Software, San Diego, CA). The competition binding assay curves were generated with the Sigma Plot 4.11 computer program (Jandel Scientific, Corta Madera, CA).

cAMP determinations. Single spleen cell suspensions were prepared from naive female B6C3F1 mice. The cells were washed once with RPMI 1640 and centrifuged at 800 g for 10 min to form a pellet. EBSS containing 5% NBCS at 1 mL/spleen in addition to Gey's solution was added to the cell pellet. The solution was swirled on ice for 5 min to lyse red blood cells. The remaining intact spleen cells were pelleted by centrifugation at 1600 g for 15 min, and the supernatant containing the lysates was discarded. The cells were washed twice in EBSS and adjusted to 1×10^7 cells/mL in RPMI 1640

containing 1% NBCS. Aliquots (1 mL) of the isolated cells were transferred to 12 × 75 mL glass tubes, and 100 μM IBMX, a phosphodiesterase inhibitor, was added. Following a 10-min incubation at 37°, cells were treated with either vehicle (1% DMSO), Δ^9-THC (22 μM) or 2-Ara-Gl (1, 10, or 20 μM) and incubated for an additional 10 min at 37°. The cell preparation was then stimulated with 50 μM forskolin for 15 min, and the reaction was stopped by the addition of acidic ethanol (1 mL, 1 N HCl/100 mL EtOH). The cells were disrupted subsequently by sonication to facilitate the release of intracellular cAMP into the extraction buffer. The cell lysate was centrifuged at 1600 *g* for 15 min to remove any remaining cell fragments, and the supernatants were collected and lyophilized. The samples were stored at −20° prior to quantitation of cAMP. Aliquots of reconstituted lyophilized cell lysates were quantitated for cAMP using a cAMP assay kit (Diagnostic Products Inc., Los Angeles, CA). This method is based on the competition between unlabeled cAMP and a fixed quantity of ^{3}H-labeled cAMP for binding to a protein that has a high specificity and affinity for cAMP, which mimics the regulatory subunit of protein kinase A. The amount of the ^{3}H-labeled cAMP protein complex formed is inversely related to the amount of unlabeled cAMP present in the assay sample. The concentration of cAMP in test samples was determined by comparison with a standard curve.

Pharmacological tests in mice. Male ICR mice (22–30 g) obtained from Dominion Laboratories (Dublin, VA) were maintained on a 14:10 hr light:dark cycle and received food and water *ad lib.* Micellar suspensions were prepared by dissolving the drugs in a 1:1 mixture of ethanol and Emulphor prior to dilution with saline to produce a 1:1:18 ratio of ethanol:Emulphor:saline (vehicle). All injections were administered i.v. in a volume of 0.1 mL/10 g body weight.

Mouse behavioral procedures. The behavioral effects of 2-Ara-Gl were evaluated as described earlier [18]. Prior to vehicle or drug administration, rectal temperature was determined by a thermistor probe (inserted 25 mm) and a telethermometer (Yellow Springs Instrument Co., Yellow Springs, OH). Mice received tail-vein injections of the vehicle or drugs and were placed into individual photocell activity chambers 5 min later. Spontaneous activity was measured for a 10-min period in a Digiscan Animal Activity Monitor (Omnitech Electronics, Inc., Columbus, OH) as the number of interruptions of 16 photocell beams per chamber. Upon removal of the mice from the activity chambers, rectal temperature was measured again (15 min after the injection), and the difference in temperature before and after injection was calculated for each animal.

In a separate series of mice, baseline tail-flick latency (sec) was measured on a standard tail-flick apparatus [19]. Following i.v. administration of drug as described above, tail-flick latency was assessed at 15 min after the injection, and the increase in the latency period (sec) for each mouse was recorded. A separate group of mice was evaluated for ring-immobility at a period of 5–10 min after i.v. drug administration, utilizing the Pertwee Ring-Test [20].

Data analysis. For the *in vivo* studies, the data are expressed as percent maximal possible effect [19] or percent of control [21] converted to probit values, and the ED_{50} was determined by unweighted least-squares linear regression analysis of the log dose versus probit plot. This analysis procedure has been used previously to describe behavioral data generated from the administration of cannabinoid drugs [22].

Electrically evoked contractions of vasa deferentia isolated from mice. Vasa deferentia were obtained from albino MF1 mice. Tissues were mounted in 4-mL organ baths at an initial tension of 0.5 g using the method described by Pertwee *et al.* [23]. The baths contained Mg^{2+}-free Krebs solution kept at 37° and bubbled with 95% O_2 and 5% CO_2. Isometric contractions were elicited by electrical field stimulation through a platinum electrode attached to the upper end of each bath and a stainless steel electrode attached to the lower end. Stimuli were generated by a Grass S48 stimulator, then amplified (Med-Lab channel attenuator), and divided to yield separate outputs to four organ baths (Med-Lab Stimusplitter). Contractions were monitored by computer (Apple Macintosh LC) using a data recording and analysis system (MacLab) that was linked via preamplifiers (Macbridge) to Dynamometer UF1 transducers (Pioden Controls Ltd.). Tissues were stimulated with 0.5 trains of three pulses of 110% maximal voltage (train frequency 0.1 Hz; pulse duration 0.5 msec). Each tissue was subjected to several periods of stimulation. The first of these began after the tissue had equilibrated but before drug administration and continued for 11 min. Drug (10 μL) was added immediately after this first stimulation period. Subsequent stimulation periods lasted for 5 min at the end of which the bath contents were washed out by overflow and a higher dose of drug was added. The time interval between each drug addition and onset of stimulation was 25 min. Concentrations of 2-Ara-Gl and anandamide producing a 50% reduction in the amplitude of electrically evoked contractions (IC_{50} values) were calculated by non-linear regression analysis using GraphPad InPlot (GraphPad software, San Diego, CA). The IC_{50} value of anandamide was calculated from data published previously [7, 23].

RESULTS

As described in detail in Materials and Methods, canine gut was extracted with methanol, and the extract was chromatographed on a silica gel column to yield a fraction that was found to bind to CB_1 in a centrifugation-based ligand binding assay [7]. At this stage of the investigation, the binding assay was of a qualitative nature, as the extract was a mixture of several, apparently related compounds. The [^{1}H]-NMR spectrum of the active fraction showed the presence of protons on double bonds (at 5.38 ppm) coupled to protons at 2.8 ppm (presumably protons allylic to two double bonds), in a ratio of *ca.* 4:2–4:3, typical of mixtures of polyunsaturated fatty acids or their derivatives (including those of arachidonic acid).

On GC-MS, three main peaks were observed, which eluted after 10:39, 12:04 and 13:35 min.

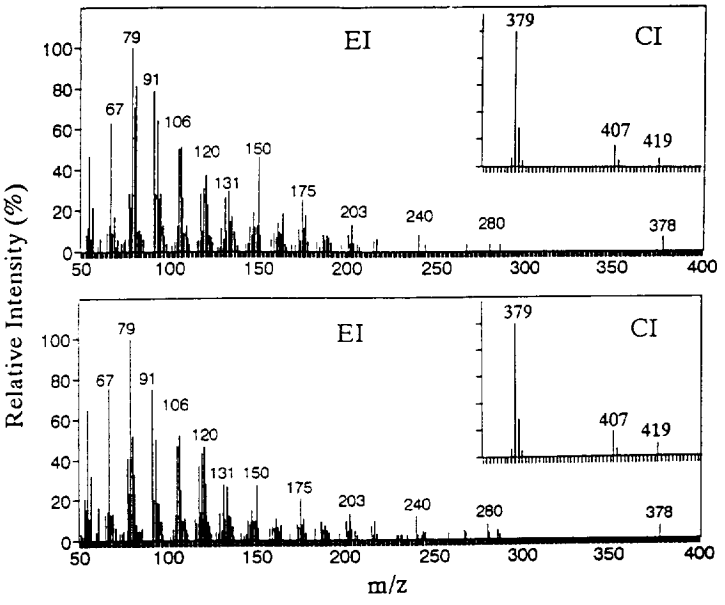


Fig. 2. Comparison of the electron impact (EI-MS) and chemical ionization (CI-MS) mass spectra of two samples of 2-arachidonyl glycerol (2-Ara-Gl). Top: identified in canine intestines. Bottom: synthetic.

Each one was examined separately. (See Fig. 2 for the EI and CI spectra of the compound eluted after 13:35 min.) The spectra suggested that the molecular weights of the three compounds were 330, 354 and 378, in view of the presence of the respective molecular M^+ ions (EI) and M + 1, M + 29 and M + 41 ions (CI with methane). Major ions in the EI spectra could be interpreted as due to acylium ions $[RCO]^+$ formed from esters of palmitic acid (16:0), linoleic acid (18:2, n-6) and arachidonic acid (20:4, n-6). On silylation with BSTFA, the three major compounds, observed prior to derivatization, were transformed into three pairs of compounds. The molecular weights of the compounds in each pair were identical, being 474, 498 and 522. The increase by 144 mass units upon derivatization corresponds to the formation of *bis*-TMS ethers, indicating the presence of two free hydroxyl groups in each compound. The above data suggested that the three pairs of compounds could be esters of palmitic acid, linoleic acid and arachidonic acid with glycerol. Direct GC-MS comparisons (retention times as well as EI and CI spectra) of the endogenous compounds (in their free and silylated forms) with commercial samples of 1- and 2-monoacylglycerols indicated that the three major compounds (one in each pair) are 2-palmityl, 2-linoleyl and 2-arachidonyl esters of glycerol. The minor components, which are present in concentrations of up to 30% of those of the major components, are the respective 1-acylglycerols. An MS comparison of synthetic 2-Ara-Gl with the endogenous constituent is presented in Fig. 2. The silylated 1-acylglycerols are easily differentiated from the silylated 2-acylglycerols by chromatography or by MS. Thus, silylated 2-acylglycerols produce characteristic fragments: $[M\text{-}RCOOH]^+$ (m/z 218) and $[RCO + 74]^+$, while $[M\text{-}RCOOCH_2]^+$ (m/z 205) and $[M\text{-}TMSOCH_2]^+$ are indicative of silylated 1-acylglycerols. The retention times of silylated 1-acylglycerols were shorter than those of silylated 2-acylglycerols: 10:54, 12:15 and 13:38 min for 1-palmityl, 1-linoleyl and 1-arachidonyl glycerols vs 11:12, 12:36 and 14:06 min for the respective 2-acylglycerols. Experiments are underway to determine whether the C-1 monoglycerides present are endogenous constituents or are formed during the extraction procedures from the 2-monoglycerides. Rearrangements of this type are well known in lipid chemistry [24].

In preliminary experiments, commercial 2-palmityl glycerol and 1-linoleyl glycerol were found to be inactive in the centrifugation-based ligand binding assay for CB_1 [7], and hence palmityl and linoleyl glycerols were not investigated further. In view of it arachidonic acid-based structure (reminiscent of the structure of anandamide), 2-Ara-Gl seemed *a priori* to be the active component of the mixture and was examined for binding activity to both CB_1 and CB_2 in suitably transfected cells.

Vogel *et al.* [11] and Felder *et al.* [12] have found that anandamide specifically binds to membranes from cells transiently (COS) or stably (Chinese hamster ovary) transfected with an expression plasmid carrying CB_1 DNA, but not to membranes from control non-transfected cells. In this system, the K_i for anandamide was 252 ± 47 nM. 2-Ara-Gl was also active in this assay, with a K_i of 472 ± 55 nM (Fig. 3). As indicated in Materials and Methods, we have now transfected into COS cells a plasmid carrying the CB_2 gene. Anandamide competed with $[^3H]$HU-243 bound to this receptor, with a K_i value of 581 ± 111 nM. 2-Ara-Gl was less active, with a K_i of 1400 ± 172 nM (Fig. 3). No binding was observed with non-transfected cells.

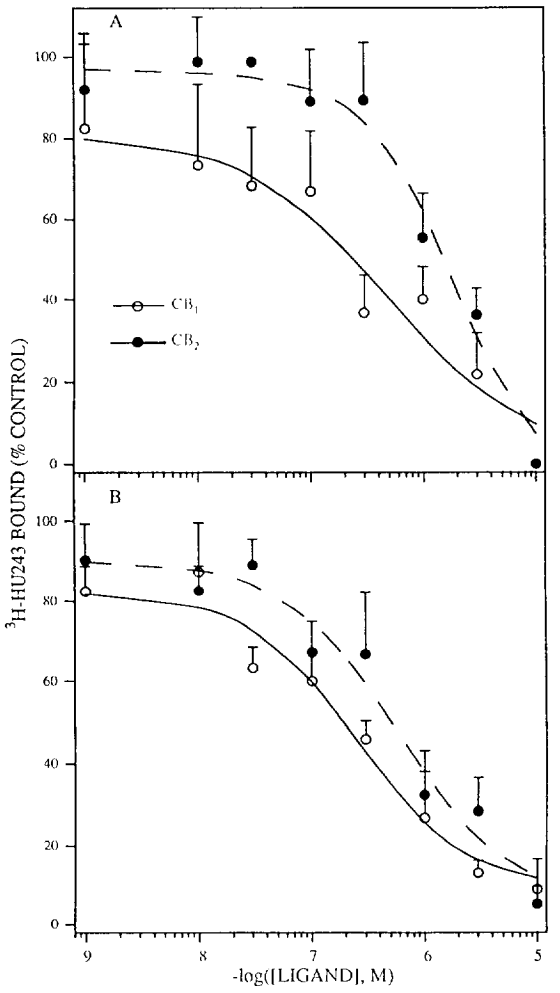


Fig. 3. Comparison of the binding of anandamide with that of 2-Ara-Gl to transfected cells that contain the brain cannabinoid receptor (CB_1) or the peripheral cannabinoid receptor (CB_2). (A) Binding of 2-Ara-Gl. (B) Binding of anandamide. Data are the means ± SEM of 3–5 experiments performed in duplicate.

2-Ara-Gl inhibited electrically evoked contractions of vasa deferentia isolated from mice (Fig. 4A). The IC_{50} value recorded was 4.8 μM, indicating the compound to be about 90 times less potent than anandamide (IC_{50} = 52 nM) [7].

Characteristic of cell types expressing cannabinoid receptors, cannabinoid treatment of spleen cell preparations has been shown to cause a marked inhibition of forskolin-stimulated adenylate cyclase activity [25]. To explore further whether 2-Ara-Gl exhibits cannabinoid-like activity, modulation of adenylate cyclase by this novel putative ligand was tested in mouse spleen cell preparations. In the presence of forskolin, 2-Ara-Gl produced a significant and concentration-related inhibition of adenylate cyclase activity, as demonstrated by a decrease in intracellular cAMP (Fig. 4B). The magnitude of inhibition ranged from approximately 20% at 1 μM to 60% at 20 μM 2-Ara-Gl, as compared with forskolin-stimulated controls. On a molar basis, 2-Ara-Gl exhibited a magnitude in inhibition of adenylate cyclase similar to that of Δ^9-THC (positive control). No significant difference was observed in intracellular cAMP between naive and vehicle controls.

When administered i.v. to mice, 2-Ara-Gl produced hypomotility, hypothermia, antinociception and catalepsy (Table 1), the same tetrad of effects produced by anandamide and Δ^9-THC [13, 15, 18]. 2-Ara-Gl was approximately equipotent to anandamide in all four behavioral assays and less potent that Δ^9-THC. All three compounds exhibited full efficacy in reducing spontaneous activity and in producing antinociception. 2-Ara-Gl appeared to be less efficacious than either Δ^9-THC or anandamide in lowering rectal temperature or in producing immobility. At a dose of 30 mg/kg, a modest 2° decrease in rectal temperature and immobility of only 26% were obtained. However, 2-Ara-Gl was lethal at a dose of 60 mg/kg. The animals died of unknown causes within 2 min of the injection.

The above-described isolation of 2-Ara-Gl was repeated with porcine brains. TLC separations of the extract were run side-by-side with authentic 2-Ara-Gl for comparison. Areas on the TLC plate that were parallel to that of 2-Ara-Gl were extracted with chloroform. We were unable to identify 2-Ara-Gl in any of the fractions extracted from these TLC plates (chromatographic behavior and GC-MS).

Canine gut was extracted following the procedures developed by us for the isolation of anandamide [7, 8]. TLC separations of the extract were run side-by-side with authentic anandamide for comparison. Areas on the TLC plate that were parallel to that of anandamide were extracted with chloroform. We were unable to identify anandamide in any of the fractions extracted from these TLC plates (chromatographic behavior and GC-MS).

DISCUSSION

Most of the recorded work on the pharmacology of cannabinoids deals with effects on the central nervous system [26, 27]. However, certainly not all cannabinoid effects are of central origin. Those on the immune system [26, 28–31] are presumably peripheral. Numerous other effects of the cannabinoids—bronchodilation [26], lowering of intraocular pressure [26], and actions on the gastrointestinal system [32], for example—could also be peripheral. There is also evidence that a cannabinoid receptor is expressed in testes [5].

The presence of RNA transcripts for cannabinoid receptors has been reported for a variety of immunologic cell types derived from both human [4] and murine [3] lymphoid organs. In a detailed study of localization of cannabinoid receptors in peripheral tissues in the rat, it was reported that specific cannabinoid receptor binding is restricted to components of the immune system [33]. The expression and functionality of cannabinoid receptors have been confirmed in mouse spleen cells by the demonstration of stereoselective immunomodulatory effects by cannabimimetic agents, by the high degree

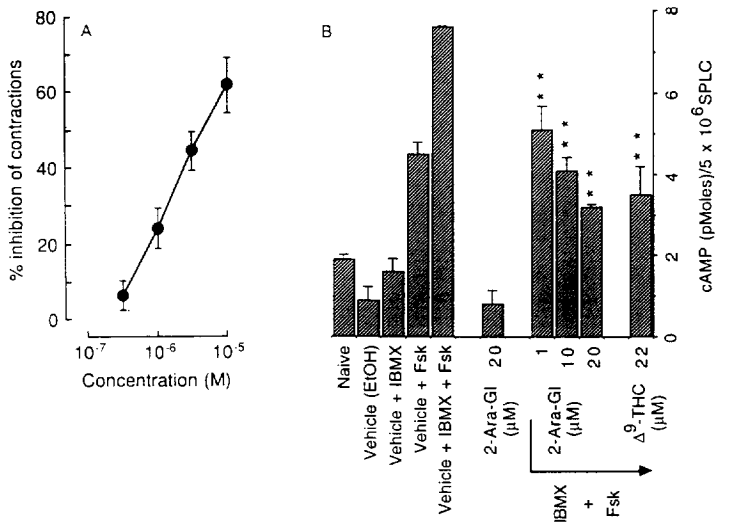


Fig. 4. Mean concentration–response curve for 2-Ara-Gl in mouse isolated vasa deferentia (A), and effect of 2-Ara-Gl on intracellular cAMP accumulation in mouse spleen cells (B). Panel A: Each symbol represents the mean value ± SEM of inhibition amplitude of electrically evoked concentrations expressed as a percentage of the amplitude of the twitch response measured immediately before the first addition of drug to the organ bath (N = 7 different vasa deferentia). Panel B: Intracellular cAMP concentrations (pmol) are expressed as the mean ± SEM for quadruplicate samples as determined for each group. Key: ** $P < 0.01$ (determined by Dunnett's *t*-test) as compared with the vehicle + forskolin (Fsk) + IBMX control group. SPLC = spleen cells.

Table 1. Comparison of the potencies and efficacies of Δ^9-THC, anandamide and 2-Ara-Gl on i.v. administration to mice*

	ED_{50} (mg/kg)			
Compound	SA†	RT‡	TF§	IMM‖
Δ^9-THC¶	0.9 (76%)**	2.3 (4.6°)	0.8 (100%)	0.9 (49%)
Anandamide††	17.9 (87%)	26.5 (3.1°)	6.2 (85%)	19.1 (88%)
2-Ara-Gl	13.2 (75%)	23.2 (2.1°)	12.3 (80%)	19.4 (26%)

* Dose–response curves were generated from at least four doses of drug. Six to twelve animals were used for each dose.
† Reduction of spontaneous activity (SA).
‡ Rectal temperature (RT).
§ Antinociception measured in the tail-flick test (TF).
‖ Immobility (IMM).
¶ Reported previously [18].
** Maximal effects in parentheses.
†† Reported previously [15].

of specific binding of [^{3}H]CP-55940 (a high affinity ligand for both CB_1 and CB_2), and most critical to the present studies, by inhibition of forskolin-stimulated cAMP accumulation with Δ^9-THC [25, 34]. It has also been shown that immune inhibition produced by cannabinoids is reversed by exogenous membrane permeable cAMP analogs (i.e. dibutyryl-cAMP and 8-bromo-cAMP) or by pretreatment of cells with pertussis toxin [34]. On the basis of the above data, we assumed that endogenous cannabinoid ligands should be present not only in the brain but also in peripheral organs. In Materials and Methods, we describe the isolation of an active fraction from a methanol extract of canine small intestines which on GC, monitored by MS, showed the presence of three compounds. Activity was qualitatively measured by inhibition of the specific binding of [^{3}H]HU-243 on synaptosomal membranes from rat brains in a centrifugal assay as previously described [7, 8, 17]. The constituents of

the active fraction could not be separated by further chromatography; however, by GC-MS analyses we identified the esters 2-palmityl glycerol, 2-linoleyl glycerol and 2-arachidonyl glycerol (2-Ara-Gl). Anandamide was not present in this active fraction, nor in any other fraction from the gut extract. It was also absent in similarly prepared extracts of canine spleen or rat testes. In preliminary tests, synthetic 2-Ara-Gl showed activity, while palmityl and linoleyl esters were inactive. As mentioned, a centrifugal rat brain membrane assay [7, 8] was employed for monitoring the extraction procedure leading to an active fraction; however, when pure 2-Ara-Gl became available, a rather high K_i value was recorded (K_i 5.85 ± 0.12 μM), which is *ca.* 100 times higher than that of anandamide [7]. In the vas deferens assay, which presumably involves CB_1 [35], 2-Ara-Gl was also *ca.* 100 times less potent than anandamide. We assumed that this could be due to esterase activity of the membrane and tissues used in the assays. Hence, we tried to find a more suitable test. Anandamide has been found to bind to membranes from cells transiently (COS) transfected with expression plasmids carrying either CB_1 or CB_2 DNA, but not to those of untransfected control cells [6, 11, 12]. We now used this binding assay for the quantitative determination of the binding characteristics of 2-Ara-Gl and for comparison with anandamide. Indeed we found that 2-Ara-Gl in this assay was only about two times less active than anandamide on binding to either CB_1 or CB_2 (Fig. 3) rather than *ca.* 100 times as found in the rat brain membrane or the vas deferens assays.

The K_i for receptor binding of anandamide varies considerably depending on the presence or absence of an amidase inhibitor in the binding assay [15, 36, 37]. Hence, if this is also the case with 2-Ara-Gl, until K_i values for this ester can be established in the presence of suitable esterase inhibitors the above-recorded constants should be considered tentative, representing an upper limit. The same caveat should be assumed for the values in the vas deferens inhibition assay and other tests reported here.

It is worth noting that the presence of an efficient mechanism for removing 2-Ara-Gl from its receptors is to be expected if this compound does indeed serve as a physiological mediator.

2-Ara-Gl is active upon i.v. administration in the now well established tetrad of pharmacological effects (Table 1) which together are typical for the cannabimimetic drugs. These observations indicate that 2-Ara-Gl crosses the blood–brain barrier under the experimental conditions. Whether these actions are of physiological relevance remains to be established.

The data reported above demonstrate that 2-Ara-Gl is an endogenous, peripheral ligand of the cannabinoid receptors. Its physiological roles remain to be established. We believe that one of them may be associated with the immune system.

Acknowledgements—We thank S. Munro for providing the plasmid containing CB_2 gene and T. Sheskin for the NMR spectra. The following agencies provided support: U.S. National Institute on Drug Abuse to R.M. (DA 8169), to B.R.M. (DA 3672, 5488), and to N.E.K. (DA 7908, 8351); Welcome Trust to R.G.P. (34925); and the Levine Fund to Z.V.

REFERENCES

1. Devane WA, Dysarz FA III, Johnson MR, Melvin LS and Howlett AC, Determination and characterization of a cannabinoid receptor in rat brain. *Mol Pharmacol* **34**: 605–613, 1988.
2. Matsuda LA, Loliat SJ, Brownstein MJ, Young AC and Bonner TI, Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* **346**: 561–564, 1990.
3. Kaminski NE, Abood ME, Kessler EK, Martin BR and Schatz AR, Identification of a functionally relevant cannabinoid receptor on mouse spleen cells that is involved in cannabinoid-mediated immune modulation. *Mol Pharmacol* **42**: 736–742, 1992.
4. Bouaboula M, Rinaldi M, Carayon P, Carillon C, Shire D, Le Fur G and Cassellas P, Cannabinoid-receptor expression in human leukocytes. *Eur J Biochem* **214**: 173–180, 1993.
5. Gerárd CM, Mollereau C, Vassart G and Parmentier M, Molecular cloning of a human cannabinoid receptor which is also expressed in testis. *Biochem J* **279**: 129–134, 1991.
6. Munro S, Thomas KL and Abu-Shaar M, Molecular characterization of a peripheral receptor for cannabinoids. *Nature* **365**: 61–64, 1993.
7. Devane WA, Hanuš L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A and Mechoulam R, Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**: 1946–1949, 1992.
8. Hanuš L, Gopher A, Almog S and Mechoulam R, Two new unsaturated fatty acid ethanolamides in brain that bind to the cannabinoid receptor. *J Med Chem* **36**: 3032–3036, 1993.
9. Mechoulam R, Hanuš L and Martin BR, The search for endogenous ligands of the cannabinoid receptor. *Biochem Pharmacol* **48**: 1537–1544, 1994.
10. Pertwee RG, Griffin G, Hanuš L and Mechoulam R, Effects of two endogenous fatty acid ethanolamides on mouse vasa deferentia. *Eur J Pharmacol* **259**: 115–120, 1994.
11. Vogel Z, Barg J, Levy R, Saya D, Heldman E and Mechoulam R, Anandamide, a brain endogenous compound, interacts specifically with cannabinoid receptors and inhibits adenylate cyclase. *J Neurochem* **61**: 352–355, 1993.
12. Felder CC, Briley EM, Axelrod J, Simpson JT, Mackie K and Devane WA, Anandamide, an endogenous cannabimimetic eicosanoid, binds to the cloned human cannabinoid receptor and stimulates receptor-mediated signal transduction. *Proc Natl Acad Sci USA* **90**: 7656–7660, 1993.
13. Fride E and Mechoulam R, Pharmacological activity of the cannabinoid receptor agonist, anandamide, a brain constituent. *Eur J Pharmacol* **231**: 313–314, 1993.
14. Weidenfeld J, Feldman S and Mechoulam R, Effect of the brain constituent anandamide, a cannabinoid receptor agonist, on the hypothalamo-pituitary-adrenal axis in the rat. *Neuroendocrinology* **59**: 110–112, 1994.
15. Smith PB, Compton DR, Welch SP, Razdan RK, Mechoulam R and Martin BR, The pharmacological activity of anandamide, a putative endogenous cannabinoid, in mice. *J Pharmacol Exp Ther* **270**: 219–227, 1994.
16. Gaoni Y and Mechoulam R, The isolation and structure of Δ^1-tetrahydrocannabinol and other neutral cannabinoids from hashish. *J Am Chem Soc* **93**: 217–224, 1991.

17. Järbe TUC, Hiltunen AJ, Mathis DA, Hanuš L, Breuer A and Mechoulam R, Discriminative stimulus effects and receptor binding of enantiomeric pairs of cannabinoids in rats and pigeons: A comparison. *J Pharmacol Exp Ther* **264**: 561–569, 1993.
18. Compton DR, Rice KC, De Costa BR, Razdan RK, Melvin LS, Johnson MR and Martin BR, Cannabinoid structure–activity relationships: Correlation of receptor binding and *in vivo* activities. *J Pharmacol Exp Ther* **265**: 218–226, 1993.
19. Dewey WL, Harris LS, Howes JF and Nuite JA, The effect of various neurohumoral modulators on the activity of morphine and the narcotic antagonists in the tail-flick and phenylquinone tests. *J Pharmacol Exp Ther* **175**: 435–442, 1970.
20. Pertwee RG, The ring test: A quantitative method for assessing the "cataleptic" effect of cannabis in mice. *Br J Pharmacol* **46**: 753–763, 1972.
21. Tallarida RJ and Murray RB, Graded dose–response. *Manual of Pharmacologic Calculations with Computer Programs*, pp. 26–31. Springer, New York, 1987.
22. Compton DR, Johnson MR, Melvin LS and Martin BR, Pharmacological profile of a series of bicyclic cannabinoid analogs: Classification as cannabimimetic agents. *J Pharmacol Exp Ther* **260**: 201–209, 1992.
23. Pertwee RG, Stevenson LA and Griffin G, Cross-tolerance between delta-9-tetrahydrocannabinol and the cannabimimetic agents, CP 55,940, WIN 55,212-2 and anandamide. *Br J Pharmacol* **110**: 1483–1490, 1993.
24. Gunston FD, Lipids. In: *Comprehensive Organic Chemistry* (Eds. Barton DHR and Ollis WD), Vol. 5, pp. 633–664. Pergamon, Oxford, 1979.
25. Schatz AR, Kessler FK and Kaminski NE, Inhibition of adenylate cyclase by Δ9-tetrahydrocannabinol in mouse spleen cells: A potential mechanism for cannabinoid-mediated immunosuppression. *Life Sci* **51**: PL25–PL30, 1992.
26. Martin BR, Cellular effects of cannabinoids. *Pharmacol Rev* **38**: 45–74, 1986.
27. Pertwee RG, The central neuropharmacology of psychotropic cannabinoids. In: *International Encyclopedia of Pharmacology and Therapeutics* (Ed. Balfour EJK), pp. 355–429. Pergamon Press, New York, 1990.
28. Zimmerman AM, Titishov N, Mechoulam R and Zimmerman S, Effect of stereospecific cannabinoids on the immune system. In: *Drugs of Abuse, Immunity and Immunodeficiency* (Eds. Friedman H, Spector S and Klein TW), pp. 71–80. Plenum Press, New York, 1991.
29. Nahas GG, Suciu-Foca N, Armand J-P and Morishima A, Inhibition of cellular immunity in marijuana smokers. *Science* **183**: 419–420, 1974.
30. Specter S, Lancz G and Friedman H, Marijuana and immunosuppression in man. In: *Drugs of Abuse and Immune Function* (Ed. Watson RR), pp. 73–85. CRC Press, Boca Raton, FL, 1990.
31. Cabral GA and Vasquez R, Effects of marijuana on macrophage function. *Adv Exp Med Biol* **288**: 93–105, 1991.
32. Loev B, Bender PE, Dowalo F, Macko E and Fowler PJ, Cannabinoids. Structure–activity studies related to 1,2-dimethylheptyl derivatives. *J Med Chem* **16**: 1200–1206, 1973.
33. Lynn AB and Herkenham M, Localization of cannabinoid receptors and nonsaturable high-density cannabinoid binding sites in peripheral tissues of the rat: Implications for receptor-mediated immune modulation by cannabinoids. *J Pharmacol Exp Ther* **268**: 1612–1623, 1994.
34. Kaminski NE, Koh WS, Yang KH, Lee M and Kessler FK, Suppression of humoral immune response by cannabinoids is partially mediated through inhibition of adenylate cyclase by a pertussis toxin-sensitive G-protein coupled mechanism. *Biochem Pharmacol* **48**: 1899–1908, 1994.
35. Pertwee RG, The evidence for the existence of cannabinoid receptors. *Gen Pharmacol* **24**: 811–824, 1993.
36. Childers SR, Sexton T and Roy MB, Effects of anandamide on cannabinoid receptors in rat brain membranes. *Biochem Pharmacol* **47**: 711–715, 1994.
37. Deutsch DG and Chin SA, Enzymatic synthesis and degradation of anandamide, a cannabinoid receptor agonist. *Biochem Pharmacol* **46**: 791–796, 1993.

Health and Quality of Life Outcomes

BioMed Central

Research

Open Access

Impairment of quality of life in parents of children and adolescents with pervasive developmental disorder

Diego Mugno*, Liliana Ruta, Valentina Genitori D'Arrigo and Luigi Mazzone

Address: Division of Child Neurology and Psychiatry, Department of Pediatrics, University of Catania, Via S. Sofia 78, 95100 Catania, Italy

Email: Diego Mugno* - progettoautismo@policlinico.unict.it; Liliana Ruta - liliana.ruta@gmail.com; Valentina Genitori D'Arrigo - valentina.genitori@libero.it; Luigi Mazzone - gigimazzone@yahoo.it

* Corresponding author

Published: 27 April 2007

Health and Quality of Life Outcomes 2007, **5**:22 doi:10.1186/1477-7525-5-22

Received: 17 October 2006
Accepted: 27 April 2007

This article is available from: http://www.hqlo.com/content/5/1/22

Abstract

Background: Little is known about the Quality of Life (QOL) in parents of children with developmental diseases as compared to other severe neurological or psychiatric disorders. Aims of the present study were: to evaluate QOL in parents of children affected by Pervasive Development Disorder (PDDs), Cerebral Palsy (CP) or Mental Retardation (MR) as compared to a control group (CG); to evaluate QOL of parents of patients with different types of PDDs, namely Autistic Disorder (AD), High Function Autism/Asperger Syndromes (HFA/AS) and Pervasive Developmental Disorder Not Otherwise Specified (PPD-NOS); and to compare the level of impairment in QOL of mothers and fathers within PDDs, CP, MR groups and between AD, HFA/AS, PDD-NOS sub-groups.

Methods: The sample consisted of 212 parents (115 mothers and 97 fathers) of 135 children or adolescents affected by PDDs, MR or CP. An additional sample of 77 parents (42 mothers and 35 fathers) of 48 healthy children was also included and used as a control group. QOL was assessed by the WHOQOL-BREF questionnaire.

Results: Compared with parents of healthy children, parents in the PDDs group reported impairment in physical activity ($p = 0.0001$) and social relationships ($p = 0.0001$) and worse overall perception of their QOL ($p = 0.0001$) and health ($p = 0.005$). Scores in the physical ($p = 0.0001$), psychological ($p = 0.0001$) and social relationships domains ($p = 0.0001$) and in the physical ($p = 0.0001$) and social relationships ($p = 0.0001$) domains were lower compared to the MR group CP group respectively. Little differences were observed between MR, CP and control groups. The level of impairment of physical ($p = 0.001$) and psychological ($p = 0.03$) well-being were higher in mothers than in fathers in the PDDs and CP groups respectively; in the other groups, and across all the other domains of QQL impairment was similar. There were no statistically significant differences in the scores between the AD, HFA/AS and PDD-NOS sub-groups, but parents in the HFA/AS sub-group seemed to display a lower QOL compared to the AD sub-group.

Conclusion: Parents of children with PDDs seem to display a higher burden, probably for a combination of environmental and genetic factors. Within this group of parents also those of HFA or AS people have higher stress. These finding must be taken into account in policy making to provide better and more specific supports and interventions for this group of diseases.

Background

Quality of Life (QOL) has been defined by the World Health Organization as individuals' perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns. It is a broad concept incorporating the person's physical health, psychological state, level of independence, social relationships, personal beliefs and their relationship to salient features of the environment [1]. As such, QOL cannot be simply equated with the terms health status, life style, life satisfaction, mental state, or well-being; rather, it is a multidimensional concept incorporating the individual's perception of these and other aspects of life [2].

Assessment of QOL is important in medical practice, to improve the doctor-patient relationship, in assessing the effectiveness and relative merits of different treatments, in health services evaluation, in research and in policy making [3].

QOL is especially relevant to conditions that are chronic and impairing, such as pervasive developmental disorder (PDD), Cerebral palsy (CP), mental retardation (MR).

PDDs, CP and MR are not rare conditions in the population. Prevalence of PPD has been estimated to be around 1–5 per 1,000 [4,5] and an increase in incidence of this disease has been reported by clinicians, schools, and service agencies worldwide [6,7] Prevalence of MR is between 3–7 subjects per 1,000 [8,9] and for CP have been reported to be between 1.5–3 per 1000 children [10].

Parents of children with developmental disabilities experience heightened stress, [11,17], impaired mental health [18], sense of devaluation and self-blame [19], impaired physical functioning, tiredness or exhaustion [20,21]. The level of Impairment in quality of life within families of children with these severe chronic conditions is likely to be moderated by a complex matrix of environmental as well as genetically-based variables such as socio-economic status, social support, parental and child characteristics and coping strategies [22,23]. The level of parental stress has been found to be related to the level of severity and disability of the children's diagnoses and to coexisting behavioral problems [24,25]. Mothers of children with PDDs reported higher levels of stress and demoralization than fathers [26,27]. Research has indicated that maternal stress in families with children with autism [28,29] is predicted by their children's co-existent behaviour problems and also by their partner's depression. It has been suggested that co-existent behaviour problems in the child predict parental stress to a higher extent than the severity of the autistic symptoms [27-29]. Furthermore mothers of children with severe mental disorder and with intellectual disability showed increased rates of physical health problems with poor perception of their health [24,30,31].

Little data are available on the QOL in parents of children with developmental diseases as compared to other severe neurological or psychiatric disorders. We assessed QOL of parents of children and adolescent affected by pervasive developmental disorders (PDDs), cerebral palsy (CP), mental retardation (MR) and normally developing (control group, CG).

Aims of this study were: 1) to evaluate QOL in parents of children suffering from PDDs, CP or MR as compared to a control group; 2) to evaluate QOL of parents of patients with different types of PDDs, namely Autistic Disorder (AD), High Function Autism/Asperger Syndromes (HFA/AS) and Pervasive Developmental Disorder not otherwise specified (PPD-NOS); 3) to compare the level of impairment in QOL of mothers and fathers within PDDs, CP, MR groups and between AD, HFA/AS, PDD-NOS subgroups. We hypothesized that parents of children of PPD might display an higher impairment of quality of life as compared to the other groups because patients with PPD usually display more and/or more severe maladaptive behaviors and have lower chance of achieving a better psychological adjustment compared to people with CP and MR. The presence of genetic liability for autism (broad autism phenotype) in family of autistic probands as well as the higher aggregation of psychiatric disorders in these families will be take into account.

Methods

Sample

The recruited sample comprised parents of 160 children or adolescent affected by PDDs, MR or CP (60 children or adolescent affected by PDDs; 60 affected by MR; 40 affected by CP). For each subject 2 questionnaires were provided to parents independently of the family status, for a total number of 320 questionnaires (120 questionnaires for the PDDs group, 120 questionnaires for the MR group, 80 questionnaires for the CP group).

An additional sample of parents of 65 healthy children was also included and used as a control group (CG), for a total number of 130 questionnaires.

Assessment of QOL

The World Health Organization (WHO), with the aid of 40 collaborating centers around the world, has developed two self-administered instruments for measuring quality of life (the WHOQOL-100 and the WHOQOL-BREF), that can be used in a variety of cultural settings whilst allowing the results from different populations and countries to be compared.

The WHOQOL-BREF contains two items from the Overall Quality of Life and General Health, and one item from each of the 24 facets included in The WHOQOL-100. Analysis of The WHOQOL-100 structure has suggested the possibility of merging two of six domains of the WHOQOL-100, thereby creating four domains of quality of life: physical, psychological, social relationships and environment [32].

The WHOQOL-BREF produces a profile with four domain scores and two individually scored items about an individual's overall perception of QOL and health (Q1 and Q2). The four domain scores are scaled in a positive direction, with a score range of 0–100, and with higher scores denoting higher QOL. The two individual items assessing overall QOL are scaled in a positive direction, with a score range of 1–5 (converted in this study into a 0–100 score), with higher scores denoting higher QOL.

Psychometric properties of the Italian version of the WHOQOL-Bref have been tested by the "*Centro Italiano Collaborativo Progetto WHOQOL*" [33].

Procedure

This study was conducted at the Department of Child Neurology and Psychiatry of the University of Catania, Italy, Participants were parents of people aged 2–18 years referred to the center during a period of 1 year (June 2005–June 2006).

The participating children underwent a comprehensive clinical diagnostic assessment based on the DSM-IV-TR criteria [34], and all diagnoses were made according to DSM-IV-TR criteria except when specified.

Potential participants could enter the study if their sons received a diagnosis of PPDs, MR or CP.

The CG consisted of mothers and fathers of typically developing people aged <18, recruited via school nurses, attending regular classes in mainstream schools, with no mental, developmental, or physical disabilities according to school medical records and not receiving ongoing prescription medication.

Exclusion criteria were inability to undergo the written parts of the questionnaire or refusal to participate. Parents of more than 1 son affected by PDDs, MR or CP were not included in the study. Questionnaires missing more than 20% of data, missing items Q1 or Q2 or missing more than two items from the domain (more than 1 for domain 3, social relationships), were discarded. Where an item was missing, the mean of other items in the domain was substituted [3].

The instrument used to assess parental QOL was distributed by the authors to the families of the study group during inpatient or outpatient visits, during home visit or by mail and email and were returned to the authors via a parental visit to the clinic, a second home visit or by mail and email. The questionnaire was distributed to the families of the CG by mail. Each parent separately filled in the QOL instrument. The questionnaires were anonymous. The cover page, gave information about the study, brief instructions and an example of how to respond to the questions. Demographic and health questions are not included in the WHOQOL-BREF and were included as a separate partially structured demographic questionnaire. The requested data were gender, age, family status, school education and health status of parents; age and gender of children. Data from the demographic questionnaire were only used to assess statistical differences between the groups on the requested information.

Data analysis

Data were assessed using Student's *t*-tests and one-way ANOVAs with post hoc Scheffé multiple comparison tests. The Statistical Package for Social Sciences (SPSS) [35] was used. Significance level $p < .05$ was regarded as statistically significant.

Results

Among the PDDs group, 51 questionnaires were not included in the study, constituting a drop-out rate of 42.5%. 39 of the 51 excluded questionnaires (76%) were not returned to the authors; 12 of the 51 excluded questionnaires (24%) were considered ineligible for the analysis because of missing data.

Among the MR group, 31 questionnaires were excluded from the study, constituting a drop-out rate of 25.8%. 19 of the 31 excluded questionnaires (62%) were not returned to the authors; 12 of the 31 questionnaires (38%) were excluded for missing data.

In the CP group, 26 questionnaires were not included in the study, constituting a drop-out rate of 32.5%. 19 questionnaires (73%) were not returned to the authors; 7 questionnaires (27%) were considered ineligible for the analysis because of missing data.

A total number of 289 questionnaires were included in the study.

The study group consisted of 212 parents (97 fathers, 115 mothers) of 135 children or adolescents affected by PDDs, MR or CP. The control group (CG) consisted of 77 parents (35 fathers and 42 mothers) of 89 healthy children.

The PDDs group consisted of 69 parents (30 fathers and 39 mothers) of 53 children and adolescent (males n. 42; females n. 11; sex ratio M/F = 3.8/1) affected by PDDs. 16 fathers and 21 mothers of 26 children and adolescents (males n. 20; females n. 6) affected by autistic disorder (AD) and IQ level<70 (n. 16, IQ between 55 and 70; n. 10, IQ between 40 and 54), 10 fathers and 12 mothers of 20 children and adolescents (males n. 17; females n. 3) affected by Asperger's disorder or high-functioning autism (HFA/AS), the latter defined as AD with IQ level>70, 4 fathers and 6 mothers of 7 children and adolescents (males n. 5; females n. 2) affected by Pervasive Developmental Disorder-Not otherwise specified (PDD-NOS) (n. 3, IQ between 55 and 69; n. 4, IQ between 40 and 54).

The MR group consisted of 89 parents (40 fathers and 49 mothers) of 55 children and adolescents (males n. 33; females n. 22; sex ratio M/F = 1.5/1) affected by MR without autism spectrum conditions. 23 fathers and 28 mothers of 32 children and adolescents (males n. 18; females n. 14) affected by mild to moderate MR, 17 fathers and 21 mothers of 23 children and adolescents (males n. 15; females n. 8) affected by severe to profound MR.

The CP group consisted of 54 parents (27 fathers and 27 mothers) of 30 children and adolescents (males n. 15; females n. 15; sex ratio M/F = 1/1) affected by CP. For this group we have adopted the classification of subtypes of cerebral palsy agreed by the Surveillance of Cerebral Palsy in Europe collaboration of cerebral palsy registers [36]. 12 fathers and 13 mothers of 12 children and adolescent (males n. 7; females n. 5) affected by 2 limbs spastic bilateral type CP (2SBCP), 9 fathers and 7 mothers of 10 children and adolescents (males n. 4; females n. 6) affected by hemiplegia type CP (HCP), 6 father and 7 mother of 8 children and adolescents (males n. 4; females n. 4) affected by 3–4 limbs spastic bilateral type CP (SBCP).

The CG consisted of 42 mothers and 35 fathers of 48 typically developing children (males n. 17; females n. 31; sex ratio M/F = 1/1.8) recruited via school nurses, attending regular classes in mainstream schools, with no mental, developmental, or physical disabilities according to school medical records and not receiving ongoing prescription medication.

Average age of parents was 40 ± 13.5 years (20–58 years). Family status was: 76% married/cohabitating, 21% separated/divorced, 3% widowed. Level of education was: 39% primary school, 44% secondary school, 17% university. None of the parents was currently ill.

Average age of children within PDDs group was 7.5 ± 5 years (range 3–17). Average age of children within the MR group was 6.3 ± 7 years (range 4–16). Average age of children within the CP group was 9 ± 5 years (range 2–16). Average age of children within the CG was 8 ± 4 years (range 4–15). There were no statistically significant differences regarding socio-demographic factors between the groups (Table 1).

Comparison between the PDDs, the MR, the CP and the control groups

Fathers in the PDDs group showed statistically significant lower scores in the social relationship domain and in the Q1 item compared to CG, and in the psychological domain compared to CP group (Table 2). Mothers in the PDDs group showed statistically significant lower scores in the Q1 and Q2 items and in the physical and social relationship domains compared to the CG, and in the psychological domain compared to the MR group. Mothers in the MR group showed statistically significant lower scores Q1 item and in the physical domain compared to the CG. Mothers in CP group showed statistically significant lower scores in the Q1 item compared to the CG (Table 2).

Table 1: Demographics characteristics of parents of children and adolescents with Pervasive development disorder (PDDs), Mental retardation (MR), Cerebral Palsy (CP) and controls CG).

	PDDs (N = 69)	MR (N = 89)	CP (N = 54)	CG (N = 77)	TOT (N = 289)
Fathers/Mothers	30/39	40/49	27/27	35/42	132/157
Age (Mean ± SD)	37 ± 12.7	43 ± 14.5	39 ± 12.5	41 ± 14.3	40 ± 13.5
Family status					
Married/Cohabitating	53 (77%)	67 (75%)	40 (74%)	60 (68%)	220 (76%)
Separated	15 (22%)	18 (20%)	12 (22%)	16 (21%)	61 (21%)
Widowed	3 (4%)	2 (2%)	2 (4%)	2 (3%)	9 (3%)
Level of Education					
Primary	26 (38%)	35 (39%)	22 (41%)	30 (39%)	113 (39%)
Secondary	30 (43%)	39 (44%)	24 (44%)	34 (44%)	127 (44%)
University	12 (17%)	14 (16%)	10 (19%)	13 (17%)	49 (17%)
Sons/Daughters	42/11	33/22	15/15	17/31	107/79
Age (Mean ± SD)	7.5 ± 5	6.3 ± 7	9 ± 5	8 ± 4	7.7 ± 5.2

Table 2: Comparison of Quality of Life (WHOQOL-BREF) between fathers and mothers of children and adolescents with Pervasive development disorder (PDDs), Mental retardation (MR), Cerebral Palsy (CP) and controls (CG).

Fathers	PDDs (N = 30)	MR (N = 40)	CP (N = 17)	CG (N = 35)	ANOVA F	p	Post-hoc contrasts
Q1 (mean ± SD)					7.28	0.000	CG>PDDs
Q2 (mean ± SD)	65.83 ± 19.12	72.16 ± 18.06	63.24 ± 20.00	74.29 ± 22.27	1.8	0.151	
Physical (mean ± SD)	65.48 ± 11.11	67.37 ± 12.83	64.92 ± 17.05	72.24 ± 14.80	1.77	0.156	
Psychological (mean ± SD)	64.58 ± 16.00	71.69 ± 12.41	76.47 ± 9.65	68.93 ± 14.93	3.05	0.031	CP>PDDs
Relationships (mean ± SD)	60.00 ± 18.36	68.37 ± 16.13	72.06 ± 9.29	75.24 ± 15.59	5.27	0.002	CG>PDDs
Environment (mean ± SD)	53.75 ± 12.70	55.61 ± 12.46	59.01 ± 12.59	56.96 ± 17.78	0.56	0.640	
Mothers	PDDs (N = 39)	MR (N = 49)	CP (N = 27)	CG (N = 42)	F	p	
Q1 (mean ± SD)	58.33 ± 21.71	63.27 ± 20.48	60.35 ± 16.53	77.98 ± 13.75	9.16	0.000	CG>PDDs, MR, CP
Q2 (mean ± SD)	55.77 ± 26.57	67.35 ± 21.77	65.29 ± 19.98	71.43 ± 21.08	3.47	0.018	CG>PDDs
Physical (mean ± SD)	53.94 ± 16.34	64.65 ± 15.91	60.87 ± 12.99	68.45 ± 15.68	6.44	0.000	CG>PDDs, MR
Psychological (mean ± SD)	57.59 ± 17.41	68.79 ± 13.40	67.20 ± 15.68	64.38 ± 15.40	4.17	0.007	MR>PDDs
Relationships (mean ± SD)	58.97 ± 23.68	69.73 ± 16.47	66.97 ± 15.99	72.22 ± 18.56	3.72	0.013	CG>PDDs
Environment (mean ± SD)	48.96 ± 14.84	57.14 ± 11.71	54.64 ± 12.60	54.24 ± 19.00	2.24	0.086	

Q1 = Overall perception of Quality of Life; Q2 = Overall perception of Health.

Comparison between fathers and mothers within the PDDs, the MR, the CP and control groups

Within the PDDs group mothers showed lower scores in the physical domain (p = 0.001) and within the CP group in the psychological domain (p = 0.03). No statistically significant differences were observed within the MR group and the CG (Table 3).

Comparison within the PDDs group: the AD, HFA/AS and PDD-NOS sub-groups

Fathers in the AD sub-group showed statistically significant lower scores in the Q1 item and in the social relationship domain compared to CG. Fathers in the HFA/AS sub-group showed statistically significant lower scores in the Q1 and Q2 items and in the social relationship domain compared to CG, and in the Q2 item compared to the PDD-NOS sub-group (Table 4). Mothers in the AD sub-group showed statistically significant lower scores in the Q1 item and in the physical domain compared to CG. Mothers in the HFA/AS sub-group showed statistically significant lower scores in the Q1 item and in the physical and social relationship domains compared to CG. Mothers in the PDD-NOS sub-group showed statistically significant lower scores Q1 item compared to CG (Table 4).

Comparison between fathers and mothers within the AD, HFA/AS, PDD-NOS

The only statistically significant differences was the lower scores of mothers in the physical domain within the AD sub-group (p = 0.02).

Discussion

Parents of children with PDDs showed a significant impairment of QOL as compared to the other groups, while little differences were observed between MR, CP and control groups. In particular, significant differences in the MR and CP groups compared to controls where present only in mothers, with impairment in the physical domain and overall perception of QOL in mothers of children with MR and in overall perception of QOL for mothers of children affected by CP. No statistically significant differences were observed between MR and CP groups.

Within the PDDs group mothers tended to have a lower QOL compared to fathers, despite the only statistically sig-

Table 3: Comparison of Quality of Life (WHOQOL-BREF) between fathers and mothers of participants with Pervasive Development Disorder (PDDs). Mental Retardation (MR). Cerebral Palsy (CP) and control group (CG).

	PDDs fathers n.30	**PDDs mothers n.39**		**MR fathers n.40**	**MR mothers n.49**		**CP fathers n.27**	**CP mothers n.27**		**Control fathers n.35**	**Control Mothers n.42**	
	Mean (SD)	Mean (SD)	***p***	Mean (SD)	Mean (SD)	***p***	Mean (SD)	Mean (SD)	***p***	Mean (SD)	Mean (SD)	***p***
Q1	59.17 (16.72)	58.33 (21.71)	N.S.	67.05 (17.7)	63.27 (20.48)	N.S.	66.18 (17.55)	60.35 (16.53)	N.S.	78.57 (16.21)	77.98 (13.75)	N.S.
Q2	65.83 (19.12)	55.77 (26.57)	N.S.	72.16 (18.06)	67.35 (21.77)	N.S.	63.24 (20)	65.29 (19.98)	N.S.	74.29 (22.27)	71.43 (21.08)	N.S.
Physical	65.48 (11.11)	53.94 (16.34)	0.001	67.37 (12.83)	64.65 (15.91)	N.S.	64.92 (17.05)	60.87 (12.99)	N.S.	72.24 (14.8)	68.45 (15.68)	N.S.
Psychological	64.58 (16)	57.59 (17.41)	N.S.	71.69 (12.41)	68.79 (13.4)	N.S.	76.47 (9.65)	67.2 (15.68)	0.03	68.93 (14.93)	64.38 (15.4)	N.S.
Relationships	60 (18.36)	58.97 (23.68)	N.S.	68.37 (16.13)	69.73 (16.47)	N.S.	72.06 (9.26)	66.97 (15.99)	N.S.	75.24 (15.59)	72.22 (18.56)	N.S.
Environment	53.75 (12.7)	48.96 (14.84)	N.S.	55.61 (12.46)	57.14 (11.71)	N.S.	59.01 (12.59)	54.64 (12.6)	N.S.	56.96 (17.78)	54.24 (19)	N.S.

Table 4: Comparison of Quality of Life (WHOQOL-BREF) between fathers and mothers of children and adolescents with Autistic Disorder (AD), Asperger's disorder or high-functioning autism (HFA/AS) and Pervasive Developmental Disorder-Not otherwise specified (PDD-NOS).

Fathers	AD (N = 16)	HFA/AS (N = 10)	PDD-NOS (N = 4)	CG (N = 35)	ANOVA F	ANOVA p	Post-hoc contrasts
Q1 (mean ± SD)	56.25 ± 19.3	60.00 ± 12.9	68.75 ± 12.5	78.57 ± 16.21	8.11	0.000	CG>AD, HFA/AS
Q2 (mean ± SD)	68.75 ± 14.43	52.50 ± 18.45	87.50 ± 14.43	74.29 ± 22.27	4.25	0.009	CG>HFA/AS; PDD-NOS>HFA/AS
Physical (mean ± SD)	65.40 ± 11.04	64.64 ± 12.65	67.86 ± 9.67	72.24 ± 14.80	1.42	0.245	
Psychological (mean ± SD)	62.24 ± 16.14	63.33 ± 16.64	77.08 ± 9.92	68.93 ± 14.93	1.47	0.231	
Relationships (mean ± SD)	59.90 ± 14.97	56.67 ± 23.17	68.75 ± 19.69	75.24 ± 15.59	4.81	0.005	CG>AD, HFA/AS
Environment (mean ± SD)	53.91 ± 14.05	50.94 ± 11.70	60.16 ± 8.98	56.96 ± 17.78	0.55	0.651	
Mothers	AD (N = 21)	HFA/AS (N = 12)	PDD-NOS (N = 6)	CG (N = 42)	F	p	
Q1 (mean ± SD)	58.33 ± 19.90	60.42 ± 22.51	54.17 ± 29.23	77.98 ± 13.75	8.01	0.000	CG>AD, HFA/AS, PDD-NOS
Q2 (mean ± SD)	54.76 ± 26.95	56.25 ± 28.45	58.33 ± 25.82	71.43 ± 21.08	2.87	0.042	
Physical (mean ± SD)	53.23 ± 17.42	53.57 ± 16.19	57.14 ± 14.98	68.45 ± 15.68	5.52	0.002	CG>AD, HFA/AS
Psychological (mean ± SD)	58.73 ± 16.66	51.74 ± 19.82	65.28 ± 13.09	64.38 ± 15.40	2.17	0.099	
Relationships (mean ± SD)	60.71 ± 22.38	52.08 ± 21.94	66.67 ± 31.62	72.22 ± 18.56	3.38	0.022	CG>HFA/AS
Environment (mean ± SD)	48.96 ± 15.76	44.79 ± 11.02	57.29 ± 16.96	54.24 ± 19.00	1.35	0.263	

Q1 = Overall perception of Quality of Life; Q2 = Overall perception of Health.

nificant difference found between fathers and mothers was in the physical domain (mothers of children with PDDs showing lower scores). More specifically, mothers of children with PDDs displayed lower physical health, impairment in social relationship and in the psychological state, and a worse overall perception of QOL and health, while fathers displayed a worse perception of their psychological state and impairment in overall QOL and in social relationship.

These findings are in accordance with previous studies, reporting parents of children with autism, particularly mothers, experience more stress than parents of typically developing children or other clinical conditions (cystic fibrosis, Down syndrome, behaviour disorders, mental retardation, learning-disability) [12,19,27,37,45].

Analyzing data within the PDDs group, the HFA/AS subgroup seems to display a lower QOL compared to the AD sub-group, because of the lower scores in more domains. In particular, the fathers' HFA/AS sub-group shows impairment in overall perception of QOL and health (the latter also compared to the PDD-NOS subgroup) and in the social relationship domain, while the fathers' AD subgroup only in overall perception of QOL and in social relationship; the mothers' HFA/AS sub-group displays impairment in overall perception of QOL, in psychical health and in social relationships while mothers' AD subgroup only in overall perception of QOL and physical health.

From an environmental stand-point, the differences in QOL between parents of children with PDDs and parents of children with other clinical diagnoses could be attributed to the environmental effects (greater stresses and burden) of having a child with such severe developmental disorders: difficult behaviors, including temper tantrum and aggressive, self-abusive, destructive, obsessive, ritualistic, impulsive and self-stimulatory behaviors; limited social skills and judgment that often resulted in being teased or rejected; the strain of not understanding their children or knowing what was wrong with them; needed constant supervision and assistance with daily living skills; financial strains; the problems associated with school and relative services; difficulty obtaining a correct diagnosis; stressful experiences with professionals; worries about the future, including living arrangements and sexuality; ineffective services and unmet needs; poor communication and coordination among services providers [46-48].

On the other hand, studies undertaken from the late 1970s indicated the presence of strong genetic influences and of a phenotype much broader than the traditional diagnostic category of autism [49]: twin and family studies [50], as well as observations on the familiality of a range of traits (social, communication and language difficulties, personality traits, vocational interests, cognitive style) linked to autism [51-63] provide direct and indirect evidences for the existence of a genetic contribution and of a genetic liability for autism spectrum disorders [64]: 'the lesser variant' [65] or 'the broad autism phenotype" [62,66].

Other studies report familial aggregation of psychiatric disorders in families of autistic individuals: obsessive-compulsive disorder, tic disorders, affective disorders (especially major depressive disorder), anxiety disorders (in particular social phobia) and personality disorders [67,68].

A few reports of psychological tests (Minnesota Multiphasic Personality Inventory, Eysenck Personality Inventory, Maudsley Personality Inventory) administered to parents of children with autism in the 1970' and 1980' indicated no significant finding [40,69,70]. However a variety of studies using self-report measures of various symptoms of depression and anxiety found increased reports of psychological distress in parents of children with autism (especially mothers) compared to parents of typical children, those with Down syndrome, or other clinical groups [15,45,71-73]. Further, researchers have recently documented increased rates among parents of children with autism of clinical depression compared to parents of children with Down syndrome [61-63]; but these studies have documented in the majority of cases of major depression, episodes of the disorder had occurred before the birth of the child with autism. The same studies also found increased rates in parents of children with autism of social phobia and other anxiety disorders and some indication of increased incidence of obsessive-compulsive disorder in the extended families.

Piven and Palmer (1999) [74] has suggested two main hypothesis to explain the finding of increased rate of depression among parents of children with autism: 1. a genetic predisposition to depression in individuals who later produce a child with autism or 2. individuals who are depressed or anxious have an increased incidence of marring/having children with a partner who is genetically predispose to produce a child with autism.

Depression seems to be associated with depression in other family members but not with the broader phenotype and with the severity of autism; its increased rate in the families of individuals with autism has not been yet explained but it does not seem to reflect a genetic liability to autism [49].

Another not clarified point is if parents of HFA or AS sons display higher stress compared to parents of children with PDD and mental retardation [75-77].

In our study, parents of children with PDDs showed a significant impairment of QOL as compared to the other groups, and parents in the HFA/AS sub-group seemed to display a lower QOL compared to the AD sub-group.

At the moment it's difficult to examine genetic and environmental influences independently.

The highest impairment of QOL that we found in parents of children with PDDs, might be the expression of this underlying genetic predisposition (both the liability to autism and the higher familiar rate of psychiatric disorders) combined with environmental precipitants.

Further researches are needed to better explain the complicated genes-environment interaction.

Results of the present study should be considered in the context of the following limitations:(1) we did not take into account important factors that might influence QOL such as the socioeconomic status of the parents; in particular no correlational or multivariate analyses with regard to predictors or determinants of parental QoL have been performed; (2) we did not assessed psychiatric comorbidity in the children; (3) this study did not evaluate the options of treatment for the children; (4) the limited number of the study group probably makes the sample not totally representative for the population of parents of children affected by PDDs, MR or CP; (5) the wide age range of the participants, may be another limitation, despite research results have been mixed as to the effect of the child's age on parental distress [37,39,71,72,74]. Some studies support the idea that mothers tend to experience distress earlier than fathers do, perhaps as a combination of childcare demands and early awareness of the child's impairments [37,39]; (6) age of diagnosis and gender of the children were not taken into consideration and no statistical analyses regarding these factors have been performed; (7) the instrument used to assess parental QOL was distributed by the authors to the families of the study group during inpatient or outpatient visits, during home visit or by mail and email and were returned to the authors via a parental visit to the clinic, a second home visit or by mail and email. The questionnaire was distributed to the families of the CG by mail. This difference in assessment methods may have contributed in an unknown way to the differences in the QoL scores between the groups.

Conclusion

Parents of children with PDDs seem to display a higher burden, probably for a combination of environmental and genetic factors. Within this group of parents also those of HFA or AS people have high stress. These finding must be taken into account in policy making to provide better and more specific supports and interventions for this group of diseases.

More attention should be given to parents' (and in particular mothers') needs. Social support and different coping strategies should be developed to respond positively to individual changing needs and in buffering parents from the stress of having a child with disability [15,44,45,78-80]. New research should be conducted to measure the effectiveness of these strategies. In addition, effective and sustainable psycho-social programs are needed to provide necessary support for the special needs of the children and their families.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

DM designed the study, collected the data, performed statistical analysis and drafted the manuscript

LR and VGD assisted in design of the study and commented on the draft paper

LM designed the study, drafted the manuscript together with DM and participated in the interpretation of data

All authors read and approved the final manuscript.

Acknowledgements

The authors thank the family associations: "Gruppo Asperger ONLUS", "Associazione di Genitori di Figli Disabili ONLUS (A.GE.DI.)", Piazza Armerina (EN) and "Un futuro per l'autismo", Lentini (SR), for their support with the questionnaires.

References

1. **The World Health Organization Quality of Life assessment (WHOQOL): position paper from the World Health Organization.** *Soc Sci Med* 1995, **41:**1403-1409.
2. **The World Health Organization Quality of Life assessment (WHOQOL): Development and general psychometric properties.** *Soc Sci Med* 1998, **46:**1569-1585.
3. **The WHOQOL Group: WHOQOL User Manual, and annexes (WHO/MSA/MHP/98.3).** World Heath Organization; 1998.
4. Gillberg C, Wing L: **Autism: not an extremely rare disorder.** *Acta Psychiatr Scand* 1999, **99(6):**399-406.
5. Fombonne E: **Epidemiological surveys of autism and other pervasive developmental disorders: An update.** *Journal of Autism and Developmental Disorders* 2003, **33:**365-382.
6. Bax M: **Autism.** *Dev Med Child Neurol* 1994, **36(8):**659-60.
7. Rice CE, Baio J, Van Naarden Braun K, Doernberg N, Meaney FJ, Kirby RS, ADDM Network: **A public health collaboration for the surveillance of autism spectrum disorders.** *Paediatr Perinat Epidemiol* 2007.
8. Kirby RS, Brewster MA, Canino CU, Pavin M: **Early childhood surveillance of developmental disorders by a birth defects surveillance system: methods, prevalence comparisons, and mortality patterns.** *J Dev Behav Pediatr* 1995, **16(5):**318-26.
9. Lakin KC, White CC, Hill BK, Bruininks RH, Wright EA: **Longitudinal change and interstate variability in the size of residential facilities for persons with mental retardation.** *Ment Retard* 1990, **28(6):**343-51.
10. Topp M, Huusom LD, Langhoff-Roos J, Delhumeau C, Hutton JL, Dolk H: **SCPE Collaborative Group. Multiple birth and cerebral palsy in Europe: a multicenter study.** *Acta Obstet Gynecol Scand* 2004, **83(6):**548-53.
11. Blacher J: **Severely Handicapped Young Children and Their Families Research in Review.** Academic Press, Orlando, FL; 1984.
12. Rodrigue JR, Morgan SB, Geffken GR: **Families of autistic children: psychological functioning of mothers.** *J Clin Child Psychol* 1990, **19:**371-379.
13. Baker BL, Blacher J, Kopp CB, Kraemer B: **Parenting children with mental retardation.** In *International Review of Research in Mental Retardation Volume 20*. Edited by: Bray NW. San Diego: Academic Press; 1997:1-45.
14. Fisman S, Wolf L: **The handicapped child: psychological effects of parental, marital, and sibling relationships.** *Psychiatr Clin North Am* 1991, **14:**199-217.
15. Wolf LC, Noh S, Fisman SN, Speechley M: **Psychological effects of parenting stress on parents of autistic children.** *J Autism Dev Disord* 1989, **19:**157-166.
16. Dyson LL: **Fathers and mothers of school-age children with developmental disabilities: parental stress, family functioning, and social support.** *Am J Ment Retard* 1997, **102:**267-279.
17. Schieve LA, Blumberg SJ, Rice C, Visser SN, Boyle C: **The relationship between autism and parenting stress.** *Pediatrics* 2007, **119(Suppl 1):**S114-21.
18. Weiss SJ: **Stressors experienced by family caregivers of children with pervasive developmental disorders.** *Child Psychiatry Hum Dev* 1991, **21:**203-216.
19. Holroyd J, Brown N, Wikler L, Simmons JQ: **Stress in families of institutionalized and noninstitutionalized autistic children.** *J Community Psychol* 1975, **3:**26-31.
20. Hedov G, Anneren G, Wikblad K: **Self-perceived health in Swedish parents of children with Down's syndrome.** *Qual Life Res* 2000, **9:**415-422.
21. Emerson E: **Mothers of children and adolescents with intellectual disability: social and economic situation, mental health status, and the self-assessed social and psychological impact of the child's difficulties.** *J Intellect Disabil Res* 2003, **47:**385-399.
22. Raina P, O'Donnell M, Schwellnus H, Rosenbaum P, King G, Brehaut J, Russell D, Swinton M, King S, Wong M, Walter SD, Wood E: **Caregiving process and caregiver burden: conceptual models to guide research and practice.** *BMC Pediatr* **4:**1. 2004 Jan 14; Review
23. Vitaliano PP, Zhang J, Scanlan JM: **Is caregiving hazardous to one's physical health? A meta-analysis.** *Psychol Bull* 2003, **129(6):**946-72.
24. Freeman NL, Perry A, Factor DC: **Child behaviors as stressors: replicating and extending the use of the CARS as a measure of stress: a research note.** *J Child Psychol Psychiatry* 1991, **32:**1025-1030.
25. Hastings RP: **Parental stress and behavior problems of children with developmental disability.** *J Intellect & Dev Disability* 2002, **27:**149-160.
26. Little L: **Differences in stress and coping for mothers and fathers of children with Asperger's syndrome and nonverbal learning disorders.** *Pediatr Nurs* 2002, **28:**565-570.
27. Allik H, Larsson JO, Smedje H: **Health-related quality of life in parents of school-age children with Asperger Syndrome or High-Functioning Autism.** *Health Qual Life Outcomes* 2006, **4:**1.
28. Hastings RP: **Child behaviour problems and partner mental health as correlates of stress in mothers and fathers of children with autism.** *J Intellect Disabil Res* 2003, **47(Pt 4–5):**231-7.
29. Hastings RP, Kovshoff H, Brown T, Ward NJ, Espinosa FD, Remington B: **Coping strategies in mothers and fathers of preschool and school-age children with autism.** *Autism* 2005, **9(4):**377-91.
30. Seltzer MM, Greenberg JS, Floyd FJ, Pettee Y, Hong J: **Life course impacts of parenting a child with a disability.** *Am J Ment Retard* 2001, **106:**265-286.
31. Magana SM, Greenberg JS, Seltzer MM: **The health and well-being of black mothers who care for their adult children with schizophrenia.** *Psychiatr Serv* 2004, **55:**711-713.
32. **Development of the World Health Organization WHOQOL-BREF Quality of Life Assessment. The WHOQOL Group.** *Psychol Med* 1998, **28:**551-558.
33. De Girolamo G, Rucci P, Scocco P, Becchi A, Coppa F, D'Addario A, Daru E, De Leo D, Galassi L, Mangelli L, Marson C, Neri G, Soldani L: **Quality of life assessment: validation of the Italian version of the WHOQOL-Brief.** *Epidemiol Psichiatr Soc* 2000, **9:**45-55.
34. American Psychiatric Association: **Diagnostic and statistical manual of mental disorders: DSM-IV-TR.** 4th edition. Washington, DC: Author; 2000.
35. SPSS base 9.0: **User's guide.** Chicago, Ill.: SPSS Inc; 1999.
36. **Surveillance of cerebral palsy in Europe: a collaboration of cerebral palsy surveys and registers. Surveillance of Cerebral Palsy (SCPE).** *Dev Med Child Neurol* 2000, **42:**816-824.
37. Bebko JM, Konstantareas MM, Springer J: **Parent and professional evaluations of family stress associ-ated with characteristics of autism.** *J Autism Dev Disord* 1987, **17:**565-576.
38. Bouma R, Schweitzer R: **The impact of chronic childhood illness on family stress: A comparison between autism and cystic fibrosis.** *J Clin Psychol* 1990, **46:**722-730.

39. Dumas JE, Wolf LC, Fisman S, Culligan A: **Parenting stress, child behavior problems, and dysphoria in parents of children with autism, Down Syndrome, behaviour disorders, and normal development.** *Exceptionality* 1991, **2:**97-110.
40. Koegel RL, Schreibman L, O'Neill RE, Burke JC: **The personality and family-interaction characteristics of parents of autistic children.** *J Consult Clin Psychol* 1983, **51:**683-692.
41. Konstantareas MM, Homatidis S, Plowright CM: **Assessing resources and stress in parents of severely dysfunctional children through the Clarke modification of Holroyd's Questionnaire on Resources and Stress.** *J Autism Dev Disord* 1992, **22:**217-234.
42. Sanders JL, Morgan SB: **Family Stress and Adjustment as Perceived by Caregivers of Children with Autism or Down Syndrome: Implications for Intervention.** *Child Fam Behav Ther* 1997, **19:**15-32.
43. Sharpley C, Bitsika V, Efremidis B: **Influence of Gender, Parental Health, and Perceived Expertise of Assistance upon Stress, Anxiety, and Depression among Parents of Children with Autism.** *J Intellect Dev Disabil* 1997, **22:**19-28.
44. Sivberg B: **Coping strategies and parental attitudes, a comparison of parents with children with autistic spectrum disorders and parents with non-autistic children.** *Int J Circumpolar Health* 2002, **61:**36-50.
45. Weiss MJ: **Harrdiness and social support as predictors of stress in mothers of typical children, children with autism, and children with mental retardation.** *Autism* 2002, **6:**115-130.
46. Midence K, O'Neill M: **The experience of parents in the diagnosis of autism: a pilot study.** In *Autism Volume 3*. Sage Publications and The National Autistic Society, London, UK; 1999:273-285.
47. Fong L, Wilgosh L, Sobsey D: **The experience of parenting an adolescent with autism.** *International Journal of Disability, Development and Education* 1993, **40:**105-113.
48. Kohler F: **Examining the services received by young children with autism and their families: A survey of parent responses.** *Focus on Autism and Other Developmental Disorders* 1999, **14:**150-159.
49. Rutter M: **Genetic studies of autism: From the 1970s into the millennium.** *Journal of Abnormal Child Psychology* 2000, **28:**3-14.
50. Lauritsen MB, Ewald H: **The genetics of autism.** *Acta Psychiatrica Scandinavica* 2001, **103:**411-427.
51. Piven J, Palmer P, Jacobi D, Childress D, Arndt S: **Broader autism phenotype: Evidence from a family history study of multiple-incidence autism families.** *American Journal of Psychiatry* 1997, **154:**185-190.
52. Piven J, Palmer P, Landa R, Santangelo S, Jacobi D, Childress D: **Personality and language characteristics in parents from multiple-incidence autism families.** *American Journal of Medical Genetics* 1997, **74:**398-411.
53. Murphy M, Bolton PF, Pickles A, Fombonne E, Piven J, Rutter M: **Personality traits of the relatives of autistic probands.** *Psychological Medicine* 2000, **30:**1411-1424.
54. Baron-Cohen S, Bolton P, Wheelwright S, Short L, Mead G, Smith A, Scahill V: **Autism occurs more often in the families of physicists, engineers and mathematicians.** *Autism* 1998, **2:**296-301.
55. Baron-Cohen S: **The extreme male brain theory of autism.** *Trends in Cognitive Sciences* 2002, **6:**248-254.
56. Happè F: **Autism: Cognitive deficit or cognitive style?** *Trends in Cognitive Sciences* 1999, **3:**216-222.
57. Landa R, Piven J, Wzorek M, Gayle JO, Chase GA, Folstein SE: **Social language use in parents of autistic individuals.** *Psychological Medicine* 1992, **22:**245-254.
58. Bailey A, Palferman S, Heavey L, Le Couteur A: **Autism: The phenotype in relatives.** *Journal of Autism and Developmental Disorders* 1998, **28:**369-392.
59. Folstein SE, Piven J: **Etiology of autism: Genetic influences.** *Pediatrics* 1991, **87:**767-773.
60. DeLong R, Nohria C: **Psychiatric family history and neurologicaldisease in autistic spectrum disorders.** *Developmental Medicine and Child Neurology* 1994, **36:**441-448.
61. Bolton PF, Pickles A, Murphy M, Rutter M: **Autism, affective and other psychiatric disorders: patterns of familial aggregation.** *Psychol Med* 1998, **28:**385-395.
62. Piven J, Chase GA, Landa R, Wzorek M, Gayle J, Cloud D, Folstein S: **Psychiatric disorders in the parents of autistic individuals.** *J Am Acad Child Adolesc Psychiatry* 1991, **30:**471-478.
63. Smalley SL, McCracken J, Tanguay P: **Autism, affective disorders, and social phobia.** *Am J Med Genet* 1995, **60:**19-26.
64. Austin EJ: **Personality correlates of the broader autism phenotype as assessed by the Autism Spectrum Quotient (AQ).** *Personality and Individual Differences* 2005, **38:**451-460.
65. Bolton P, Macdonald H, Pickles A, Rios P, Goode S, Crowson M, Bailey A, Rutter M: **A case-control family history study of autism.** *J Child Psychol Psychiatry* 1994, **35:**877-900.
66. Piven J: **The broad autism phenotype: a complementary strategy for molecular genetic studies of autism.** *Am J Med Genet* 2001, **105:**34-35.
67. Mouridsen SE, Rich B, Isager T, Nedergaard NJ: **Psychiatric disorders in the parents of individuals with infantile autism: a case-control study.** *Psychopathology* **40(3):**166-171. 2007 Feb 22
68. Yirmiya N, Shaked M: **Psychiatric disorders in parents of children with autism: a meta-analysis.** *J Child Psychol Psychiatry* 2005, **46(1):**69-83.
69. Cantwell DP, Baker L, Rutter M: **Families of autistic and dysphasic children – I. Family life and interaction patterns.** *Arch Gen Psychiatry* 1978, **36:**682-688.
70. McAdoo WG, DeMyer MK: **Personality characteristics of parents.** In *Autism: a reappraisal of concepts and treatment* Edited by: Rutter M, Schopler E. New York: Plenum Press; 1978:251-267.
71. Moes D, Koegel RL, Schreibman L, Loos LM: **Stress profiles for mothers and fathers of children with autism.** *Psychol Rep* 1992, **71:**1272-1274.
72. Bristol MM: **Mothers of children with autism or communication disorders: successful adaptation and the double ABCX model.** *J Autism Dev Disord* 1987, **17:**469-486.
73. Gray DE, Holden WJ: **Psychosocial Well-Being among the Caregivers of Children with Autism.** *Australia and New Zealand Journal of Developmental Disabilities* 1992, **18:**83-93.
74. Piven J, Palmer P: **Psychiatric disorder and the broad autism phenotype: evidence from a family study of multiple-incidence autism families.** *Am J Psychiatry* 1999, **156:**557-563.
75. Konstantareas MM, Homatidis S: **Assessing child symptom severity and stress in parents of autistic children.** *J Child Psychol Psychiatry* 1989, **30:**459-470.
76. Schuntermann P: **Pervasive developmental disorder and parental adaptation: previewing and reviewing atypical development with parents in child psychiatric consultation.** *Harv Rev Psychiatry* 2002, **10(1):**16-27.
77. Gabriels RL, Cuccaro ML, Hill DE, Ivers BJ, Goldson E: **Repetitive behaviors in autism: relationships with associated clinical features.** *Res Dev Disabil* 2005, **26:**169-181.
78. Tunali B, Power TG: **Coping by redefinition: cognitive appraisals in mothers of children with autism and children without autism.** *J Autism Dev Disord* 2002, **32:**25-34.
79. Henderson D, Vandenberg B: **Factors influencing adjustment in the families of autistic children.** *Psychol Rep* 1992, **71:**167-171.
80. Bristol MM, Gallagher JJ, Schopler E: **Mothers and Fathers of Young Developmentally Disabled and Nondisabled Boys: Adaptation and Spousal Support.** *Dev Psychol* 1988, **24:**441-451.

References

Sharpley CF, Bitsika V, & Efremidis B. (1997). Influence of gender, parental health, and perceived expertise of assistance upon stress, anxiety, and depression among parents of children with autism. *Journal of Intellectual & Developmental Disability*, *22*(1), 19–28. https://doi.org/10.1080/13668259700033261

INFLUENCE OF GENDER, PARENTAL HEALTH, AND PERCEIVED EXPERTISE OF ASSISTANCE UPON STRESS, ANXIETY, AND DEPRESSION AMONG PARENTS OF CHILDREN WITH AUTISM[1]

A survey of 219 parents of children with autism was administered on a confidential and anonymous basis. As well as tapping information about the nature of the child's disorder, parental well-being (anxiety, depression), parents' daily level of stress arising from parenting, their confidence in handling their child's major difficulty, and the frequency of being stretched beyond their limits were included as dependent variables. Independent variables were: gender of parents, age of child and age of onset, parental health, access to other family members, and level of understanding of those family members of the child's problems. Data indicated that, although social support has previously been posited as an alleviating factor for parental stress, this may be a result of the perceived expertise of the family member who provides respite care for the parents. Issues of self-efficacy, training in behaviour management, and provision of home-based care for parents are discussed.

There is little doubt that parenting a child with autism is extremely demanding. Because of (a) the relatively poor understanding of autism by the general public when compared with other disabilities such as Down syndrome (Fisman, Wolf, & Noh, 1989), leading to a marked antipathy for the typical behaviour exhibited by children with autism (Koegel et al., 1992), and also because of (b) the socially inappropriate and aggressive nature of much autistic behaviour, parents of children with autism often report high levels of anxiety, depression, and everyday stress from parenting (DeMeyer, 1979; Harris, 1984). This is further exacerbated when parents realise that there is no cure for autism (Liwag, 1989), and that services which can be of real assistance are often insufficient to meet parents' needs. In addition, as noted by Holroyd, Brown, Wikler, and Simmons (1975), the extra time which parents have to devote to their child with autism can sometimes make other children feel neglected, or cause conflict between siblings, thus exacerbating parental stress. Parenting a child with autism has also been shown to have detrimental effects on marital relations (Piven, Chase, Landa, & Wzorek, 1991).

As mentioned above, three of the most stressful factors in regard to parenting a child with autism are the permanency of the condition, the lack of acceptance of autistic behaviour by society and sometimes other family members, and very low levels of social support (Gray & Holden, 1992; Konstantareas & Homatidis, 1989). Gray and Holden concluded that social support and gender of parent influenced the adaptation of parents to the behaviour of their children with autism. In terms of gender, mothers have been shown to suffer higher levels of anxiety and depression than fathers of children with autism (Freeman, Perry, & Factor, 1991; Gray & Holden, 1992; Moes, Koegel, Schreibman, & Loos, 1992). This may be because mothers usually assume greater responsibility for the day-to-day care of these children and are less likely to be in fulltime outside employment, thereby lacking in the social support which is often available from workmates. In fact, Konstantareas and Homatidis (1989) noted that mothers' stress levels rose and fell in an inverse relationship with their perceived level of social support, leading Koegel et al. (1992) to argue for the development and implementation of social support systems for parents of children with autism. The relationship between social support and anxiety in these parents was also noted by Gray and Holden (1992) in their study of Australian parents with children who had autism, although those authors concluded that there was a need for further investigation of the nature of the role which social support played in alleviating parental anxiety and depression. This suggestion complies with a deal of previous data which demonstrate a buffering relationship between social support and stress in more general situations (e.g., House, Landis, & Umberson, 1988; Lin, Woefel, & Light, 1985; Wheaton, 1985).

Although respite care is available to most parents of children with autism via government agencies, it is generally infrequent. The most common form of "respite" from everyday parenting is through members of the immediate family, and it is these grandparents, aunts, uncles, and siblings to whom parents of children with autism look primarily for social and emotional support. However, the value of social support may well lie in the nature of the support and/or respite care offered to parents by these relatives. If this is the case, then the perceived expertise of those relatives in understanding and handling the child's behaviour could well be the underlying causal connection between this form of social support and parental well-being which has been previously reported in the literature. Thus, the present study examined the effect of those immediate family members who provided respite care to parents according to whether those family members had (in the parents' view) a clear understanding of the child's needs and behaviour.

Others factor which have been shown to exacerbate the level of stress experienced by parents of children with autism are: (a) the age of the child, with parents of older children showing higher levels of stress than parents of younger children (Holroyd, Brown, Wikler, & Simmons, 1975); and (b) the age of diagnosis, with a significant direct correlation reported between age of diagnosis and level of depression in parents (Gray & Holden, 1992).

The present study was designed to determine whether parental stress, anxiety and depression arising from parenting an autistic child could be shown to be related to gender or alleviated by social support. Previous findings regarding the later variable were tested by defining social support in terms of the presence/absence of a clear understanding of the child's disorder by those immediate family members who provided respite from the day-to-day demands of parenting. In addition, because stress has been shown to contribute to illness (Everly, 1990), thus perhaps leading to a selfperpetuating cycle of stress-related ill health and parental anxiety, the presence of illness among parents and its influence upon

parental stress, anxiety and depression was also examined here. Finally, age of the child with autism and age of diagnosis were also examined as possible determining factors of parental distress.

METHOD

Participants

A total of 1076 questionnaires were sent out in a newsletter from Autism Victoria to 511 families with a child with autism in Victoria and another 27 families from interstate. Two questionnaires were sent to each family (one for each parent) because no attempt was made to determine if families were two- or single-parent families, so as to protect their anonymity. From these 1076 questionnaires, 219 (20.3%) parents (141 females) returned the questionnaire in useable form. Of these, 213 (97.3%) were parents of the autistic child in question, and six were step-parents. Although the return rate was lower than hoped, it is not unduly less than common in anonymous questionnaire surveys of this sort. The generalisability of the data obtained cannot be guaranteed, and there may be some response bias present, but there is no reason to conclude that it is not acceptable according to the usual standards applied in such survey research.

Instruments

In terms of the dependent variables to be tested, standardised instruments were used for anxiety and depression. However, one limitation of these instruments is that they are primarily developed to be applied across a range of situations, with a range of individuals. In order to examine the effects of the above mentioned independent variables on parental well-being, three extra questions were designed and used here, and these are described below.

As well as a demographic questionnaire designed to tap various aspects of their child's disorder, age, gender, schooling, and family structure, parents were asked several questions about their own background (sex, age, employment status of people in family, relationship to child if not parent, if they suffered from any illness or disability and whether medication was being taken because of this) and what form of assistance was available from within and outside the immediate family. In particular, parents were asked to judge whether the assistance they received from within or outside the family was provided by people who had a "clear understanding of your child's difficulties and needs". The wording of this item was tested for reliability with a small sample of parents (n = 10), confirming that it was clearly understood by them as a measure of their confidence in the expertise of the assistance available to them and was therefore suitable for inclusion in the study.

Parents were also asked about the major difficulty they were currently experiencing with their child, and their major concern for their child's future, and completed the Zung Self-Rating Anxiety Scale (SAS: Zung, 1971) and the Zung Self-Rating Depression Scale (SDS: Zung, 1965). Both the SAS and the SDS possess validity because of their construction on the basis of DSM-II criteria (i.e., both somatic and cognitive/emotional symptoms such as worrying, feeling hopeless, sleeplessness, lack of enjoyment of life, muscle tremors, stomach churning, sweating, and increased frequency of urination). Reliability data for the SAS is satisfactory at 0.71 (split half: Zung, 1971), and a coefficient alpha of 0.85 has been reported (Zung, 1980), indicating acceptable levels of internal consistency. Similarly, the SDS has been shown to possess split half reliability of 0.73 (Zung, 1973) and alpha coefficients between 0.86 and 0.90 (Schaefer, Brown, Watson, Plenel, DeMotts, Howard, Petrik & Balleweg, 1985).

As mentioned above, three questions were designed to assess parents' stress in more specific ways than the SAS and SDS. These questions were designed after consultation with a small group of parents of children with autism and some teachers and psychologists who worked with these children. Parents were asked to rate, on 10-point Likert scales, (a) their degree of confidence about handling their child's current major difficulty, and (b) their average daily level of stress arising from parenting. Parents were also asked whether there were times when "the caring for your child stretches you beyond your personal limits" and, if so, how many times they felt this way per month. Together with the SAS and SDS, these measures constituted the dependent variables used to assess parental well-being, according to the influence of the independent variables listed above. Because of the different nature of the independent variables (i.e., age of child, age of onset, gender of parent, presence and expertise of social/ familial support, parental illness or disability), these were tested for their effects on the dependent variables of parental well-being via a series of MANOVAs. This procedure was designed to elicit the most comprehensive analysis of the independent variables (i.e., by allowing for stronger conclusions than those obtainable from regression procedures used by some previous researchers such as Gray and Holden, 1992), while minimising the chance of Type I and Type II errors.

RESULTS

(a) The children

Children of the parents sampled ranged from age three years to 33 years, with most (75 %) being less than nine years of age. The age of diagnosis also ranged widely (from 1 to 21 years), but most (79.8%) had been diagnosed between two and five years of age. As might be expected from the wider literature, most children (82.3%) were male. Nearly all (90.9%) of the children had siblings, and in 17.3% of cases, these siblings also had a disorder including both intellectual and physical conditions and disorders. The most common educational setting for these children of school age was a specialist school (48.6%), but over one-third (37.7%) were in mainstream schools. Of those who were in mainstream schools, 88.6% had some level of assistance from a teacher's aide. Half (50%) of the respondents received respite care, which was mostly external to the family home (36.3%), although 32.7% received in-home respite care, and another 30% received a combination of these two types of respite care for their children. Nearly two-thirds (59.1%) of parents received some form of other assistance with their child, this most commonly being provided by government agencies (47.7%), followed by private or non-government sources (27.6%), or a combination of these.

The major current child-management difficulties experienced by parents were: behavioural (35.9%), toilet training (7.7%), communication (7.3%), and learning difficulties (5.5%), clearly indicating that everyday management of their children's behaviour was a major source of stress for these parents. The degree of confidence which parents experienced in handling these major current difficulties varied from low to very high, ("average" = 26.8%, "above average" = 43.5%, and "below average" = 29.7%).

(b) The parents

Most (64.4%) respondents were female, and nearly all (97.3%) were natural parents of a child with autism, with the rest being step-parents of such a child. In terms of the ability of these parents to deal effectively with their children's behaviour, 81.9% reported that they were sometimes stretched beyond their limits. At these times, they were "unable to cope" (52.2%), "anxious or stressed" (13.3%),

"depressed" (9.9%), felt "isolated and lonely" (3.0%), "blamed themselves" (1.5%), or just "felt awful" (.5%). Nearly half (46.4%) of those parents who reported feeling stretched beyond their limits said they felt this way from 1 to 5 times per month, with 18.7% feeling stretched beyond their limits between 6 and 10 times/month. However, 11.1% of parents felt this way more than 15 times each month (i.e., about every two days on average), suggesting that feeling being stretched beyond their limits was a relatively frequent experience for more than one in every ten parents. Parents experienced a wide range of levels of daily stress from parenting their child with autism, ranging from "very low" (6.6%), "low" (3.2%), "average" (36.9%), "high" (40.0%), and "very high" (10.5%), with a clear skew to the above average side of the distribution. In addition, although 41.8% of parents scored in the "moderate anxiety" range on the SAS, 18.6% were "highly anxious", and a further 9.1% were "severely anxious" according to Zung's (1971) recommended scoring methods. These data represent a higher prevalence rate for anxiety than the 9% reported by Zung (1980) for a normal adult population. For depression, 13.2% of parents reported symptoms of "moderate depression" and 5.9% fell into the "severely depressed" category, giving a total "depressed" category prevalence of 18.1%, higher than the figure of about 15%

reported by Byrne (1980) for a general Australian population. It is therefore sensible to ask what were the factors which distinguished those parents who had high levels of daily stress, anxiety and depression from those who had lower levels of these unwanted outcomes of parenting. All of the analyses reported below were conducted via MANOVA.

(i) Gender of parent

Females were more anxious (M SAS score = 46) than males (M SAS score = 41: $F(1,157) = 6.233$, $p < .05$), and more depressed (M SDS score = 50) than males (M SDS score = 45: $F(1,157) = 6.647$, $p < .05$). Females also reported a nonsignificantly higher frequency of feeling stretched beyond their limits per month (7.1 times) than males (4.7 times), and a higher daily level of stress from parenting (6.5) than males (5.9). However, females also reported that they had nonsignificantly greater levels of confidence in handling their child's major problem (5.9) than males did (5.4).

(ii) Access to other family members for assistance in child care

Of the parents sampled here, 61.5% reported that they had access to other family members for assistance in child care. The sources of this assistance were most often grandparents (52%), followed by aunts and uncles (23.8%) and older siblings (19.4%), although there were no significant differences in parental well-being according to the relationship between parents and the family members who gave assistance. Access to other family members for child care (versus no such access) was associated with nonsignificantly lower frequency per month of feeling stretched beyond personal limits (access parents = 6.04, non-access parents = 6.51), anxiety (SAS scores: access parents = 34.86 vs non-access parents = 36.06) and depression (SDS scores: access parents = 37.97 vs non-access parents = 39.82) scores, and daily level of stress from parenting (access parents = 6.12 vs non-access parents = 6.53); and with a higher level of confidence in handling their child's current major difficulty (access parents = 6.28, non-access parents = 4.89).

(iii) Level of understanding of child's difficulties by other family members to whom the parents have access for assistance

A further investigation of the effects of support within the immediate family (specifically the relationship between parental well-being and the presence of perceived expertise as caregivers to a child with autism of those family members who were accessible) was carried out by asking parents if they considered that the other family members to whom they had access had a "clear understanding of your child's difficulties and needs". As mentioned above, this item had been developed and tested on a sample of parents before inclusion in the questionnaire. Although there were no significant differences in the parental well-being dependent variables according to the relationship between the family members and parents (i.e., whether they were grandparents, aunts, etc.), and no significant interaction between that relationship and having a clear understanding of the child's' difficulties and needs, there was a significant main effect for the presence/absence in assistant care-givers of a "clear understanding of the child's difficulties and needs" ($F(5,89) = 3.103$, $p < .05$), with significant univariate effects for SAS scores ($F(1,93) = 5.004$, $p < .05$), SDS scores ($F(1,93) = 5.954$) $p < .05$), and confidence in parents' ability to handle their child's current major difficulties ($F(1,93) = 5.922$, $p < .05$). All of these differences were in the expected direction, suggesting that a higher level of parentally-perceived understanding of the autistic child's difficulties and needs by assistance-giving family members is significantly associated with lower parental stress arising from caring for a child with autism, and that this relationship is independent of the identity of those family members.

(iv) Parental health

Those parents who suffered from an illness or disability which, in their view, "hinders your ability to be an active parent to your child" with autism had significantly higher SAS scores ($F(1,161) = 9.014$, $p < .01$), SDS scores ($F(I,161) = 10.844$, $p < .005$) and daily level of stress from parenting ($F(1,161) = 7.245$)$p < .01$) than parents with no such illness or disability.

(v) Age of child and age of diagnosis

Neither of these two independent variables (age of child now and age when autism was first diagnosed) showed any significant main or univariate effects on any of the dependent variables.

DISCUSSION

As previously reported by several authors, parents of a child with autism showed elevated levels of anxiety and depression when compared with the normal population, with the most common source of stress arising from parenting being that of their child's behavioural problems. Levels of daily stress arising from parenting were skewed towards the high end of the distribution, and more than 80% reported being sometimes stretched beyond their limits. Females reported higher levels than males on all of these measures of outcomes from parenting stress, but also showed higher levels of confidence in handling their child's major problem than did males, perhaps reflecting greater day-to-day exposure to those problems and therefore a greater degree of equanimity and proficiency in handling them.

Perhaps to be expected, parents with a major illness or disability reported higher levels of anxiety, depression and daily stress than non-disabled or ill parents. While any task is more difficult when we are ill, the particularly demanding task of parenting a child with autism apparently is similarly exacerbated for those parents who are not well themselves, reinforcing the need for effective and frequent home help for those parents with a disability or illness.

Nearly two-thirds of the parents sampled had access to other family members for assistance in child care. Although the differences were nonsignificant at traditional levels, there were consistently lower scores on depression, anxiety, daily level of stress and frequency of being stretched beyond their limits for those parents who did have access to family members for child care than for those parents with no such access. These parents also reported higher levels of confidence in handling their child's major difficulty.

There was no significant difference between parental well-being according to the relationship parents had with the immediate family member who gave assistance, suggesting that this is not the prime causal variable determining the value of such assistance. Instead, there was a significant main effect according to the level of understanding which parents felt that their immediate family member had of the child's problems. That is, parents who believed that the family members giving assistance had a clear understanding of the child's difficulties and needs were also less anxious, depressed and had higher levels of confidence in their own ability to handle their child's major difficulties. Thus, perceived expertise (by the assisting family member) in handling the child's problems generalised to the parent's own belief in their ability to similarly deal with those problems. This finding is an important step in understanding how to better prepare parents to handle the day-to-day demands of parenting a child with autism. As is well demonstrated across a wide range of tasks, self-efficacy (or belief in one's ability to perform a task successfully) is the major predictor of future successful performance of that task (Bandura, 1986). If parental confidence in their ability to handle their child's major problem is directly influenced by their confidence in another family member's understanding of their child's problems, then this raises an important issue for training parents and care-givers in how to deal effectively with children with autism. Clearly, while it is vital to train parents in behaviour management of their child with autism, it appears to be similarly important to train those immediate family members in the same skills, thus contributing to the parents' well-being and their confidence, self-efficacy, and future ability to deal effectively with the demands of parenting a child with autism.

Several implications arise from these findings. First, while general social support is no doubt of value to parents of children with autism, it may be of most value when the immediate family members who give assistance have a clear understanding of the child's difficulties. Thus, immediate family members who give assistance could be included in training workshops originally designed for parents, enabling sharing of expertise as well as building closer family links and contributing to a stronger general base of well-informed social support for parents. Similarly, inclusion of these family members who provide assistance in any conferences or meetings between counsellors or support workers and parents may alleviate the isolation of parents and improve the eventual expertise of the care which children with autism receive in their home settings. Second, the presence of parental illness or disability appears to be an additional impediment to parents of children with autism, and needs to be taken into account when developing training for parents or planning the delivery of services to those parents so that the assistance given goes beyond the "home help" domestic chores model and also includes assistance in child care. Counsellors and support workers need to be aware that parental illness and disability are more than a discomfort to parents, but may actually impede caring for the child with autism. Therefore, the incorporation of some advice and services to the parents themselves regarding their own illness/disability may be advantageous in reducing the effects of these on parenting skills.

Several recommendations are made for future research. While standardised instruments should always be included in any evaluation of well-being, it is also clearly valuable to incorporate specifically designed measures which tap the particular concerns of the population sampled in language that is relevant to that population. In addition, because parenting is a skills-based task like many other life demands, the role of self-efficacy in predicting ability to perform this important task should be further addressed. It may also be valuable to measure the relative effectiveness of training programmes which do/do not incorporate self-efficacy enhancement as a part of the training given to parents. Another avenue for future research could be the relative effectiveness of assistance provided by family members versus governmental agencies. Finally, the role which immediate family members can play in parental wellbeing and confidence has been shown here. To further elucidate that role, an intervention-based study incorporating the comparison of training for those family members (as well as parents) versus no such training could add to our understanding of these important issues in parenting children with autism.

Acknowledgement

We wish to express our gratitude to Autism Victoria and to Amanda Golding in particular for her assistance with aspects of the data collection.

REFERENCES

Bandura, A. (1986). Social foundations of thought and action. Englewood Cliffs: Prentice-Hall.

Byrne, D. G. (1980). The prevalence of symptoms of depression in an Australian general population. Australian and New Zealand Journal of Psychiatry, 14, 65-71.

DeMeyer, M. (1979). Parents and children in autism. New York: John Wiley & Sons.

Everly, G. (1990). A clinical guide to the treatment of the human stress response. New York: Plenum.

Fisman, S., Wolf, L., & Noh, S. (1989). Marital intimacy in parents of exceptional children. Canadian Journal of Psychiatry, 34, 519-525.

Freeman, N., Perry, A., & Factor, D. (1991). Child behaviours as stressors: Replicating and extending the use of the CARS as a measure of stress: A research note. Journal of Child Psychology and Psychiatry and Allied Disciplines, 32, 1025-1030.

Gray, D., & Holden, W., (1992). Psycho-social well being among the parents of children with autism. Australia and New Zealand Journal of Developmental Disabilities, 18, 83-93.

Harris, S. (1984). The family and the autistic child: A behavioral perspective. Family Relations, 33, 127-134.

Holroyd, J., Brown, N., Wikler, L., & Simmons, J. (1975). Stress in families of institutionalised and non institutionalised autistic children. Journal of Community Psychology, 3, 26-31.

House, J. S., Landis, K.R., & Umberson, D. (1988). Social relationships and health. Science, 241, 540-545.

Koegel, R., Schreibman, L., Loos, L., Dirlich-Wilhelm, H., Dunlap, G., Robbins, F., & Plienis, A. (1992). Consistent stress profiles in mothers of children with autism. Journal of Autism and Developmental Disorders, 22, 205-216.

Konstantareas, M., & Homatidis, S. (1989). Assessing child symptom severity and stress in parents of autistic children. Journal of Child Psychology and Psychiatry and Allied Disciplines, 30, 459-470.

Lin, N., Woefel, M. W., & Light, S.C. (1985). The buffering effect of social support subsequent to an important life event. Journal of Health and Social Behavior, 26, 247-263.

Liwag, M. (1989). Mothers and fathers of autistic children: An exploratory study of family stress and coping. Philippine Journal of Psychology, 22, 3-16.

Moes, D., Koegel, R., Schreibman, L, & Loos, L. (1992). Stress profiles for mothers and fathers of children with autism. Psychological Reports, 71, 1272-1274.

Piven, J., Chase, G., Landa, R., & Wzorek, M. (1991). Psychiatric disorders in the parents of autistic individuals. Journal of the American Academy of Child and Adolescent Psychiatry, 30, 471-478.

Schaefer, A., Brown, J., Watson, C., Plenel, D., DeMotts, J., Howard, M., Petrik, N., & Balleweg, B. (1985). Comparison of the validities of the Beck, Zung, and MMPI depression scales. Journal of Consulting and Clinical Psychology, 53, 415-418.

Wheaton, B. (1985). Models for the stress-buffering functions of coping resources. Journal of Health and Social behavior, 26, 352-364.

Zung, W. (1965). A self-rating depression scale. Archives of General Psychiatry, 12, 63-70.

Zung, W. (1971). A rating instrument of anxiety disorders. Psychosomatics, 12, 371-379.

Zung, W. (1973). From art to science: The diagnosis and treatment of depression. Archives of General Psychiatry, 29, 328-337.

Zung, W. (1980). How normal is anxiety? Current concepts. Durham, N.C.: Upjohn.

~~~~~~~~

By CHRISTOPHER F SHARPLEY , VICKI BITSIKA and BILL EFREMIDIS Monash University

---

Source: Journal of Intellectual & Developmental Disability
~~~~~~~~

Isolation and Structure of a Brain Constituent That Binds to the Cannabinoid Receptor

Article *in* Science · January 1993

DOI: 10.1126/science.1470919 · Source: PubMed

10 authors, including:

William Devane
Ollscoil na Gaillimhe – University of Galway
24 PUBLICATIONS **13,698** CITATIONS
SEE PROFILE

Lumir Hanus
School of Pharmacy, Ein Kerem Campus, Hebrew University of Jerusalem
196 PUBLICATIONS **21,312** CITATIONS
SEE PROFILE

Aviva Breuer
Hebrew University of Jerusalem
49 PUBLICATIONS **11,628** CITATIONS
SEE PROFILE

Roger G Pertwee
University of Aberdeen
332 PUBLICATIONS **47,325** CITATIONS
SEE PROFILE

Reprint Series
18 December 1992, Volume 258, pp. 1946–1949

SCIENCE

Isolation and Structure of a Brain Constituent That Binds to the Cannabinoid Receptor

William A. Devane,*† Lumir Hanuš, Aviva Breuer, Roger G. Pertwee, Lesley A. Stevenson, Graeme Griffin, Dan Gibson, Asher Mandelbaum, Alexander Etinger, and Raphael Mechoulam†

Isolation and Structure of a Brain Constituent That Binds to the Cannabinoid Receptor

William A. Devane,*† Lumir Hanuš, Aviva Breuer, Roger G. Pertwee, Lesley A. Stevenson, Graeme Griffin, Dan Gibson, Asher Mandelbaum, Alexander Etinger, Raphael Mechoulam†

Arachidonylethanolamide, an arachidonic acid derivative in porcine brain, was identified in a screen for endogenous ligands for the cannabinoid receptor. The structure of this compound, which has been named "anandamide," was determined by mass spectrometry and nuclear magnetic resonance spectroscopy and was confirmed by synthesis. Anandamide inhibited the specific binding of a radiolabeled cannabinoid probe to synaptosomal membranes in a manner typical of competitive ligands and produced a concentration-dependent inhibition of the electrically evoked twitch response of the mouse vas deferens, a characteristic effect of psychotropic cannabinoids. These properties suggest that anandamide may function as a natural ligand for the cannabinoid receptor.

The psychoactive constituent of cannabis, Δ^9-tetrahydrocannabinol (Δ^9-THC) (*1*), binds to a specific G protein–coupled receptor in the brain (*2*). Sequence information on the cannabinoid receptor is available from cloned rat (*3*) and human (*4*) genes, but thus far it has not provided insight into the protein's physiological role(s). The abundance and anatomical localization of the receptor in the brain (*5*), together with the behavioral effects of Δ^9-THC (*6*), are consistent with roles in the control of

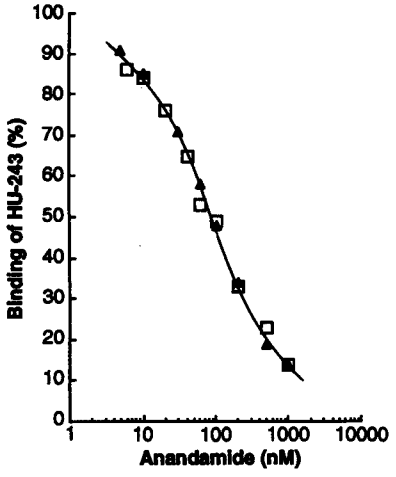


Fig. 1. Competitive inhibition of [^{3}H]HU-243 binding by natural anandamide. Synaptosomal membranes were prepared from the brains of Sprague-Dawley male rats (430 to 470 g) after removal of the brainstem. The [^{3}H]HU-243 probe (45 to 55 pM) was incubated with synaptosomal membranes (3 to 4 μg) for 90 min at 30°C with the indicated concentrations of anandamide or with the vehicle alone (fatty-acid–free bovine serum albumin at a final concentration of 0.5 mg/ml). Bound and free radioligand were separated by centrifugation [see (*8*) for full details of the assay]. The data were normalized to 100% of specific binding, which was determined with 50 nM unlabeled HU-243. Specific binding accounted for 77 to 82% of the total radioactivity bound to the membranes. Data points represent the average of triplicate determinations from two independent experiments. The K_i value was determined from the Ligand program (*26*).

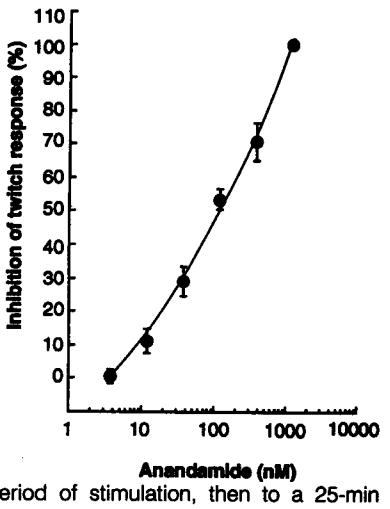


Fig. 2. Inhibition of the vas deferens twitch response by natural anandamide. Data are presented as the percentage of inhibition ± SEM (n = 6 or 7) for each concentration. Vas deferentia from MF1 mice were mounted in siliconized organ baths (4 ml) under an initial tension of 0.5 g. The baths contained Mg^{2+}-free Krebs solution (*13*), which was kept at 37°C and bubbled with 95% O_2 and 5% CO_2. The tissues were stimulated supramaximally with 0.5-s trains of three pulses (train frequency, 0.1 Hz; pulse duration, 0.5 ms). Isometric contractions were evoked by electrical field stimulation through electrodes attached at the upper and lower ends of each bath and were registered on a polygraph recorder (Grass model 7D) with Pye Ether UF1 transducers. The effect of natural anandamide on the twitch response was measured 30 min after its administration. As in previous experiments with cannabinoids (*13*), only one dose was added to each tissue and a pattern of intermittent stimulation was used. Each tissue was subjected to an 11-min period of stimulation, then to a 25-min stimulation-free period, and finally to a second, 5-min period of stimulation. Natural anandamide was added in a volume of 40 μl at the end of the first 11-min period of stimulation. The anandamide was dispersed in Tween-80 and saline (*13*). Tween-80 alone did not inhibit the twitch response (n = 6) at the maximum concentration used in the baths (0.63 μg/ml).

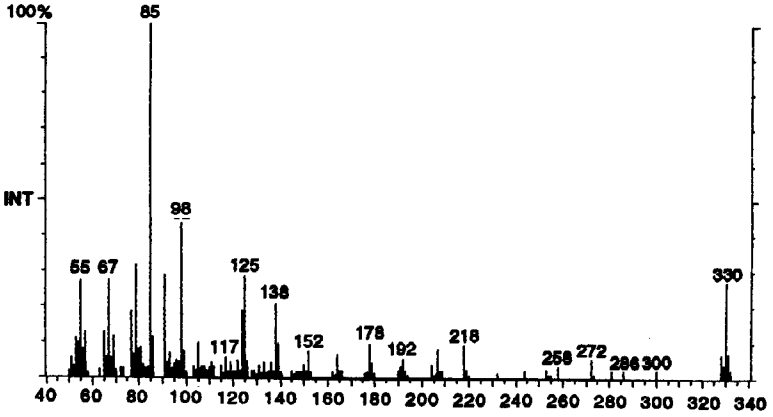


Fig. 3. GC-MS spectrum of anandamide in an ion-trap instrument [for details, see (*12*)]. Anandamide undergoes thermal dehydration under these conditions; hence the spectrum shown is that of the $M^{+\cdot}$ ion of the corresponding 2-oxazoline.

movement, memory, emotions, and pain modulation, among other activities. Although a specific endogenous ligand for the cannabinoid receptor has not yet been identified, its existence is predicted from the strict structural requirements for receptor activation by exogenous ligands (*7*). Moreover, the 97.3% sequence conservation displayed by the rat and human cannabinoid receptors (*4*) suggests that this endogenous ligand(s) may also be conserved across species.

To screen for endogenous cannabinoid compounds, we tested the ability of fractions from porcine brain extracts to displace a radiolabeled cannabinoid probe in a centrifugation-based ligand binding assay. The probe, [^{3}H]HU-243 (11-hydroxy-hexahydrocannabinol-3-dimethylheptyl homolog), has a dissociation constant of 45 pM in rat synaptosomal membranes (*8*). Organic solvent extracts of porcine brain were first chromatographed according to standard protocols for the separation of lipids (*9*). Because many of the initial fractions inhibited the binding of [^{3}H]HU-243 to the cannabinoid receptor, we paid particular attention to the binding of [^{3}H]HU-243 to the siliconized polypropylene microfuge tubes in which the assay was conducted. Normally, about 15 to 20% of the added [^{3}H]HU-243 adheres to the microfuge tube, with the amount increasing slightly when unlabeled cannabinoid drugs displace the radioligand from the receptor. When monitoring the three-way equilibrium of [^{3}H]HU-243 among the synaptosomal receptors, the solution, and the microfuge tube, we observed that all the crude brain fractions that inhibited the binding of [^{3}H]HU-243 to the receptors also inhibited the binding of the radioligand to the microfuge tube. We speculated that these fractions contained compounds that sequestered the radioligand in the solution, perhaps in micelles formed by lipids. After analyzing the binding behavior of [^{3}H]HU-243 at different concentrations of the brain fractions, we chose those fractions that inhibited binding to the receptor but had the least effect on the adherence of the radioligand to the microfuge tube. Several promising fractions were further purified by low- and medium-pressure column chromatography and by thin-layer chromatography (TLC). Both normal-phase and reversed-phase systems were used (*10*).

From these fractions we purified a compound (final yield = 0.6 mg from 4.5 kg of brain) that we named anandamide (*11*). Anandamide showed one spot on TLC and eluted as one main peak on gas chromatography (GC) when a mass spectrometer was used as a detector (*12*). Anandamide inhibited the specific binding of [^{3}H]HU-243 to

W. A. Devane, L. Hanuš, A. Breuer, D. Gibson, R. Mechoulam, Department of Natural Products, Medical Faculty, Hebrew University, Jerusalem 91120, Israel. R. G. Pertwee, L. A. Stevenson, G. Griffin, Department of Biomedical Sciences, Marischal College, University of Aberdeen, Aberdeen AB9 1AS, United Kingdom. A. Mandelbaum and A. Etinger, Department of Chemistry, Technion–Israel Institute of Technology, Haifa 32000, Israel.

*Present address: Laboratory of Cell Biology, National Institute of Mental Health, Building 36, Room 3A-15, Bethesda, MD 20892.

†To whom correspondence should be addressed.

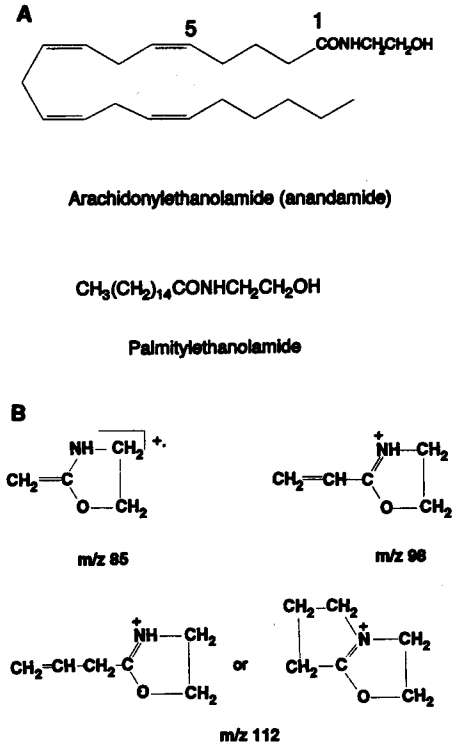


Fig. 4. Structures of anandamide and palmitylethanolamide (**A**) and the dihydro- and tetrahydrooxazole ion fragments (**B**) formed from these compounds on thermal dehydration under GC-MS conditions. The *m/z* 112 ion is formed only from palmitylethanolamide.

synaptosomal membranes in a manner typical of competitive ligands (*8*), with an inhibition constant (K_i) value (± standard error of the mean; $n = 3$) of 52 ± 1.8 nM (Fig. 1). In this system, the K_i of Δ^9-THC was 46 ± 3 nM (*8*).

To determine whether anandamide had cannabimimetic pharmacological activity, we investigated its ability to inhibit the twitch response of isolated murine vas deferentia. This assay was chosen because previous studies had shown it to be a suitable and sensitive model for investigating the mode(s) of action of psychotropic cannabinoids (*13*). Anandamide produced a concentration-dependent inhibition of the twitch response, decreasing twitch heights by 50% at a concentration of 90 nM (Fig. 2). The inhibition was not reversed by addition of the opioid antagonist naloxone at a concentration of 300 nM. Both the potency of anandamide and the degree of inhibition it produced in this functional assay were comparable to those observed in the receptor binding assay. However, like other bioassays for cannabinoids, the mouse vas deferens assay lacks specificity. Thus, although the naloxone experiment indicated that anandamide did not exert its inhibitory effect on the vas deferens through opioid receptors, we cannot firmly conclude that the inhibition was mediated through the cannabinoid receptor.

The structure of anandamide was established by mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy. A variety of MS measurements were performed on the purified material (*12*). Direct-exposure chemical ionization (isobutane-DCI) mass spectrum indicated a molecular weight of 347 (*m/z* 348 MH^+ ion). High-resolution MS measurements suggested the elemental composition $C_{22}H_{37}NO_2$ (*m/z* 347.2762), which is consistent with the presence of five double bonds or rings. Collision-induced dissociation (CID) measurements of the *m/z* 348 MH^+ ion (obtained under isobutane-DCI) gave rise to the following significant fragments: *m/z* 287, 245, 203, 62 (highest abundance), and 44. The only reasonable composition of the most abundant *m/z* 62 fragment ion is C_2H_8NO, which corresponds to protonated ethanolamine $HOCH_2CH_2NH_3^+$. The *m/z* 44 ion may be formed by dehydration of the *m/z* 62 fragment. The *m/z* 287 $[MH-61]^+$ fragment ion corresponds to the loss of ethanolamine (C_2H_7NO) from MH^+. Additional data were obtained from the GC-MS and CID measurements of the trimethylsilyl (TMS) derivative of the purified material (*14*). Together, these results suggested that anandamide is an ethanolamide of a tetraenic C_{20} fatty acid.

Supporting evidence for this general structure was found in the behavior of anandamide under GC-MS conditions (*12*). Thermal dehydration gave rise to *m/z* 329 $M^{+\cdot}$ ion upon electron ionization (EI) and to *m/z* 330 MH^+ ion under CI. Both self-CI *m/z* 330 MH^+ and *m/z* 329 $M^{+\cdot}$ were formed under EI conditions in an ion-trap instrument (*12*) (Fig. 3). A similar dehydration under GC-MS conditions was also observed for synthetic palmitylethanolamide (*15*), and it presumably leads to the formation of 2-oxazoline derivatives (*16*). The fragmentation patterns of the dehydration products of anandamide and palmitylethanolamide were similar in the low mass range of the EI mass spectra and included *m/z* 85 (McLafferty rearrangement ion) and *m/z* 98 (product of a γ-cleavage) (Fig. 4). The EI mass spectrum of dehydrated palmitylethanolamide exhibited an *m/z* 112 ion that corresponded to a δ-cleavage fragment. The absence of this ion from the EI mass spectrum obtained in the GC-MS analysis of anandamide suggests the presence of the first double bond in the tetraenic acid at position 5 (as in arachidonylethanolamide, which would not be expected to yield a δ-cleavage product) (Fig. 4).

Because of the small amount of natural anandamide available, we were able to record ^1H NMR spectra only (*17*). The peaks attributed to double-bond protons (δ 5.30 to 5.45, multiplet) were coupled with those of protons that have the chemical shifts of doubly allylic protons (δ 2.75 to 2.90, multiplet). Such doubly allylic protons are typically found in all-*cis*, nonconjugated polyunsaturated fatty acids such as linoleic and arachidonic acids (*18*). Three pairs of protons were observed between δ 2.01 and 2.27, which we attributed to two allylic methylene groups and one methylene group α to a carbonyl moiety. Only one methyl group was observed [0.88, t (triplet)]. The peaks observed for two protons at 3.42 (N-CH_2, t), two protons at 3.72 (O-CH_2, t), and two protons at 2.20 ($COCH_2$, t) were similar in chemical shifts and spin-coupling patterns to peaks observed in the NMR spectrum of synthetic palmitylethanolamide. The peaks for N-CH_2 and O-CH_2 were coupled.

A juxtaposition of the various analytical data led us to conclude that the structure of anandamide is that of arachidonyl ethanolamide [5,8,11,14-eicosatetraenamide, (*N*-2-hydroxyethyl)-(all-Z)]. This conclusion was confirmed by synthesis (*19*). Synthetic arachidonylethanolamide was identical with the natural product on TLC, NMR (300 MHz), and GC-MS (retention time and fragmentation pattern) (Fig. 3). Synthetic anandamide bound to the cannabinoid receptor with a K_i of 39 ± 5.0 nM ($n = 3$).

Classification of a substance as a neuromediator requires fulfillment of numerous criteria, such as receptor specificity and appropriate cellular localization. Although our present results, therefore, do not establish that anandamide is a neuromediator, it is noteworthy that three related lipids, palmitylethanolamide, arachidonic acid methyl ester, and phytanic acid, did not inhibit the binding of [^{3}H]HU-243 to the cannabinoid receptor in concentrations up to 1 μM (*15*). Howlett *et al.* (*20*) have investigated the effect of 136 natural and synthetic ligands for various receptors, including 24 eicosanoid derivatives, on binding of the cannabinoid probe [^{3}H]CP-55940 to rat brain membranes, with essentially negative results. These observations indicate that binding to the cannabinoid receptor by vertebrate constituents is thus far limited to anandamide. We have, however, detected additional porcine brain constituents that display cannabinoid-like activity in both the binding and the vas deferens assays. These constituents appear to differ from anandamide in potency, efficacy, and rate of onset of action (*15*).

The identification of anandamide as a potential ligand for the cannabinoid receptor reaffirms the biological importance of fatty acid amides and their derivatives. In 1957, palmitylethanolamide from egg yolk was shown to be an anti-inflammatory agent (*21*), and, in 1965, fatty acid amides of ethanolamine were shown to be endogenous products of mammalian brain tissue

(*22*). More recently, a fatty acid amide isolated from bovine mesentery was found to be angiogenic (*23*), and synthetic arachidonamide was found to inhibit leukotriene biosynthesis (*24*). Our results raise the possibility that anandamide is formed via an as-yet-uncharacterized route of arachidonic acid metabolism leading to compounds that act, at least in part, through the cannabinoid receptor.

Finally, our results may help to clarify the biological significance of previously reported interactions between cannabinoids and eicosanoids (*25*).

REFERENCES AND NOTES

1. Y. Gaoni and R. Mechoulam, *J. Am. Chem. Soc.* **86**, 1646 (1964).
2. W. A. Devane, F. A. Dysarz III, M. R. Johnson, L. S. Melvin, A. C. Howlett, *Mol. Pharmacol.* **34**, 605 (1988); W. A. Devane, thesis, St. Louis University, St. Louis, MO, (1989).
3. L. A. Matsuda, S. J. Lolait, M. J. Brownstein, A. C. Young, T. I. Bonner, *Nature* **346**, 561 (1990).
4. C. M. Gérard, C. Mollereau, G. Vassart, M. Parmentier, *Biochem. J.* **279**, 129 (1991).
5. M. Herkenham *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 1932 (1990); M. Herkenham *et al.*, *J. Neurosci.* **11**, 563 (1991).
6. R. G. Pertwee, *Pharmacol. Ther.* **36**, 189 (1988).
7. R. Mechoulam *et al.*, *Experientia* **44**, 762 (1988); R. Mechoulam, W. A. Devane, R. Glaser, in *Marijuana/Cannabinoids: Neurobiology and Neurophysiology*, L. Murphy and A. Bartke, Eds. (CRC Press, Boca Raton, FL, 1992), pp. 1–33.
8. W. A. Devane *et al.*, *J. Med. Chem.* **35**, 2065 (1992).
9. Porcine brains were homogenized in chloroform and/or methanol and centrifuged at 13,000*g*. The organic solvent extract was fractionated over a silica column (70 to 230 μm, Kieselgel 60, Merck), according to elution schemes used to separate the major classes of lipids [C. C. Sweeley, *Methods Enzymol.* **14**, 254 (1969); J. C. Dittmer and M. A. Wells, *ibid.*, p. 482].
10. The TLC plates (analytical, RP-18, Merck) were eluted with methanol-dichloromethane (4:1) and developed twice. The first solvent front was at 3.1 cm, and the second was at 7.4 cm. The R_f value was 0.65 [W. A. Devane, L. Hanuš, R. Mechoulam, *Proceedings of the Fifth Nordic Neuroscience Meeting*, Publ. Univ. Kuopio Med. (1991), p. 198]. Anandamide eluted from a silica column (Kieselgel 60, 40 to 63 μm, Merck) with methanol-chloroform (2:98). It eluted from a reversed-phase column (RP-C_{18}, 40 to 63 μm, Sigma) with methanol-water (88:12).
11. The term "anandamide" was coined from the Sanskrit word "ananda," meaning bliss, and from the chemical nature of the compound.
12. GC-MS analyses were carried out with a Finnigan ITS-40 system and with a Finnigan TSQ-70B triple-stage quadrupole mass spectrometer coupled to a Varian 3400 gas chromatograph. Separations were performed on a DB-5 (0.25-μm film) capillary column that was 30 m in length and had an internal diameter (i.d.) of 0.25 mm. The column temperature was programmed to increase from 60 to 280°C at a rate of 20°C per minute. The compounds were injected into the GC in methylene chloride. The electron energy in EI measurements was 70 eV with one scan per second. The isobutane DCI measurements were carried out with a TSQ-70B mass spectrometer under standard conditions. High-resolution mass spectral measurements were performed with a Varian-MAT 711 double-focusing mass spectrometer. The CID measurements were carried out with the TSQ-70B triple-stage mass spectrometer. The collision energy was 50 eV, and argon was used as the target gas at an indicated pressure of 0.4 mtorr.
13. R. G. Pertwee, L. A. Stevenson, D. B. Elrick, R. Mechoulam, A. D. Corbett, *Br. J. Pharmacol.* **105**, 980 (1992).
14. The GC-MS and CID measurements of the TMS derivative of anandamide gave m/z 419 M^+ and 404 $[M\text{-}CH_3]^+$ fragment ions, which indicated formation of a mono-TMS ether. The CID spectrum of the m/z 404 ion exhibited two major fragment ions at m/z 118 and 172, corresponding to $Me_2Si^+OCH_2CH_2NH_2$ and $Me_2Si^+OCH_2CH_2NHCOCH{=}CH_2$, respectively.
15. W. A. Devane *et al.*, unpublished data.
16. H. Wenker, *J. Am. Chem. Soc.* **57**, 1079 (1935).
17. The one- and two-dimensional proton NMR spectra (in $CDCl_3$) were obtained on a Varian VXR300s spectrometer equipped with a 5-mm computer switchable probehead and on an AMX 400.133 Bruker spectrometer with a 5-mm reverse probehead. The chemical shifts are presented in parts per million. The Bruker library program (NOESY) was used for the two-dimensional measurements.
18. W. W. Christie, *Lipid Analysis* (Pergamon, Oxford, ed. 2, 1982), pp. 81–82; D. J. Frost and J. Barzilay, *Anal. Chem.* **43**, 1316 (1971).
19. Arachidonyl chloride, which was prepared from arachidonic acid and oxalyl chloride according to a published procedure (*24*), was dissolved in methylene chloride and added at 0°C under a nitrogen atmosphere to ethanolamine. The ethanolamine was present in a tenfold molar excess and was also dissolved in methylene chloride. After 15 min the reaction mixture was washed with water, dried, and the product purified by silica column chromatography (eluted with 2% methanol in chloroform) to give arachidonylethanolamide (an oil, ~90% yield) that was 97% pure as judged by GC-MS.
20. A. C. Howlett, D. M. Evans, D. B. Houston, in *Marijuana/Cannabinoids: Neurobiology and Neurophysiology*, L. Murphy and A. Bartke, Eds. (CRC Press, Boca Raton, FL, 1992), pp. 35–72.
21. F. A. Kuehl, Jr., T. A. Jacob, O. H. Ganley, R. E. Osmond, M. A. P. Meisinger, *J. Am. Chem. Soc.* **79**, 5577 (1957).
22. N. R. Bachur, K. Masek, K. L. Melmon, S. Udenfriend, *J. Biol. Chem.* **240**, 1019 (1965).
23. K. Wakamatsu, T. Masaki, F. Itoh, K. Kondo, K. Sudo, *Biochem. Biophys. Res. Commun.* **168**, 423 (1990).
24. E. J. Corey, J. R. Cashman, S. S. Kantner, S. W. Wright, *J. Am. Chem. Soc.* **106**, 1503 (1984).
25. S. H. Burstein, in (*20*), pp. 73–91 and references therein; R. G. Pertwee, *ibid.*, pp. 165–218; J. W. Fairbairn and J. T. Pickens, *Br. J. Pharmacol.* **72**, 401 (1981); A. Sklenovsky, J. Navratil, Z. Chmela, Z. Krejči, L. Hanuš, *Acta Univ. Palacki. Olomuc. Fac. Med.* **122**, 83 (1989).
26. P. J. Munson and D. Rodbard, *Anal. Biochem.* **107**, 220 (1980).

27. Supported by grants to R.M. from the National Institute for Drug Abuse (DA 6481) and the Amdursky Fund of the Friends of the Hebrew University and by a grant to R.G.P. from the Wellcome Trust. We thank I. Ringel (Jerusalem) for the initial NMR work, S. Levin (Jerusalem) for advice on chromatography, and J. Katzir and A. Idina (Haifa) for dedicated technical help.

1 September 1992; accepted 6 November 1992

the FEBS Journal

MINIREVIEW

Modulating the endocannabinoid system in human health and disease – successes and failures

Pál Pacher and George Kunos

Laboratory of Physiologic Studies, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD, USA

Keywords
cannabinoids; clinical trials; disease; endocannabinoid system; human; pharmacology; therapeutic potential

Correspondence
P. Pacher, Laboratory of Physiological Studies, National Institutes of Health/ NIAAA, 5625 Fishers Lane, MSC-9413, Bethesda, MD 20892-9413, USA
Fax: +1 301 480 0257
Tel: +1 301 443 4830
E-mail: pacher@mail.nih.gov

(Received 14 January 2013, revised 11 March 2013, accepted 19 March 2013)

doi:10.1111/febs.12260

The discovery of the endocannabinoid system, comprising the G-protein coupled cannabinoid 1 and 2 receptors ($CB_{1/2}$), their endogenous lipid ligands or endocannabinoids, and synthetic and metabolizing enzymes, has triggered an avalanche of experimental studies implicating the endocannabinoid system in a growing number of physiological/pathological functions. These studies have also suggested that modulating the activity of the endocannabinoid system holds therapeutic promise for a broad range of diseases, including neurodegenerative, cardiovascular and inflammatory disorders; obesity/metabolic syndrome; cachexia; chemotherapy-induced nausea and vomiting; and tissue injury and pain, amongst others. However, clinical trials with globally acting CB_1 antagonists in obesity/metabolic syndrome, and other studies with peripherally-restricted $CB_{1/2}$ agonists and inhibitors of the endocannabinoid metabolizing enzyme in pain, have introduced unexpected complexities, suggesting that a better understanding of the pathophysiological role of the endocannabinoid system is required to devise clinically successful treatment strategies.

Introduction

Although *Cannabis sativa* (the marijuana plant) is one of the most ancient medicinal plants in the history of medicine [1], the clinical use of synthetic cannabinoids or medicinal plant extracts has been largely empirical and limited to a few specific indications related to pain, wasting disorders, and chemotherapy-induced nausea and vomiting, as a result of their socially undesirable psychoactive properties [2]. The discovery of endocannabinoids (ECs), which mimic some of the effects of synthetic cannabinoids *in vivo*, their G-protein coupled receptors, as well as their synthetic and metabolizing enzymes, has prompted preclinical studies aiming to explore the role of the endocannabinoid system (ECS) in health and disease [2–4]. These studies have been greatly facilitated by the introduction of mice deficient in cannabinoid receptors or EC degrading enzymes, as well as selective cannabinoid receptor ligands and inhibitors of EC metabolism. The results of these studies have implicated the ECS in a variety of physiopathological processes, both in the peripheral and central nervous systems and in various peripheral organs [2]. Such studies have further suggested that modulating ECS activity may have therapeutic potential in almost all diseases affecting humans, including obesity/metabolic syndrome [5]; diabetes and diabetic complications [6]; neurodegenerative [7,8], inflammatory [9], cardiovascular [10–12], liver [13,14], gastrointestinal [15] and skin [16] diseases; pain [17,18]; psychiatric disorders [19,20]; cachexia [2]; cancer [21,22]; and chemotherapy-induced nausea and vomiting [23], amongst many others [2].

Abbreviations
2-AG, 2-arachidonoylglycerol; AEA, anandamide or arachidonoyl ethanolamide; $CB_{1/2}$, cannabinoid receptor 1 or 2; CBD, cannabidiol; CNS, central nervous system; EC, endocannabinoid; ECS, endocannabinoid system; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; MS, multiple sclerosis; THC, Δ9-tetrahydrocannabinol; $TRPV_1$, transient receptor potential cation channel subfamily V member 1.

These investigations have also uncovered the remarkable complexity of the ECS, as exemplified by differences in the therapeutic profile of activating/inhibiting the same receptor in the central nervous system (CNS) or in peripheral tissues, by the intriguing overlap between EC and eicosanoid signalling, or by the often opposite effects mediated by cannabinoid 1 and 2 receptors ($CB_{1/2}$) receptors in disease models [2–4,6,24]. Similar complexities have emerged in clinical trials targeting the ECS. Although globally acting (i.e. brain-penetrant) CB_1 antagonists/inverse agonists were shown to have therapeutic efficacy in obesity/metabolic syndrome, they elicited anxiety/depression in a small proportion of subjects, which has led to their withdrawal from the market worldwide and halted their further therapeutic development [5,25,26]. The first human trial with peripherally-restricted mixed $CB_{1/2}$ agonist(s) for pain failed as a result of cardiovascular and metabolic side effects and hepatotoxicity [27,28]. Amplifying the ECS tone by inhibiting EC metabolism was ineffective in alleviating osteoarthritic pain in human subjects [29,30]. Thus, we need to better understand the pathophysiological function of the ECS in humans, as well as refine the indications and design of clinical trials, so that it is possible to successfully translate recent progress in cannabinoid biology into clinically effective treatment strategies.

The present minireview discusses preclinical evidence implicating the ECS in human disease, and reviews the treatment strategies that target the ECS for therapeutic gain in humans. Because of limitations of space, reference is also made to recent overviews on specific subjects, rather than to original papers.

The ECS

Δ9-Tetrahydrocannabinol (THC), the putative psychoactive ingredient of marijuana, and its endogenous counterparts, anandamide (arachidonoyl ethanolamide) (AEA) and 2-arachidonoylglycerol (2-AG), exert their primary effects through $CB_{1/2}$ receptors; 2-AG favours CB_2, whereas AEA binds with higher affinity to CB_1 [2], although, at higher concentrations, it may also modulate transient receptor potential cation channel subfamily V member 1 ($TRPV_1$) and other receptors. Signalling by cannabinoid receptors is complex because it may involve both G protein-dependent pathways, such as inhibition of adenyl cyclase or the modulation of ion channel function, and G protein-independent mechanisms, including the activation of various mitogen-activated protein kinases (p44/42 mitogen-activated protein kinases, p38, extracellular signal-regulated kinase and c-Jun N-terminal kinase) or ceramide signalling [2,31,32].

CB_1 receptors, the most abundant G-protein coupled receptor in the mammalian brain, mediate the socially undesirable psychoactive effects of cannabis. Although their expression was initially considered to be restricted to the brain, more recent studies have identified CB_1 receptors in almost all peripheral tissues and cell types, albeit at much lower densities than in the brain, and documented their important regulatory functions [2,3,5]. CB_2 receptors are largely restricted to immune and haematopoetic cells, although functionally relevant expression has been found in specific regions of the brain and in the myocardium, gut, endothelial, vascular smooth muscle and Kupffer cells, exocrine and endocrine pancreas, bone, and reproductive organs/cells, as well as in various tumours [4]. Both cannabinoid receptors may undergo rapid internalization and intracellular trafficking upon agonist exposure [33,34].

In the CNS, AEA and 2-AG are synthesized 'on demand' and released to act as retrograde transmitters on CB_1 receptors [35–37]. They are not stored and are rapidly degraded after exerting a transient and localized effect [38]. The synthesis of ECs largely depends on the intracellular Ca^{2+}-concentration. AEA is mainly formed via a two step-pathway, involving a Ca^{2+}-dependent *N*-acyltranferase and *N*-acylphosphatidylethanolamine-hydrolyzing phospholipase D, whereas diacylglycerol lipase and phospholipase Cβ are mainly responsible for the biosynthesis of 2-AG [3,37]. The existence of additional, parallel biosynthetic pathways for AEA has also been proposed [39,40].

AEA and 2-AG are removed from the extracellular space by a process of cellular uptake and metabolism; however, the putative transporter(s) involved have not yet been cloned, and are the subject of much controversy [41–43]. AEA is degraded primarily by fatty acid amide hydrolase (FAAH) and 2-AG is degraded by monoacylglycerol lipase (MAGL) [3,44], although additional enzymes have also been implicated in the degradation of both AEA and 2-AG [45,46]. Endocannabinoids may also be metabolized by cyclooxygenases, lipooxygenases and cytochrome P450, leading to the formation of bioactive metabolites that may activate CB receptor-independent mechanisms [24,47]. It is also important to note that FAAH and MAGL are also responsible for the degradation of numerous potentially bioactive lipids. Thus, the biological consequences of the inhibition of these enzymes are not necessarily a result of enhanced EC levels. Some of the enzymes involved in EC synthesis/degradation may exist in several forms and their activity may vary in

different tissues or even in different regions of the same tissue [3,37,48–52].

In addition to AEA and 2-AG, several other EC-like molecules have been discovered, although their activities have not been studied in sufficient detail [53,54]. Interestingly, recent studies have identified novel peptide allosteric negative modulators of CB_1 receptors [55], the biological significance of which is yet to be determined. Additionally, the anti-inflammatory lipid lipoxin A4 may be an endogenous allosteric enhancer of CB_1 receptors [56]. A comprehensive overview of the ECS is beyond the scope of the present minireview; instead, several detailed reviews are available on this subject [3,24,37,57].

The ECS in health and disease

Despite the ubiquitous expression of the various components of the ECS, their genetic ablation or pharmacological blockade in normal, healthy animals has minimal functional consequences, which suggests that the ECS has minimal or no tonic activity under normal physiological conditions [2,4]. On the other hand, an increase or decrease in ECS tone is associated with various pathological states, as a result of the altered expression of CB receptors, endocannabinoid metabolizing enzymes and/or synthetic pathways, in a tissue-specific and time-dependent manner. Examples of selected pathologies in which dysregulation of the ECS was reported (in most cases, up-regulation of $CB_{1/2}$ and/or an increase in tissue levels of ECs) are shown in Table 1, and have been summarized in more detail elsewhere [2–4,58,59]. In some cases, altered ECS activity is transient and forms part of the body's compensatory response to a particular insult, thus reducing symptoms and/or slowing progression of the disease (e.g. in neuropathic pain); in other cases, activation of the ECS may be pathogenic (e.g. in various forms of shock or diabetic complications) or may reflect a deficiency (e.g. in various tumours) of unknown significance [2].

From a therapeutic standpoint, the identification of regional or tissue-specific changes in CB receptors is important because their possible selective targeting may mitigate unwanted side effects [59,60]. However, these changes can serve as a basis for successful drug development only as long as they are determined using appropriate tools (e.g. specific antibodies), the specificity of which needs to be carefully validated [4,61]. It is also very important to understand the underlying mechanisms of these alterations; for example, is the increase in the tissue level of an EC the result of its increased biosynthesis or a decrease in its enzymatic degradation?

Cardiovascular consequences of targeting the ECS in health and disease

Because many promising drugs fail in clinical development as a result of cardiovascular side effects, it is important to briefly overview the cardiovascular consequences of modulating the ECS. ECs exert complex cardiovascular effects that are dominated by a decrease in blood pressure and myocardial contractility, mediated primarily by CB_1 receptors located in the myocardium, vasculature and neurones in the central and autonomic nervous systems [2,62]. In cultured human coronary artery endothelial cells [63] and cardiomyocytes [64], CB_1 activation promotes stress signalling and cell death, and decreases contractility [10,12]. By contrast, activation of cardiovascular CB_2 receptors does not have adverse haemodynamic consequences [11]. CB_1, CB_2 or FAAH knockout mice have normal blood pressure, myocardial contractility and/or baroreflex sensitivity, indicating the minimal role of the ECS in normal cardiovascular regulation [2]. However, in several pathological conditions (e.g. shock, heart failure, cardiomyopathies, advanced liver cirrhosis), the ECS may become activated to promote hypotension/cardiodepression through cardiovascular CB_1 receptors [2,10]. CB_1 receptor signalling may also promote disease progression in preclinical models of heart failure [64–66] and atherosclerosis [67,68], and contributes to increased cardiovascular risk (e.g. plasma lipid alterations, abdominal obesity, hepatic steatosis, insulin and leptin resistance) in obesity/metabolic syndrome and diabetes, both in rodents and humans [5,69–71]. By contrast, CB_2 signalling in the heart and vasculature may activate cardioprotective mechanisms and limit inflammation [11].

Acute or chronic use of marijuana may decrease or increase the heart rate and decrease blood pressure depending on the duration of the use, dose and route of administration [2,10]. An elevated resting heart rate is a known independent risk factor for cardiovascular disease in healthy men and women [72]. A recent controlled study at the National Institute on Drug Abuse evaluated the development of tolerance to the effects of oral synthetic THC in 13 healthy male daily cannabis smokers who were residing on a secure research unit over a period of 6 days [73]. Despite the development of tolerance to the subjective intoxicating effect of THC, no tolerance was observed to its hypotensive and tachycardic effects [73]. Another recent study of 72 young male cannabis users and 72 matched controls reported an increased heart rate variability in cannabis users [74]. Surinabant, a selective CB_1 antagonist, has

Table 1. Examples of the dysregulation of the ECS in disease. C, canine; H, human; P, pig; R, rodent. ND, not determined.

Disease, sample	Expression/changes in $CB_{1/2}$	Changes in endocannabinoid levels	Proposed role of CB receptors in disease	Reference
Myocardial infarction (ischaemia-reperfusion injury) (R, P, H)	Myocardium, in human epicardial adipose tissues of ischaemic hearts, up-regulation of CB_1 and protein kinase A, accompanied by CB_2 and FAAH down-regulation, increased inducible NOS/endothelial NOS ration and reduced cell survival signalling	Increase in circulating immune cells or in serum of obese patients with adverse cardiovascular events; Elevated endocannabinoid plasma levels are strongly associated with coronary dysfunction in obese human subjects	CB_2: decrease in leukocyte infiltration and enhancement of pro-survival pathways; CB_1: contribution to cardiovascular dysfunction, cell death/dysfunction in human endothelial cells and cardiomyocytes; central hypothermia (the latter is only in rodents and can be protective)	11,12,76, 85–87,90, 184–187
Heart failure, cardiomyopathies (R, H)	Myocardium, cardiomyocytes, endothelial cells	Myocardium, cardiomyocytes, circulating immune cells and platelets	CB_2: attenuation of inflammation/injury; CB_1: promotion of cardiac dysfunction and cell death in cardiomyocytes and endothelial cells	64,65,186, 188–192
Atherosclerosis, restenosis (R, H)	Infiltrating and other immune cells, vascular smooth muscle and endothelium	Serum, atherosclerotic plaques	CB_2: context-dependent attenuation or promotion of vascular inflammation (monocyte chemotaxis, infiltration and activation) and factors of plaque stability; attenuation of vascular smooth muscle proliferation; CB_1: increase of vascular inflammation and/or plaque vulnerability	67,84,133,134, 193–198
Stroke, spinal cord injury (R, H)	Brain, microglia, infiltrating immune cells, endothelium	Serum, brain	CB_2: attenuation of inflammation (endothelial activation, leukocyte infiltration), and tissue injury, attenuation of motor and autonomic deficits in a mouse model of spinal cord injury; CB_1: promotes hypothermia-dependent protection but, if hypothermia is compensated, ineffective or enhances injury	90,199–206
Cirrhotic cardiomyopathy (R, H)	ND	Myocardium, circulating immune cells and platelets	CB_2: attenuation of hypotension by decreasing liver inflammation; CB_1: contribution to cardiovascular dysfunction	189–192
Septic shock by live bacteria (R, H)	ND	Serum	CB_2: decrease or increase in inflammation and tissue injury most likely by affecting bacterial load; CB_1: contribution to cardiovascular collapse	10,207–210
Hepatic ischaemia-reperfusion injury (R, P, H)	Inflammatory immune cells, activated endothelium	Liver, serum, hepatocytes, Kupffer and endothelial cells	CB_2: attenuation of inflammation (endothelial activation, leukocyte chemotaxis, infiltration and activation), oxidative stress, and tissue injury; CB_1: promotion of liver injury	135,138,211–213
Obesity, non-alcoholic fatty liver disease, diabetic complications (R, H)	Hepatocytes, inflammatory cells, adipocytes, certain neurones, sites of diabetic complications (kidneys, retina and myocardium)	Liver, adipose tissue, brain, skeletal muscle, diabetic kidneys, hearts, retinas, serum	CB_2: enhancement of high fat diet-induced steatosis and inflammation or attenuation of obesity associated one with age; CB_1: increase in fat storage, decrease in metabolism, promotion of insulin and leptin resistance and inflammation in adipose tissue and in the liver	5,6,70,101,108, 214–221

Table 1. (Continued).

Disease, sample	Expression/changes in $CB_{1/2}$	Changes in endocannabinoid levels	Proposed role of CB receptors in disease	Reference
Liver fibrosis, cirrhosis, alcohol-induced liver injury (R, H)	Activated stellate cells, inflammatory cells, hepatocytes, Kupffer cells	Liver, serum, inflammatory cells	CB_2: attenuation of fibrosis and injury/inflammation; CB_1: increase in fibrosis/injury	14,136,137,191, 222,223
Pancreatitis (R, H)	Pancreas	Inflamed pancreas	CB_2: attenuation of inflammation; CB_1: context-dependent effect	145,146,148,224
Inflammatory bowel disease, colitis, diverticulitis (R, H)	Epithelial cells, infiltrating inflammatory cells, enteric nerves	Inflamed gut	Attenuation of inflammation and visceral sensitivity	130,151,225–229
Nephropathy (R, H)	Kidney, human proximal tubular cells, podocytes	Kidney	CB_2: attenuation of inflammation (chemokine signalling and chemotaxis, inflammatory cell infiltration and endothelial activation) and oxidative stress; CB_1: promotion of inflammation/injury	105,219,220, 230–233
Neurodegenerative/ neuroinflammatory disorders (multiple sclerosis, Alzheimer's, Parkinson's and Huntington's disease, spinal cord injury) (R, H)	Microglia, inflammatory cells, brain lesions, neurones?	Brain, spinal fluid	CB_2: attenuation of inflammation (microglia activation, secondary immune cell infiltration), facilitation of neurogenesis; CB_1: attenuation of excitotoxicity, hypothermia; context-dependent effect on injury/inflammation	2,7,91,92,152, 205,234–250
Pain (R)	Inflammatory cells, certain neurones	Site of induced chronic inflammatory pain	CB_2: attenuation of inflammatory pain via unknown mechanism(s); CB_1: attenuation of various forms of pain by inhibiting neurotransmission	17,95,96,251–266
Psychiatric disorders (anxiety and depression, schizophrenia) (R, H)	Glial, inflammatory cells, neurones?	Blood, cerebrospinal fluid, brain (increased in schizophrenia, but decreased in brain in depression)	CB_2: largely unexplored, in rodent models of depression/anxiety, it may modulate CNS inflammation and either attenuate or promote anxiety like behaviour; CB_1: context-dependent effect on anxiety, improved sleep	19,267–277
Rheumatoid arthritis (H)	ND	Synovial fluid, synovia	CB_2: attenuation of the autoimmune inflammatory response; CB_1: attenuation of pain	278
Cancer (R, H)	In various tumours or cancer cells	Various tumours	$CB_{1/2}$: context-dependent attenuation or promotion of tumour growth (apoptosis, angiogenesis, proliferation, etc.)	279–282, 2,22,149, 155,157

recently been reported to inhibit THC-induced central nervous system and heart rate effects in humans, providing proof of principle that those effects were indeed mediated by CB_1 receptor activation [75]. At the 20th International Cannabinoid Research Society meeting in Sweden, AstraZeneca presented data from the first clinical studies investigating two novel, peripherally-restricted, orally active mixed $CB_{1/2}$ agonists (AZD1940 and AZD1704). The study was terminated as a result of adverse cardiovascular effects, weight gain and mild hepatotoxicity [27,28].

An increasing number of case reports associates marijuana smoking with the precipitation of acute coronary syndrome [76]. Alarmingly, this occurs mostly in young healthy subjects without any previous cardiovascular disease [77,78]. A retrospective study assessed the risk of acute coronary syndrome after exposure to marijuana smoke. It was found that the risk of myocardial infarction was highest during the first hour of exposure [79]. The effect of marijuana use on mortality after acute myocardial infarction was assessed in a prospective study involving 1913 adults who were hospitalized with myocardial infarction at 45 US hospitals between 1989 and 1994, with a median follow-up of 3.8 years. The results indicated that marijuana use may pose an increased risk of infarction in susceptible individuals with coronary heart disease [80]. A more recent study evaluated the consequences of marijuana use and long-term mortality among survivors of acute myocardial infarction, and found that habitual marijuana use among patients presenting with acute myocardial infarction was associated with an apparent increase in mortality rate (29% higher) over the subsequent 18 years, although this did not reach statistical significance because of the limited sample size [81]. In the absence of large-scale, long-term controlled studies with repeated measures of marijuana use, a firm conclusion on the long-term impact of cannabis use on cardiovascular mortality cannot be drawn. Nevertheless, the above findings are of concern. Because THC is a relatively weak CB_1 agonist compared to many synthetic ligands, and also activates cardioprotective CB_2 receptors and is a potent antioxidant, it may be predicted that the uncontrolled spread and use of mixtures of potent synthetic CB_1 agonists (spice, K2, etc.) employed as recreational drugs would lead to significantly greater cardiovascular morbidity. Indeed, in a recent case series in healthy children, myocardial infarction was precipitated by synthetic cannabinoid use [82], and another study reported tachycardia, loss of consciousness and diffuse pain in two adolescents [83].

What is the situation regarding the ECS and cardiovascular pathology? As noted previously, EC/CB_1 receptor signalling has been implicated as a pathogenic factor in rodent models of cardiovascular diseases, including atherosclerosis, shock and various forms of cardiomyopathy. However, ECs were also reported to exert protective effects, based mostly on *ex vivo* and indirect studies, via CB_2 and CB-receptor independent mechanisms. Clearly, selective CB_2 agonists exert beneficial effects in rodent models of myocardial infarction by limiting inflammatory cell infiltration (in cardiomyocytes, the expression of CB_2 is very low, if any) [11]. To analyze the role of the ECS more directly, a recent study employed FAAH knockout mice with a 2.5- to three-fold increase in myocardial AEA content. When such mice were used to induce various experimental models of cardiomyopathy, they displayed increased mortality, tissue injury and neutrophil infiltration in the heart, which could be partially rescued by CB_1 antagonists [66]. Consistent with this report, a recent study showed that FAAH deficiency enhanced intraplaque neutrophil recruitment in atherosclerotic mice and increased a pro-inflammatory immune response [84]. These findings indicate that the primary cardiovascular effects of elevated EC tone are deletorious and are mediated by CB_1 receptors.

In obese human subjects, increased plasma levels of AEA and 2-AG were strongly associated with coronary circulatory dysfunction, suggesting that plasma EC levels may be used as biomarkers of cardiovascular risk in obesity [85]. In another study, increased plasma AEA and 2-AG levels positively correlated with impaired coronary endothelial function in obese subjects [86]. In samples of epicardial fat from ischaemic human hearts, the up-regulation of CB_1 was accompanied by down-regulation of CB_2 and FAAH compared to non-ischaemic hearts [87]. CB_1 receptor density was significantly higher in atherosclerotic coronary artery sections from patients with unstable angina compared to those with stable angina [67]. A G1359A polymorphism in the CB_1 receptor gene was also associated with coronary artery disease in the Chinese Han population, although the effect of this polymorphism on receptor function is unknown [88]. Both ECs were reported to inhibit human cardiac Kv4.3 channels at fairly low concentrations in ovary cells expressing Kv4.3 or in human cardiomyocytes in a receptor-independent manner [89], a harbinger of pro-arrhythmic risk.

Thus, it is clear that the activation of CB_1 receptors by synthetic ligands or ECs is associated with adverse cardiovascular consequences, which must be given very careful consideration during the preclinical/clinical development of new drugs targeting the ECS.

Activation of $CB_{1/2}$ receptors: THC, synthetic agonists and cannabinoid extracts

THC (dronabinol; Marinol; Solvay Pharmaceuticals, Brussels, Belgium) and its synthetic analogue nabilone (Cesamet; Valeant Pharmaceuticals, Irvine, CA, USA) have been approved by the Food and Drug Administration for treatment of chemotherapy-induced nausea and vomiting and for stimulating appetite in wasting disorders (e.g. AIDS, tumour cachexia, etc). Sativex (GW Pharmaceuticals, Salisbury, Wiltshire, UK), an oromucosal spray containing THC and the nonpsychoactive plant cannabinoid, cannabidiol (CBD), has recently been approved in Canada, the UK and several other European countries for the symptomatic relief of neuropathic pain and spasticity associated with multiple sclerosis, and as an adjunctive analgesic treatment for adults with advanced cancer. However, the therapeutic utility of THC and its synthetic analogues are limited because of their unwanted psychotropic effects mediated by central CB_1 receptors. The present minireview summarizes only the clinically most relevant indications.

Earlier preclinical studies suggested that ECs or plant-derived cannabinoids exert neuroprotective effects in the CNS by: (a) modulating excitability and calcium homeostasis via effects on various ion channels (Ca^{2+}, Na^+, K^+), intracellular Ca^{2+} stores and gap junctions and *N*-methyl-D-aspartate receptors; (b) attenuating excitatory glutamatergic transmissions and modulating synaptic plasticity via presynaptic CB_1 receptors; (c) inducing CB_1 receptor-mediated hypothermia; (d) exerting antioxidant effects; and (e) modulating immune responses and the release of proinflammatory mediators by CB_1, CB_2 and non-CB_1/CB_2 receptors on microglia, astrocytes, macrophages, neutrophils, lymphocytes and neurones [2]. Numerous recent studies have suggested that many of the previously described protective effects of synthetic CB_1 ligands were attributable to centrally-mediated hypothermia and/or receptor-independent antioxidant/anti-inflammatory effects of the compounds, and that ECs through the activation of CB_1 receptors may also promote tissue injury and neurodegeneration (e.g. in stroke and other forms of I/R injury) [6,90–92].

Historical documents reveal that one of the earliest uses of cannabis was to treat pain [93]. Studies in modern times initially focused on CB_1 receptors and demonstrated beneficial effects of cannabinoids in rodent models of acute and chronic pain. The results suggested that the observed antinociceptive effects have complex mechanisms involving actions in the CNS, spinal cord and peripheral sensory nerves [2,94]. Recent evidence also implicates CB_2 receptors in the antihyperalgesic activity of cannabinoids [95,96]; however, the exact mechanisms and cellular targets are elusive because of a lack of reliable antibodies for CB_2 [4].

In humans, the analgesic activity of THC and other cannabinoids is less clear-cut because cannabinoids are relatively weak analgesics compared to opiates, even when they do show efficacy [2]. The clinical data on THC, CBD and their combinations have been comprehensively reviewed elsewhere [97,98]. The primary focus of these studies has been the safety/efficacy and symptom relief (e.g. bladder incontinence, limb spasticity, pain and sleep quality) in multiple sclerosis (MS) or other pain-related conditions. Three studies have demonstrated that cannabis extract in MS patients improved urinary incontinence [98]. A number of controlled and blinded trials evaluating the efficacy of oral or sublingual cannabis/Sativex on spasticity in MS found that, at doses lacking overt psychoactivity, these drugs show no or minimal efficacy, as assessed by the objective outcomes using the Ashworth scale. However, the treatment consistently improved subjective, patient-assessed endpoints (spasms, pain, spasticity, sleep quality). Follow-up studies using a patient assessed numeric rating scale for spasticity showed significant benefits of Sativex compared to placebo [98]. It could be argued that some of the benefits observed were a result of mood improvement (patients feel subjective improvement) but, because only some of the symptoms were improved (spasticity, pain and sleep quality), this may not be the case. In patients treated with THC for 1 year, improvements using the Ashworth scale were reported [98]. Zhornitsky and Potvin [97] performed a meta-analyses of the data from 33 studies with CBD alone or in various combinations with THC, with the rationale for combining THC and CBD being to attenuate the psychoactive effects of THC by CBD, based on empirical evidence obtained in some studies. Among these studies, 16 had been conducted in healthy subjects and 17 in clinical populations, including four in MS, three in neuropathic and cancer pain, four in schizophrenia and bipolar mania, two in social anxiety disorder, and one each in cancer-related anorexia, Huntington's disease, insomnia and epilepsy [97]. It was concluded that, depending on the study and on the THC/CBD ratio, CBD may prolong/intensify or inhibit THC-induced effects. In some of these studies, THC or CBD+THC was more effective at reducing pain, although, in other studies, CBD alone also exerted (or completely lacked) analgesic properties. Notably, several of these studies used

multiple pain assessment scores, and the treatments were effective when evaluated by some but not by other scales [97]. In one of the studies in which the oral administration of CBD+THC in MS was not effective in improving symptoms, immunological analysis unexpectedly revealed a certain pro-inflammatory effect of the drug [97]. The preliminary clinical evidence was concluded to suggest that high-dose oral CBD may have therapeutic benefits in social anxiety disorder, insomnia and epilepsy, although it may also cause mental sedation [97].

Taken together, the above studies in MS show consistent improvements in subjective rather than quantitative symptomatic outcome measures (including pain), which supports the beneficial effects of cannabinoid-based medicines in neuropathic pain associated with MS. The relatively poor efficacy observed in some clinical studies may be attributable to pharmacokinetic problems such as first-pass effects via the liver and slow absorption via the oral route of administration, which may also limit the success of self-titration [98]. In most of these studies, formulations containing THC frequently caused generally mild to moderate side effects. However, with individual dose-titration, which can be better achieved by using the oromucosal Sativex spray, side effects can be further attenuated. Initial dose-titration may also help in the early selection of responders and exclusion of nonresponders. Future clinical studies should explore how cannabinoid-based medicines affect MS progression. In light of the preclinical data, the combination of THC with CBD appears to be the most promising, given the neuroprotective effects of CBD observed in numerous preclinical studies [99].

There is considerable interest in developing THC-based medicines for other forms of pain, such as pain associated with cancer or diabetic neuropathy. However, under these conditions, we should also carefully weigh the potential effect of the treatment on cancer and/or diabetes progression. Regarding cancer, although numerous studies suggest that THC may slow down the growth/progression of certain types of cancers in preclinical models, others suggest that THC may in fact promote cancer growth, and cannabinoid receptor deletion or inhibition is beneficial [2,4,22]. In addition, the results of a clinical study evaluating the association between ECS activity and survival and pain in pancreatic cancer indicate that, although patients with high CB_1 receptor expression in enlarged nerves in pancreatic ductal adenocarcinoma had a lower combined pain score (intensity, frequency, duration), they had significantly shorter survival [100]. For CBD, the evidence more clearly suggests potential benefits in multiple preclinical tumour models [99]. In the case of diabetes and diabetic complications, there is strong evidence (both preclinical and clinical) indicating that CB_1 activation promotes primary diabetes and also contributes to all diabetic complications (including neuropathy), and that CB_1 antagonists can prevent or reverse these changes, as well as insulin resistance [6,69,101].

Interestingly, analysis of cross-sectional data from the National Health and Nutrition Examination Survey (NHANES III, 1988–1994) indicated that marijuana use was independently associated with a lower prevalence of diabetes mellitus [102], and glucose tolerance and insulin sensitivity were found to be unchaged in chronic marijuana smokers [103]. In view of the demonstrated ability of acute marijuana smoking to induce insulin resistance [104], these findings may reflect desensitization of peripheral CB_1 receptors in chronic users. Further clinical studies are needed to analyze the differential mechanisms involved in the acute and chronic effects of marijuana use on glycaemic control.

Nevertheless, in light of the overwhelming preclinical and clinical evidence suggesting that CB_1 receptor activation contributes to diabetes development and its complications (cardiovascular, neuropathy, retinopathy, and nephropathy) [6], and a recent study by the Centers for Disease Control and Prevention associating cases of acute kidney injury with synthetic cannabinoid use [105], the use of THC would be risky from a clinical point of view in patients with established diabetes. Diabetic patients also have impaired immune functions and wound healing, which could be adversely affected by immunosuppressive/immunomodulatory drugs such as THC. By contrast, CBD demonstrated beneficial effects as a result of its anti-inflammatory and antioxidant properties both in preclinical models of primary diabetes and in models of all major diabetic complications, which is encouraging for its potential testing in diabetic patients [6].

As noted above, THC and its synthetic analogue Nabilone are used to treat chemotherapy-induced nausea and vomiting, as well as to stimulate appetite in cachexia associated with AIDS or terminal tumours [2]. In the case of AIDS, recent controlled studies in nonhuman primates showed unexpectedly that chronic THC administration before and during simian immunodeficiency virus infection ameliorates disease progression, and also attenuates viral load and tissue inflammation, significantly reducing the morbidity and mortality of virus-infected macaques [106], which is very encouraging.

There is considerable preclinical and clinical evidence showing that the combination of THC with

opioids or nonsteroidal anti-inflammatory drugs may enhance their efficacy in pain and also limit their side effects [2,95,96]. It has become clear that cannabinoid analgesia is predominantly mediated via peripheral CB_1 receptors in nociceptors [107], providing the rationale for selectively targeting peripheral CB_1 receptors by peripherally-restricted (brain impermeable) agonists, thereby eliminating the undesirable CNS consequences of CB_1 stimulation [71]. Astra Zeneca (London, UK) has developed two novel peripherally-restricted, orally bioavailable $CB_{1/2}$ agonists (AZD1940 and AZD1704). Despite their mixed agonist activity at CB_1 and CB_2 receptors, the analgesic efficacy in rodent models was mainly driven by CB_1 receptors, as validated through the use of CB_1 selective antagonist and knockout mice [27]. The clinical efficacy of AZD1940 as a pain reliever was tested in two single-dose, phase II studies (human capsaicin and third molar extraction models) and in a multiple ascending doses study performed in subjects with chronic low-back pain. The two single-dose, phase II studies showed no efficacy at the primary endpoints (pain intensity and heat pain threshold for capsaicin study) [28]. In the multiple ascending dose study where AZD1940 was administered for 12 days, repeated dosing led to slow compound accumulation, significant weight gain and elevation of hepatic transaminases. AZD1704 also induced profound hypotensive effects [28]. Thus, the analgesic efficacy of peripherally-restricted CB_1 agonists remains to be established in humans. Although their cardiovascular and metabolic side effects confirm the role of CB_1 receptors in these functions in humans, they further limit their usefulness as therapeutic agents. The above studies of Astra Zeneca with novel, peripherally-restricted, orally bioavailable $CB_{1/2}$ agonists did not indicate CB_2 involvement in preclinical models of analgesia, whereas other studies suggest that CB_2 activation may attenuate certain types of pain [95,96]. CB_2-selective peripherally-restricted agonists (instead of mixed $CB_{1/2}$ agonists) may offer the better optimization of dosing in humans because metabolic and cardiovascular side effects are less likely to occur.

Inhibition of the CB_1 receptors: global and peripherally-restricted CB_1 antagonists

Recent preclinical studies have provided compelling evidence that ECs modulate food intake, energy balance, glucose and lipid metabolism through CB_1 receptors expressed in the brain and various peripheral tissues, such as fat, liver and skeletal muscle [5,70,108,109]. Treatment with brain-penetrant CB_1 receptor antagonists/inverse agonists resulted in improvements of multiple cardiovascular risk factors both in preclinical studies and in clinical trials in obese/overweight subjects [110–116]. Parallel preclinical studies clearly demonstrated that reduced food intake was not the primary mechanism responsible for the weight-reducing effect of CB_1 antagonists, and suggested that peripheral energy metabolism might be directly under EC control [5]. These studies demonstrated that ECs promote lipogenesis in adipose tissue and liver but inhibit fatty acid oxidation and mitochondrial biogenesis, whereas CB_1 antagonists exert the opposite effects [5]. Meanwhile, clinical trials have revealed that a small but statistically significant fraction of subjects treated with the CB_1 inverse agonist rimonabant exhibited anxiety, depression and/or suicidal ideations, eventually leading to the withdrawal of rimonabant from the market in over 50 countries and discontinuation of the therapeutic development of this class of compounds [117].

By that time, there were several lines of evidence strongly suggesting that selective inhibition of peripheral CB_1 receptors may preserve much of the metabolic benefit of global CB_1 blockade at the same time as minimizing side effects as a result of the blockade of CB_1 receptors in the CNS [5]. A proof of principle study by Tam *et al.* [118] demonstrated that chronic treatment of DIO mice with AM6545 (the first high-affinity, selective, peripherally-restricted neutral CB_1 antagonist) improved glucose tolerance, insulin sensitivity and the plasma lipid profile, and also reversed fatty liver, although it was less effective than its parent compound rimonabant in reducing body weight because it did not affect caloric intake. The same study also provided evidence for the importance of CB_1 receptors in hepatocytes in the development of diet-induced insulin resistance. A subsequent study provided additional mechanistic insight by demonstrating that CB_1-mediated hepatic insulin resistance involves ER stress-dependent impairment of insulin signalling, as well as reduced insulin clearance [119]. In a follow-up study, a highly potent, selective and brain impermeable CB_1 receptor inverse agonist, JD5037, was even more effective in improving metabolic parameters in mouse models of obesity, and it not only improved cardiometabolic risk, but also had antiobesity and hypophagic effects by reversing leptin resistance [101]. This compound is currently undergoing toxicology screening as a prelude to its clinical testing.

As discussed above, we have learned important lessons from the first clinical trials aiming to attenuate pain with the peripherally-restricted mixed $CB_{1/2}$ agonists, which were terminated because of excessive weight

gain, hepatotoxicity and cardiovascular adverse effects. Interestingly, this side-effect profile strongly supports the rationale for the development and therapeutic use of peripherally-restricted CB_1 antagonists in humans [27,28].

Activation of CB_2 receptors by selective agonists

Overwhelming evidence for the therapeutic potential of EC/CB_2 receptor signalling in some of the major pathologies affecting humans has been reviewed recently [4]. An important consideration for the therapeutic development of selective CB_2 receptor agonists is the absence of psychoactive effects, coupled with the anti-inflammatory and tissue protective activity of these ligands in numerous preclinical disease models [4].

CB_2 receptors are predominantly expressed in peripheral blood immune cells where the level of their expression is strongly modulated by pro-inflammatory and other stimuli, largely depending on the experimental conditions [120]. Initial studies focusing on the immunomodulatory effects of THC and other cannabinoid ligands *in vivo* in rodents and *in vitro* in human immune cell cultures demonstrated immunosuppressive effects in T and B lymphocytes, natural killer cells and macrophages, which most likely involved both CB_1 and CB_2 receptors, as well as CB receptor-independent mechanisms [9,120,121]. ECs were also found to modulate T and B cell proliferation and apoptosis, immune cell activation and inflammatory cytokine production, chemotaxis and inflammatory cell migration, and macrophage-mediated killing of sensitized cells [9,120,122]. These generally inhibitory effects were ligand- and cell type-dependent and were also influenced by the experimental conditions used [9,120,123,124]. A complicating factor is the agonist-induced rapid internalization and trafficking of CB_2 receptors *in vitro*, which can confound any interpretation of the results [33,34]. The effects of ECs or synthetic analogues on microglia activation/migration also appear to be largely experimental condition-dependent [123].

One important recent development has been the identification of low levels of CB_2 receptor expression in tissues previously considered to be devoid of these receptors. These include specific regions of the brain [125–127], spinal cord and dorsal root ganglia [17,95,128], neurones in the myenteric and submucosal plexus of the enteric nervous system [129–131], myocardium or cardiomyocytes [64,65,132], human vascular smooth muscle and endothelium [25,133–135], activated hepatic stellate cells [136,137], Kupffer cells [138], reproductive organs/cells [139,140], colonic epithelial cells [141], bone [142–144], mouse and human exocrine and endocrine pancreas [145–148], and various human tumours [149]. Further studies are needed to fully explore the function of CB_2 receptors at these sites.

More importantly, disease-induced changes (usually increases) in CB_2 receptor expression have been reported (Table 1), and synthetic CB_2 receptor agonists exerted protective effects in a variety of preclinical disease models and pathological conditions [4], ranging from cardiovascular disorders [11], various forms of ischaemic-reperfusion injury [90], gastrointestinal and liver inflammation [13,150,151], autoimmune and neurodegenerative disorders [7,152–154], kidney disorders [4], bone disorders [143,144], cancer [149,155–157], and pain [17,95].

As for the therapeutic potential of CB_2 agonists, it is important to note that, although, under conditions of a sterile inflammatory response, CB_2 agonists may limit injury, in pathogen-induced inflammation, the immunosuppressive effects of the CB_2 receptor activation may enhance or even inflict tissue damage, and may also lead to accelerated cancer growth in certain types of tumours [4]. To successfully target CB_2 in selected human diseases, it is imperative to identify the exact cellular location and disease-induced, time-dependent changes in the expression of CB_2 receptors. This will necessitate the development of improved research tools, such as more reliable and specific antibodies. This is particularly important because, in many injury models, CB_2 agonists appear to be most effective when given before the initiation of the insult, and may lose their efficacy or even promote inflammation when given at later time [4]. Thus, a better understanding of the underlying pathology and its effects on CB_2 expression is required for the development of meaningful therapeutic approaches. Before going to clinical development for a particular indication, it is also important to confirm previous preclinical findings with novel and more selective CB_2 agonists, because currently available ligands may not be entirely specific. Better knowledge of the pharmacokinetics and metabolism of ligands is also essential, particularly given the bell-shaped dose–response often seen with recently available CB_2 agonists in various disease models [4]. The reason for the latter may be that, when used at higher doses, currently used CB_2 agonists may also activate CB_1 receptors, particularly when the relative expression of CB_1 over CB_2 is high. Our understanding of the complexities of CB_2 receptor signalling is still limited, and important interspecies differences in CB_2 receptor signalling and in the pharmacology of CB_2 ligands must also be considered [158].

Problems with the use of peripherally-restricted $CB_{1/2}$ agonists for pain relief as a result of cardiovascular and metabolic side effects have been discussed above. A plausible alternative could be the testing of peripherally-restricted selective CB_2 agonists for analgesia in humans because such compounds would be expected to be devoid of cardiometabolic liabilities. However, the preclinical data with AZD1940 and AZD1704 indicate that the analgesic efficacy of this class of compounds was mainly driven by the CB_1 receptor [27] which, if confirmed in humans, would limit the promise of this approach. Nevertheless, the therapeutic development of selective CB_2 receptor ligands (agonists or inverse agonists/antagonists depending on the pathology and its stage) is still a promising strategy for a number of disease conditions, provided that the issues discussed above are successfully resolved [4].

Inhibition of EC metabolism, cellular uptake or biosyntheses

The hypothesis behind the therapeutic inhibition of EC degradation was that increasing EC tissue levels would be less likely to cause psychoactive effects than would the use of synthetic CB_1 ligands (endocannabinoids are biosynthesized and degraded in a site and time-dependent manner), whereas the beneficial effects of $CB_{1/2}$ activation, such as analgesia, would be maintained [159]. In support of this, FAAH knockout mice or mice treated with a FAAH inhibitor have elevated AEA levels in the brain and other tissues, are supersensitive to exogenous AEA, and exhibit CB_1 receptor-mediated hypoalgesia [160,161] and reduced anxiety, although they do not display catalepsy, an indicator of psychoactivity in humans [162]. The antinociceptive effect of FAAH inhibitors, likely mediated through increases in AEA and PEA levels that activate $CB_{1/2}$, peroxisome proliferator-activated receptor α and/or TRPV1 [163], was investigated in acute and chronic rodent models of pain [164]. Most of the initial results were based on using URB597, which irreversibly inhibits FAAH both in the CNS and periphery [164]. Recent studies with a peripherally-restricted FAAH inhibitor, URB937, showed efficacy in neuropathic and inflammatory pain [165], confirming that the analgesic effects of AEA are initiated at the peripheral sites [107]. However, similar to direct-acting peripheral $CB_{1/2}$ agonists, URB597 has both hypotensive [166] and diabetogenic effects [167] mediated by CB_1 receptors, and FAAH knockout mice are also prone to diet-induced obesity and diabetes [168]. The diabetogenic effect of URB597 has been attributed to blocking FAAH in the liver, and the novel FAAH inhibitor AM3506, which does not block FAAH in the liver as a result of its rapid uptake and metabolism by hepatocytes, was found to be devoid of glycaemic side effects in rodents [167]. FAAH antagonism may also promote fat accumulation and insulin resistance through centrally-mediated hypothyroidism [169].

The analgesic effects of FAAH inhibition in preclinical models prompted the development of PF-04457845, an irreversible FAAH inhibitor with excellent analgesic efficacy in animal models [29,170], which was selected for clinical development. In a randomized, placebo-controlled, phase II clinical trial PF-04457845 was recently evaluated in patients with osteoarthritic pain of the knee [30]. The results clearly demonstrated that PF-04457845 inhibited FAAH activity in white blood cells and raised the concentrations of various fatty acid amides 3.5-10 fold, which persisted for up to 2 weeks after discontinuation of the drug, and did not affect cognitive function in test subjects. However, the study failed to show any analgesic efficacy of PF-04457845, whereas the nonsteroidal anti-inflammatory drug naproxen, used as a positive control, was effective [30]. These results were also highlighted and discussed in a recent editorial [171].

A promising alternative indication for the therapeutic use of FAAH antagonists is post-traumatic stress syndrome. The FAAH inhibitor AM3506 was recently found to be effective in increasing fear extinction in a CB_1 receptor-dependent manner in a mouse model of post-traumatic stress syndrome, and human carriers of a low-expressing FAAH variant displayed quicker habituation of amygdala reactivity to threat, as detected by brain imaging [172].

The main rationale for the development of MAGL inhibitors, which metabolize 2-AG, is similar to the rationale for FAAH inhibitors. Numerous recent studies have demonstrated that MAGL inhibition or genetic deletion exerts anti-emetic [173], antineoplastic [174], and anxiolytic and antinociceptive effects in rodents [175], and also protects against brain injury [176,177], acute liver injury/inflammation [138] and colitis either via enhancing $CB_{1/2}$ signalling or by attenuating eicosanoid synthesis in specific tissues, such as the brain and liver [178], or by a combination of both. In the case of cancer, MAGL inhibition modulates fatty acid release for the synthesis of protumourigenic signalling lipids [174], as reviewed recently [179,180].

Although the above preclinical findings are indeed exciting, they also highlight important limitations. (a) Raising the tissue levels of ECs may promote the formation of cyclooxygenase-, lipoxygenase- and

cytochrome P450-derived pro-inflammatory metabolites [47,181]. (b) Some of the prostaglandins that were attenuated by MAGL inhibitors have well documented tissue protective functions. (c) Although the dual effect of MAGL inhibition on attenuating eicosanoid and enhancing EC signalling can be beneficial in certain tissues (e.g. the brain and liver) where MAGL links the EC and eicosanoid systems through the hydrolysis of 2-AG, in other tissues, it can promote inflammation and injury (e.g. in the myocardium) through the non-CB mechanisms described above (the cardiotoxicity of COX-2 inhibitors is well documented in humans). (d) Chronic MAGL inhibition leads to functional antagonism of the ECS [175]. (e) As previously discussed, very strong preclinical and clinical evidence suggests that, in cardiovascular disease and diabetes/diabetic complications, endocannabinoids (through CB_1 and most likely through the first two mechanisms described above) promote cardiovascular injury. (f) There is growing evidence that ECs exert pro-inflammatory effects in various disease models through both CB_1-dependent and -independent mechanisms [6]. This is supported by a recent study demonstrating that the inhibition of EC synthesis is anti-inflammatory in macrophages [182]. (g) Various isoforms of metabolizing enzymes (e.g. FAAH) may have distinct functions [52], and the functional properties of rodent and human FAAH may also be different [183]. (h) Most of the benefits observed with inhibitors of FAAH or MAGL were reported in acute models; the safety of chronic inhibition of these enzymes has not yet been determined, particularly in pathological situations. (i) The use of irreversible inhibitors of FAAH and MAGL could be a disadvantage for accurate dose titration and would make it difficult to treat toxicity [164].

Conclusions and future directions

Recent clinical studies show that cannabinoid-based medicines with controlled doses of plant-derived cannabinoids can provide symptomatic relief in a subset of patients suffering from pain and spasticity associated with MS and certain other types of pain, and there is hope (based on preclinical studies) that these medications would also positively modulate disease progression. Synthetic cannabinoids are also useful in subset of patients with wasting disorders and chemotherapy-induced nausea and vomiting. There are numerous promising new targets (plant-derived cannabinoids, peripherally-restricted CB_1 antagonists, selective CB_2 agonists, inhibitors of endocannabinoid metabolism/transport) 'in waiting', as discussed in the present

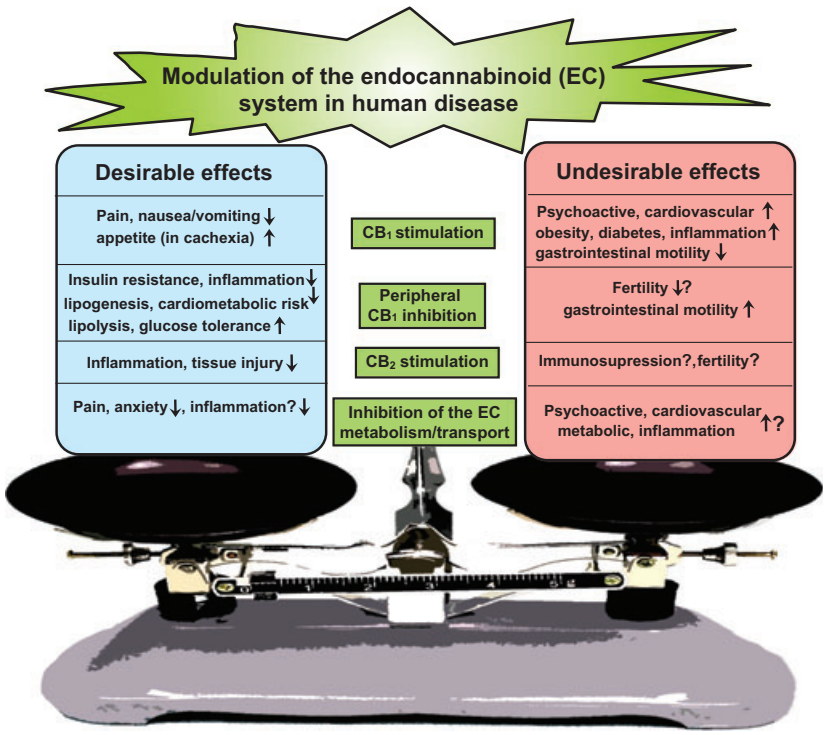


Fig. 1. Cannabinoid therapeutics: finding the right balance.

Table 2. Potential approaches/directions for future success.

Therapeutic approach (target)	Possible directions/approaches for success	Possibly therapeutic indications in humans (realistic)	Potential/expected adverse effects
THC based medicines, cannabinoid based extracts (CB_1, CB_2 and unrelated antioxidant anti-inflammatory mechanisms)	Optimization of route of administration, dosing and indication Better selection criteria for trials, identification of potential positive responders by initial titration Placebo-controlled trials to establish short- and long-term efficacy in given indications Long-term controlled studies to determine possible disease-modifying effects (e.g. in multiple sclerosis) and adverse consequences (e.g. immune and/or cardiovascular effects, etc.) Combination approaches in pain to achieve better efficacy and fewer side effects (e.g. with opioids, nonsteroid anti-inflammatory drugs, etc.) Optimization of the extract composition for improved benefit/risk profile	Symptomatic relief in certain forms of pain and spasticity (as in neurodegenerative disorders such as multiple sclerosis) Stimulation of appetite in patients with wasting disorders Attenuation of chemotherapy-induced nausea and vomiting Topical administration in certain skin disorders? Nonpsychoactive constituents of marijuana, such as CBD or their analogues, may have therapeutic utility in certain forms of acute tissue injury, inflammatory disorders, diabetes and diabetic complications	In the case of THC-containing formulations, effects related to CB_1 stimulation at higher doses (e.g. psychoactive, cardiovascular, metabolic side effects) and potential modulation of immune responses
Peripherally restricted CB_1 agonists (peripheral CB_1)	Evaluation of the feasibility of the topical/local use of peripherally restricted CB_1 agonists in certain forms of pain and skin conditions (e.g. pruritus)	Topical/local use in certain forms of pain and skin conditions/ diseases? (the systematic administration/use is not likely because of the established adverse cardiovascular and metabolic consequences of this approach)	Cardiovascular Metabolic Kidney Gastrointestinal (decreased motility) Pro-inflammatory?
Peripherally restricted or global CB_2 agonists (peripheral CB_2)	Re-evaluation of human indications based on previous failures of trials with mixed peripherally restricted $CB_{1/2}$ agonists Search for new indications More preclinical and clinical research to understand the significance of tissue and time specific changes in CB_2 receptor expression in pathological conditions Development of novel, specific and orally available ligands for proof of the principle studies; evaluation of toxicology and pharmacokinetics	Various forms of acute tissue injuries associated with inflammation (stroke, myocardial infarction, traumatic injury, organ transplantation, etc.) Various forms of inflammatory diseases if the anti-inflammatory effects are confirmed in humans	Most likely related to effects on immune and haematopoietic system Effects on fertility?
Peripherally restricted CB_1 antagonists, inverse agonists (peripheral CB_1)	Development and testing of new ligands, toxicology and safety studies in rodents, large animals, and humans Proof of the principle studies in large animals and humans	Diabetes and diabetic complications, Cardiometabolic syndrome Kidney disease?	Gastrointestinal (increased motility) Effects on fertility?
Inhibition of EC metabolism, cellular uptake or biosynthesis ($CB_{1/2}$, $TRPV_1$ and nuclear receptors, prostaglandin and leukotriene signalling)	Preclinical research to identify the putative endocannabinoid transporter(s), and to better understand the tissue, time, and disease-specific metabolism of endocannabinoids to various other bioactive mediators (e.g. prostaglandins, leukotriens, etc.) Re-evaluation of human indications based on previous failures of trials with FAAH inhibitors in pain Search for new indications, better and more selective ligands	Pain? Certain disorders associated with anxiety? Certain forms of acute tissue injury?	Similar, but acutely less pronounced than with CB_1 agonists. However, long-term use may be associated with adverse effects similar to cyclooxygenase 2 inhibitors (e.g. cardiovascular). Pro-inflammatory effects in certain cases?

minireview. However, it is clear that, for the successful translation of preclinical findings to clinical practice, a better understanding of the pathological role of the ECS in various diseases, of the potential side effects of targeting this system, and of endocannabinoid pharmacology is required, coupled with the development of improved research tools to dissect these processes (Fig. 1 and Table 2).

Future studies should focus on a rigorous evaluation of the CB receptor dependent/independent and hypothermia-independent effects of THC in preclinical models (e.g. in tissue injury, cancer, inflammation, etc.) using global and tissue/cell specific knockout mice and also aim to identify potential novel targets/mechanisms of action of THC and other plant-derived cannabinoids, coupled with the identification of nonpsychoactive constituents in cannabis extracts with potential therapeutic effects. Novel highly selective, orally available nontoxic cannabinoid ligands should be developed and evaluated in preclinical disease models. Large animal studies (e.g. canine, pig, primate) should confirm the efficacy of cannabinoid ligands obtained in rodent disease models before initiating human trials. The development of specific novel antibodies for $CB_{1/2}$ receptors and endocannabinoid metabolic enzymes (FAAH, MAGL, diacylglycerol lipase α/β) validated by using positive and negative controls is essential for accurately assessing the time-dependent changes in $CB_{1/2}$ receptors and metabolic enzyme expression in diseased animal and human tissues, with the aim of understanding the human relevance of these changes. Our limited knowledge should be expanded to enable an understanding of $CB_{1/2}$ receptor trafficking, signalling and their interspecies differences. The development of reliable radioligands suitable for human imaging studies and research could contribute to a better understanding of the role of ECS in human health and disease.

Acknowledgements

This study was supported by funds from the Intramural Research Program of NIAAA to P.P. and G.K. The authors apologize to colleagues whose important work could not be cited because of space limitations.

References

1 Hanus LO (2009) Pharmacological and therapeutic secrets of plant and brain (endo)cannabinoids. *Med Res Rev* **29**, 213–271.
2 Pacher P, Batkai S & Kunos G (2006) The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol Rev* **58**, 389–462.
3 Di Marzo V (2008) Targeting the endocannabinoid system: to enhance or reduce? *Nat Rev Drug Discov* **7**, 438–455.
4 Pacher P & Mechoulam R (2011) Is lipid signaling through cannabinoid 2 receptors part of a protective system? *Prog Lipid Res* **50**, 193–211.
5 Kunos G & Tam J (2011) The case for peripheral CB (1) receptor blockade in the treatment of visceral obesity and its cardiometabolic complications. *Br J Pharmacol* **163**, 1423–1431.
6 Horvath B, Mukhopadhyay P, Hasko G & Pacher P (2012) The endocannabinoid system and plant-derived cannabinoids in diabetes and diabetic complications. *Am J Pathol* **180**, 432–442.
7 Centonze D, Finazzi-Agro A, Bernardi G & Maccarrone M (2007) The endocannabinoid system in targeting inflammatory neurodegenerative diseases. *Trends Pharmacol Sci* **28**, 180–187.
8 Skaper SD & Di Marzo V (2012) Endocannabinoids in nervous system health and disease: the big picture in a nutshell. Philosophical transactions of the Royal Society of London. *Series B, Biol Sci* **367**, 3193–3200.
9 Klein TW (2005) Cannabinoid-based drugs as anti-inflammatory therapeutics. *Nat Rev Immunol* **5**, 400–411.
10 Pacher P, Mukhopadhyay P, Mohanraj R, Godlewski G, Batkai S & Kunos G (2008) Modulation of the endocannabinoid system in cardiovascular disease: therapeutic potential and limitations. *Hypertension* **52**, 601–607.
11 Steffens S & Pacher P (2012) Targeting cannabinoid receptor CB(2) in cardiovascular disorders: promises and controversies. *Br J Pharmacol* **167**, 313–323.
12 Montecucco F & Di Marzo V (2012) At the heart of the matter: the endocannabinoid system in cardiovascular function and dysfunction. *Trends Pharmacol Sci* **33**, 331–340.
13 Lotersztajn S, Teixeira-Clerc F, Julien B, Deveaux V, Ichigotani Y, Manin S, Tran-Van-Nhieu J, Karsak M, Zimmer A & Mallat A (2008) CB2 receptors as new therapeutic targets for liver diseases. *Br J Pharmacol* **153**, 286–289.
14 Tam J, Liu J, Mukhopadhyay B, Cinar R, Godlewski G & Kunos G (2011) Endocannabinoids in liver disease. *Hepatology* **53**, 346–355.
15 Izzo AA & Camilleri M (2008) Emerging role of cannabinoids in gastrointestinal and liver diseases: basic and clinical aspects. *Gut* **57**, 1140–1155.
16 Biro T, Toth BI, Hasko G, Paus R & Pacher P (2009) The endocannabinoid system of the skin in health and disease: novel perspectives and therapeutic opportunities. *Trends Pharmacol Sci* **30**, 411–420.
17 Guindon J & Hohmann AG (2008) Cannabinoid CB2 receptors: a therapeutic target for the treatment of

inflammatory and neuropathic pain. *Br J Pharmacol* **153**, 319–334.
18 Guindon J & Hohmann AG (2009) The endocannabinoid system and pain. *CNS Neurol Disord Drug Targets* **8**, 403–421.
19 Mechoulam R & Parker LA (2013) The Endocannabinoid System and the Brain. *Annu Rev Psychol* **64**, 21–47.
20 Hillard CJ, Weinlander KM & Stuhr KL (2012) Contributions of endocannabinoid signaling to psychiatric disorders in humans: genetic and biochemical evidence. *Neuroscience* **204**, 207–229.
21 Guindon J & Hohmann AG (2011) The endocannabinoid system and cancer: therapeutic implication. *Br J Pharmacol* **163**, 1447–1463.
22 Velasco G, Sanchez C & Guzman M (2012) Towards the use of cannabinoids as antitumour agents. *Nat Rev Cancer* **12**, 436–444.
23 Parker LA, Rock EM & Limebeer CL (2011) Regulation of nausea and vomiting by cannabinoids. *Br J Pharmacol* **163**, 1411–1422.
24 Piscitelli F & Di Marzo V (2012) 'Redundancy' of endocannabinoid inactivation: new challenges and opportunities for pain control. *ACS Chem Neurosci* **3**, 356–363.
25 Pacher P & Steffens S (2009) The emerging role of the endocannabinoid system in cardiovascular disease. *Semin Immunopathol* **31**, 63–77.
26 Di Marzo V (2008) Play an adagio with a Stradivarius: the right patient for CB1 receptor antagonists? *Nat Clin Pract Cardiovasc Med* **5**, 610–612.
27 Groblewski T, Hong X, Lessard E, St-Onge S, Yang H, Panetta R, Cao CQ, Swedberg MD, Cebers G, Nyberg S *et al*. (2010) Pre-clinical pharmacological properties of novel peripherally-acting CB1-CB2 agonists. Proccedings of 20th Annual Symposium of the International Cannabinoid Research Society, Lund, Sweden, 2010.
28 Groblewski T, Karlsten R, Segerdhal M, Kalliomäki J, Jonzon B, Bielenstein M, Cebers G, Swedberg M, Annas A, Christoph G *et al*. (2010) Peripherally-acting CB1-CB2 agonists for pain: do they still hold promise? Proceedings of the 20th Annual Symposium of the International Cannabinoid Research Society, Lund, Sweden, 2010.
29 Li GL, Winter H, Arends R, Jay GW, Le V, Young T & Huggins JP (2012) Assessment of the pharmacology and tolerability of PF-04457845, an irreversible inhibitor of fatty acid amide hydrolase-1, in healthy subjects. *Br J Clin Pharmacol* **73**, 706–716.
30 Huggins JP, Smart TS, Langman S, Taylor L & Young T (2012) An efficient randomised, placebo-controlled clinical trial with the irreversible fatty acid amide hydrolase-1 inhibitor PF-04457845, which modulates endocannabinoids but fails to induce effective analgesia in patients with pain due to osteoarthritis of the knee. *Pain* **153**, 1837–1846.
31 Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, Felder CC, Herkenham M, Mackie K, Martin BR *et al*. (2002) International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol Rev* **54**, 161–202.
32 Pertwee RG, Howlett AC, Abood ME, Alexander SP, Di Marzo V, Elphick MR, Greasley PJ, Hansen HS, Kunos G, Mackie K *et al*. (2010) International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB1 and CB2. *Pharmacol Rev* **62**, 588–631.
33 Atwood BK, Wager-Miller J, Haskins C, Straiker A & Mackie K (2012) Functional selectivity in CB2 cannabinoid receptor signaling and regulation: implications for the therapeutic potential of CB2 ligands. *Mol Pharmacol* **81**, 250–263.
34 Kleyer J, Nicolussi S, Taylor P, Simonelli D, Furger E, Anderle P & Gertsch J (2012) Cannabinoid receptor trafficking in peripheral cells is dynamically regulated by a binary biochemical switch. *Biochem Pharmacol* **83**, 1393–1412.
35 Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A & Mechoulam R (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**, 1946–1949.
36 Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martin BR, Compton DR *et al*. (1995) Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol* **50**, 83–90.
37 Wang J & Ueda N (2009) Biology of endocannabinoid synthesis system. *Prostaglandins Other Lipid Mediat* **89**, 112–119.
38 Di Marzo V, Bifulco M & De Petrocellis L (2004) The endocannabinoid system and its therapeutic exploitation. *Nat Rev Drug Discov* **3**, 771–784.
39 Liu J, Wang L, Harvey-White J, Huang BX, Kim HY, Luquet S, Palmiter RD, Krystal G, Rai R, Mahadevan A *et al*. (2008) Multiple pathways involved in the biosynthesis of anandamide. *Neuropharmacology* **54**, 1–7.
40 Simon GM & Cravatt BF (2008) Anandamide biosynthesis catalyzed by the phosphodiesterase GDE1 and detection of glycerophospho-N-acyl ethanolamine precursors in mouse brain. *J Biol Chem* **283**, 9341–9349.
41 Fowler CJ (2012) Anandamide uptake explained? *Trends Pharmacol Sci* **33**, 181–185.
42 Fowler CJ (2013) Transport of endocannabinoids across the plasma membrane and within the cell. *FEBS J* **280**, 1895–1904.

43 Kaczocha M, Vivieca S, Sun J, Glaser ST & Deutsch DG (2012) Fatty acid-binding proteins transport N-acylethanolamines to nuclear receptors and are targets of endocannabinoid transport inhibitors. *J Biol Chem* **287**, 3415–3424.
44 Cravatt BF & Lichtman AH (2003) Fatty acid amide hydrolase: an emerging therapeutic target in the endocannabinoid system. *Curr Opin Chem Biol* **7**, 469–475.
45 Ueda N, Tsuboi K & Uyama T (2010) N-acylethanolamine metabolism with special reference to N-acylethanolamine-hydrolyzing acid amidase (NAAA). *Prog Lipid Res* **49**, 299–315.
46 Ueda N, Tsuboi K, Uyama T & Ohnishi T (2011) Biosynthesis and degradation of the endocannabinoid 2-arachidonoylglycerol. *BioFactors* **37**, 1–7.
47 Rouzer CA & Marnett LJ (2011) Endocannabinoid oxygenation by cyclooxygenases, lipoxygenases, and cytochromes P450: cross-talk between the eicosanoid and endocannabinoid signaling pathways. *Chem Rev* **111**, 5899–5921.
48 Cravatt BF & Lichtman AH (2002) The enzymatic inactivation of the fatty acid amide class of signaling lipids. *Chem Phys Lipids* **121**, 135–148.
49 McKinney MK & Cravatt BF (2005) Structure and function of fatty acid amide hydrolase. *Annu Rev Biochem* **74**, 411–432.
50 Fezza F, De Simone C, Amadio D & Maccarrone M (2008) Fatty acid amide hydrolase: a gate-keeper of the endocannabinoid system. *Subcell Biochem* **49**, 101–132.
51 Palkovits M, Harvey-White J, Liu J, Kovacs ZS, Bobest M, Lovas G, Bago AG & Kunos G (2008) Regional distribution and effects of postmortal delay on endocannabinoid content of the human brain. *Neuroscience* **152**, 1032–1039.
52 Fu J, Bottegoni G, Sasso O, Bertorelli R, Rocchia W, Masetti M, Guijarro A, Lodola A, Armirotti A, Garau G *et al*. (2012) A catalytically silent FAAH-1 variant drives anandamide transport in neurons. *Nat Neurosci* **15**, 64–69.
53 Di Marzo V & De Petrocellis L (2010) Endocannabinoids as regulators of transient receptor potential (TRP) channels: A further opportunity to develop new endocannabinoid-based therapeutic drugs. *Curr Med Chem* **17**, 1430–1449.
54 Hanus LO & Mechoulam R (2010) Novel natural and synthetic ligands of the endocannabinoid system. *Curr Med Chem* **17**, 1341–1359.
55 Bauer M, Chicca A, Tamborrini M, Eisen D, Lerner R, Lutz B, Poetz O, Pluschke G & Gertsch J (2012) Identification and quantification of a new family of peptide endocannabinoids (Pepcans) showing negative allosteric modulation at CB1 receptors. *J Biol Chem* **287**, 36944–36967.
56 Pamplona FA, Ferreira J, Menezes de Lima O Jr, Duarte FS, Bento AF, Forner S, Villarinho JG, Bellochio L, Wotjak CT, Lerner R *et al*. (2012) Anti-inflammatory lipoxin A4 is an endogenous allosteric enhancer of CB1 cannabinoid receptor. *Proc Natl Acad Sci USA* **109**, 21134–21139.
57 Ueda N, Tsuboi K & Uyama T (2013) Metabolism of endocannabinoids and related N-acylethanolamines: Canonical and alternative pathways. *FEBS J* **280**, 1874–1894.
58 Di Marzo V (2008) Endocannabinoids: synthesis and degradation. *Rev Physiol Biochem Pharmacol* **160**, 1–24.
59 Miller LK & Devi LA (2011) The highs and lows of cannabinoid receptor expression in disease: mechanisms and their therapeutic implications. *Pharmacol Rev* **63**, 461–470.
60 Pertwee RG (2012) Targeting the endocannabinoid system with cannabinoid receptor agonists: pharmacological strategies and therapeutic possibilities. Philosophical Transactions of the Royal Society of London. *Series B, Biol Sci* **367**, 3353–3363.
61 Atwood BK & Mackie K (2010) CB2: a cannabinoid receptor with an identity crisis. *Br J Pharmacol* **160**, 467–479.
62 Pacher P, Batkai S & Kunos G (2005) Cardiovascular pharmacology of cannabinoids. *Handb Exp Pharmacol* **168**, 599–625.
63 Rajesh M, Mukhopadhyay P, Hasko G, Liaudet L, Mackie K & Pacher P (2010) Cannabinoid-1 receptor activation induces reactive oxygen species-dependent and -independent mitogen-activated protein kinase activation and cell death in human coronary artery endothelial cells. *Br J Pharmacol* **160**, 688–700.
64 Mukhopadhyay P, Rajesh M, Batkai S, Patel V, Kashiwaya Y, Liaudet L, Evgenov OV, Mackie K, Hasko G & Pacher P (2010) CB1 cannabinoid receptors promote oxidative stress and cell death in murine models of doxorubicin-induced cardiomyopathy and in human cardiomyocytes. *Cardiovasc Res* **85**, 773–784.
65 Mukhopadhyay P, Batkai S, Rajesh M, Czifra N, Harvey-White J, Hasko G, Zsengeller Z, Gerard NP, Liaudet L, Kunos G *et al*. (2007) Pharmacological inhibition of CB1 cannabinoid receptor protects against doxorubicin-induced cardiotoxicity. *J Am Coll Cardiol* **50**, 528–536.
66 Mukhopadhyay P, Horvath B, Rajesh M, Matsumoto S, Saito K, Batkai S, Patel V, Tanchian G, Gao RY, Cravatt BF *et al*. (2011) Fatty acid amide hydrolase is a key regulator of endocannabinoid-induced myocardial tissue injury. *Free Radic Biol Med* **50**, 179–195.
67 Sugamura K, Sugiyama S, Nozaki T, Matsuzawa Y, Izumiya Y, Miyata K, Nakayama M, Kaikita K,

Obata T, Takeya M *et al*. (2009) Activated endocannabinoid system in coronary artery disease and antiinflammatory effects of cannabinoid 1 receptor blockade on macrophages. *Circulation* **119**, 28–36.
68 Dol-Gleizes F, Paumelle R, Visentin V, Mares AM, Desitter P, Hennuyer N, Gilde A, Staels B, Schaeffer P & Bono F (2009) Rimonabant, a selective cannabinoid CB1 receptor antagonist, inhibits atherosclerosis in LDL receptor-deficient mice. *Arterioscler Thromb Vasc Biol* **29**, 12–18.
69 Kunos G, Osei-Hyiaman D, Liu J, Godlewski G & Batkai S (2008) Endocannabinoids and the control of energy homeostasis. *J Biol Chem* **283**, 33021–33025.
70 Di Marzo V (2008) The endocannabinoid system in obesity and type 2 diabetes. *Diabetologia* **51**, 1356–1367.
71 Kunos G, Osei-Hyiaman D, Batkai S, Sharkey KA & Makriyannis A (2009) Should peripheral CB(1) cannabinoid receptors be selectively targeted for therapeutic gain? *Trends Pharmacol Sci* **30**, 1–7.
72 Cooney MT, Vartiainen E, Laatikainen T, Juolevi A, Dudina A & Graham IM (2010) Elevated resting heart rate is an independent risk factor for cardiovascular disease in healthy men and women. *Am Heart J* **159**, 612–619e3.
73 Gorelick DA, Goodwin RS, Schwilke E, Schwope DM, Darwin WD, Kelly DL, McMahon RP, Liu F, Ortemann-Renon C, Bonnet D *et al*. (2013) Tolerance to effects of high-dose oral {Delta}9-tetrahydrocannabinol and plasma cannabinoid concentrations in male daily cannabis smokers. *J Anal Toxicol* **37**, 11–16.
74 Schmid K, Schonlebe J, Drexler H & Mueck-Weymann M (2010) The effects of cannabis on heart rate variability and well-being in young men. *Pharmacopsychiatry* **43**, 147–150.
75 Klumpers LE, Roy C, Ferron G, Turpault S, Poitiers F, Pinquier JL, van Hasselt JG, Zuurman L, Erwich FA & van Gerven JM (2012) Surinabant, a selective CB(1) antagonist, inhibits THC-induced central nervous system and heart rate effects in humans. *Br J Clin Pharmacol* doi:10.1111/bcp.12071.
76 Singla S, Sachdeva R & Mehta JL (2012) Cannabinoids and atherosclerotic coronary heart disease. *Clin Cardiol* **35**, 329–335.
77 Pratap B & Korniyenko A (2012) Toxic effects of marijuana on the cardiovascular system. *Cardiovasc Toxicol* **12**, 143–148.
78 Leblanc A, Tirel-Badets A, Paleiron N, Castellant P, Cornily JC, Andre M, Grassin F, Feuvrier Y, Blanchard C, Zagnoli F *et al*. (2011) Cannabis and myocardial infarction without angiographic stenosis in young patient: guilty or not guilty? A case report. *Annales de Cardiologie et d'Angeiologie* **60**, 154–158.
79 Mittleman MA, Lewis RA, Maclure M, Sherwood JB & Muller JE (2001) Triggering myocardial infarction by marijuana. *Circulation* **103**, 2805–2809.
80 Mukamal KJ, Maclure M, Muller JE & Mittleman MA (2008) An exploratory prospective study of marijuana use and mortality following acute myocardial infarction. *Am Heart J* **155**, 465–470.
81 Frost L, Mostofsky E, Rosenbloom JI, Mukamal KJ & Mittleman MA (2013) Marijuana use and long-term mortality among survivors of acute myocardial infarction. *Am Heart J* **165**, 170–175.
82 Mir A, Obafemi A, Young A & Kane C (2011) Myocardial infarction associated with use of the synthetic cannabinoid K2. *Pediatrics* **128**, e1622–e1627.
83 Heath TS, Burroughs Z, Thompson AJ & Tecklenburg FW (2012) Acute intoxication caused by a synthetic cannabinoid in two adolescents. *J Pediatr Pharmacol Ther* **17**, 177–181.
84 Lenglet S, Thomas A, Soehnlein O, Montecucco F, Burger F, Pelli G, Galan K, Cravatt B, Staub C & Steffens S (2013) Fatty acid amide hydrolase deficiency enhances intraplaque neutrophil recruitment in atherosclerotic mice. *Arterioscler Thromb Vasc Biol* **33**, 215–223.
85 Quercioli A, Pataky Z, Vincenti G, Makoundou V, Di Marzo V, Montecucco F, Carballo S, Thomas A, Staub C, Steffens S *et al*. (2011) Elevated endocannabinoid plasma levels are associated with coronary circulatory dysfunction in obesity. *Eur Heart J* **32**, 1369–1378.
86 Quercioli A, Pataky Z, Montecucco F, Carballo S, Thomas A, Staub C, Di Marzo V, Vincenti G, Ambrosio G, Ratib O *et al*. (2012) Coronary vasomotor control in obesity and morbid obesity: contrasting flow responses with endocannabinoids, leptin, and inflammation. *JACC Cardiovasc Imaging* **5**, 805–815.
87 Cappellano G, Uberti F, Caimmi PP, Pietronave S, Mary DA, Dianzani C, Micalizzi E, Melensi M, Boldorini R, Nicosia G *et al*. (2013) Different expression and function of the endocannabinoid system in human epicardial adipose tissue in relation to heart disease. *Can J Cardiol* **29**, 499–509.
88 Liu R & Zhang Y (2011) G1359A polymorphism in the cannabinoid receptor-1 gene is associated with coronary artery disease in the Chinese Han population. *Clin Lab* **57**, 689–693.
89 Amoros I, Barana A, Caballero R, Gomez R, Osuna L, Lillo MP, Tamargo J & Delpon E (2010) Endocannabinoids and cannabinoid analogues block human cardiac Kv4.3 channels in a receptor-independent manner. *J Mol Cell Cardiol* **48**, 201–210.
90 Pacher P & Hasko G (2008) Endocannabinoids and cannabinoid receptors in ischaemia-reperfusion

injury and preconditioning. *Br J Pharmacol* **153**, 252–262.
91 Bisogno T & Di Marzo V (2010) Cannabinoid receptors and endocannabinoids: role in neuroinflammatory and neurodegenerative disorders. *CNS Neurol Disord Drug Targets* **9**, 564–573.
92 Fowler CJ, Rojo ML & Rodriguez-Gaztelumendi A (2010) Modulation of the endocannabinoid system: neuroprotection or neurotoxicity? *Exp Neurol* **224**, 37–47.
93 Mechoulam R & Hanus L (2000) A historical overview of chemical research on cannabinoids. *Chem Phys Lipids* **108**, 1–13.
94 Hohmann AG & Suplita RL II (2006) Endocannabinoid mechanisms of pain modulation. *AAPS J* **8**, E693–E708.
95 Anand P, Whiteside G, Fowler CJ & Hohmann AG (2009) Targeting CB2 receptors and the endocannabinoid system for the treatment of pain. *Brain Res Rev* **60**, 255–266.
96 Rahn EJ & Hohmann AG (2009) Cannabinoids as pharmacotherapies for neuropathic pain: from the bench to the bedside. *Neurotherapeutics* **6**, 713–737.
97 Zhornitsky S & Potvin S (2012) Cannabidiol in humans-the quest for therapeutic targets. *Pharmaceuticals* **5**, 529–552.
98 Baker AL, Thornton LK, Hides L & Dunlop A (2012) Treatment of cannabis use among people with psychotic disorders: a critical review of randomised controlled trials. *Curr Pharm Des* **18**, 4923–4937.
99 Izzo AA, Borrelli F, Capasso R, Di Marzo V & Mechoulam R (2009) Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. *Trends Pharmacol Sci* **30**, 515–527.
100 Michalski CW, Oti FE, Erkan M, Sauliunaite D, Bergmann F, Pacher P, Batkai S, Muller MW, Giese NA, Friess H *et al.* (2008) Cannabinoids in pancreatic cancer: correlation with survival and pain. *Int J Cancer* **122**, 742–750.
101 Tam J, Cinar R, Liu J, Godlewski G, Wesley D, Jourdan T, Szanda G, Mukhopadhyay B, Chedester L, Liow JS *et al.* (2012) Peripheral cannabinoid-1 receptor inverse agonism reduces obesity by reversing leptin resistance. *Cell Metab* **16**, 167–179.
102 Rajavashisth TB, Shaheen M, Norris KC, Pan D, Sinha SK, Ortega J & Friedman TC (2012) Decreased prevalence of diabetes in marijuana users: cross-sectional data from the National Health and Nutrition Examination Survey (NHANES) III. *BMJ Open* **2**, e000494.
103 Muniyappa R, Sable S, Ouwerkerk R, Mari A, Gharib AM, Walter M, Courville A, Hall G, Chen KY, Volkow ND *et al.* (2013) Metabolic effects of chronic cannabis smoking. *Diabetes Care* **36**, 1–8.
104 Hollister LE & Reaven GM (1974) Delta-9-tetrahydrocannabinol and glucose tolerance. *Clin Pharmacol Ther* **16**, 297–302.
105 Murphy TD, Weidenbach KN, Houten CV, Gerona RR, Moran JH, Kirschner RI, Maraffa JM, Stork CM, Birkhead GS, Newman A *et al.* (2013) Acute kidney injury associated with synthetic cannabinoid use – multiple States, 2012. *MMWR Morb Mortal Wkly Rep* **62**, 93–98.
106 Molina PE, Winsauer P, Zhang P, Walker E, Birke L, Amedee A, Stouwe CV, Troxclair D, McGoey R, Varner K *et al.* (2011) Cannabinoid administration attenuates the progression of simian immunodeficiency virus. *AIDS Res Hum Retroviruses* **27**, 585–592.
107 Agarwal N, Pacher P, Tegeder I, Amaya F, Constantin CE, Brenner GJ, Rubino T, Michalski CW, Marsicano G, Monory K *et al.* (2007) Cannabinoids mediate analgesia largely via peripheral type 1 cannabinoid receptors in nociceptors. *Nat Neurosci* **10**, 870–879.
108 Osei-Hyiaman D, DePetrillo M, Pacher P, Liu J, Radaeva S, Batkai S, Harvey-White J, Mackie K, Offertaler L, Wang L *et al.* (2005) Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. *J Clin Invest* **115**, 1298–1305.
109 Pagotto U, Marsicano G, Cota D, Lutz B & Pasquali R (2006) The emerging role of the endocannabinoid system in endocrine regulation and energy balance. *Endocr Rev* **27**, 73–100.
110 Despres JP, Golay A & Sjostrom L (2005) Effects of rimonabant on metabolic risk factors in overweight patients with dyslipidemia. *N Engl J Med* **353**, 2121–2134.
111 Van Gaal LF, Rissanen AM, Scheen AJ, Ziegler O & Rossner S (2005) Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. *Lancet* **365**, 1389–1397.
112 Scheen AJ, Van Gaal LG, Despres JP, Pi-Sunyer X, Golay A & Hanotin C (2006) Rimonabant improves cardiometabolic risk profile in obese or overweight subjects: overview of RIO studies. *Rev Med Suisse* **2**, 1916–1923.
113 Despres JP, Ross R, Boka G, Almeras N & Lemieux I (2009) Effect of rimonabant on the high-triglyceride/low-HDL-cholesterol dyslipidemia, intraabdominal adiposity, and liver fat: the ADAGIO-Lipids trial. *Arterioscler Thromb Vasc Biol* **29**, 416–423.
114 Nissen SE, Nicholls SJ, Wolski K, Rodes-Cabau J, Cannon CP, Deanfield JE, Despres JP, Kastelein JJ, Steinhubl SR, Kapadia S *et al.* (2008) Effect of rimonabant on progression of atherosclerosis in

patients with abdominal obesity and coronary artery disease: the STRADIVARIUS randomized controlled trial. *JAMA* **299**, 1547–1560.
115 Rosenstock J, Hollander P, Chevalier S & Iranmanesh A (2008) SERENADE: the Study Evaluating Rimonabant Efficacy in Drug-naive Diabetic Patients: effects of monotherapy with rimonabant, the first selective CB1 receptor antagonist, on glycemic control, body weight, and lipid profile in drug-naive type 2 diabetes. *Diabetes Care* **31**, 2169–2176.
116 Hollander PA, Amod A, Litwak LE & Chaudhari U (2010) Effect of rimonabant on glycemic control in insulin-treated type 2 diabetes: the ARPEGGIO trial. *Diabetes Care* **33**, 605–607.
117 Di Marzo V & Despres JP (2009) CB1 antagonists for obesity–what lessons have we learned from rimonabant? *Nat Rev Endocrinol* **5**, 633–638.
118 Tam J, Vemuri VK, Liu J, Batkai S, Mukhopadhyay B, Godlewski G, Osei-Hyiaman D, Ohnuma S, Ambudkar SV, Pickel J *et al*. (2010) Peripheral CB1 cannabinoid receptor blockade improves cardiometabolic risk in mouse models of obesity. *J Clin Investig* **120**, 2953–2966.
119 Liu J, Zhou L, Xiong K, Godlewski G, Mukhopadhyay B, Tam J, Yin S, Gao P, Shan X, Pickel J *et al*. (2012) Hepatic cannabinoid receptor-1 mediates diet-induced insulin resistance via inhibition of insulin signaling and clearance in mice. *Gastroenterology* **142**, 1218–1228.
120 Klein TW, Newton C, Larsen K, Lu L, Perkins I, Nong L & Friedman H (2003) The cannabinoid system and immune modulation. *J Leukoc Biol* **74**, 486–496.
121 Cabral GA & Staab A (2005) Effects on the immune system. *Handb Exp Pharmacol* **168**, 385–423.
122 Cencioni MT, Chiurchiu V, Catanzaro G, Borsellino G, Bernardi G, Battistini L & Maccarrone M (2010) Anandamide suppresses proliferation and cytokine release from primary human T-lymphocytes mainly via CB2 receptors. *PLoS ONE* **5**, e8688.
123 Miller AM & Stella N (2008) CB2 receptor-mediated migration of immune cells: it can go either way. *Br J Pharmacol* **153**, 299–308.
124 Buckley NE (2008) The peripheral cannabinoid receptor knockout mice: an update. *Br J Pharmacol* **153**, 309–318.
125 Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K, Stella N, Makriyannis A, Piomelli D, Davison JS *et al*. (2005) Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science* **310**, 329–332.
126 Onaivi ES (2006) Neuropsychobiological evidence for the functional presence and expression of cannabinoid CB2 receptors in the brain. *Neuropsychobiology* **54**, 231–246.
127 Viscomi MT, Oddi S, Latini L, Pasquariello N, Florenzano F, Bernardi G, Molinari M & Maccarrone M (2009) Selective CB2 receptor agonism protects central neurons from remote axotomy-induced apoptosis through the PI3K/Akt pathway. *J Neurosci* **29**, 4564–4570.
128 Beltramo M (2009) Cannabinoid type 2 receptor as a target for chronic – pain. *Mini Rev Med Chem* **9**, 11–25.
129 Wright KL, Duncan M & Sharkey KA (2008) Cannabinoid CB2 receptors in the gastrointestinal tract: a regulatory system in states of inflammation. *Br J Pharmacol* **153**, 263–270.
130 Marquez L, Suarez J, Iglesias M, Bermudez-Silva FJ, Rodriguez de Fonseca F & Andreu M (2009) Ulcerative colitis induces changes on the expression of the endocannabinoid system in the human colonic tissue. *PLoS ONE* **4**, e6893.
131 Duncan M, Mouihate A, Mackie K, Keenan CM, Buckley NE, Davison JS, Patel KD, Pittman QJ & Sharkey KA (2008) Cannabinoid CB2 receptors in the enteric nervous system modulate gastrointestinal contractility in lipopolysaccharide-treated rats. *Am J Physiol Gastrointest Liver Physiol* **295**, G78–G87.
132 Bouchard JF, Lepicier P & Lamontagne D (2003) Contribution of endocannabinoids in the endothelial protection afforded by ischemic preconditioning in the isolated rat heart. *Life Sci* **72**, 1859–1870.
133 Rajesh M, Mukhopadhyay P, Hasko G, Huffman JW, Mackie K & Pacher P (2008) CB2 cannabinoid receptor agonists attenuate TNF-alpha-induced human vascular smooth muscle cell proliferation and migration. *Br J Pharmacol* **153**, 347–357.
134 Rajesh M, Mukhopadhyay P, Batkai S, Hasko G, Liaudet L, Huffman JW, Csiszar A, Ungvari Z, Mackie K, Chatterjee S *et al*. (2007) CB2-receptor stimulation attenuates TNF-alpha-induced human endothelial cell activation, transendothelial migration of monocytes, and monocyte-endothelial adhesion. *Am J Physiol Heart Circ Physiol* **293**, H2210–H2218.
135 Rajesh M, Pan H, Mukhopadhyay P, Batkai S, Osei-Hyiaman D, Hasko G, Liaudet L, Gao B & Pacher P (2007) Cannabinoid-2 receptor agonist HU-308 protects against hepatic ischemia/reperfusion injury by attenuating oxidative stress, inflammatory response, and apoptosis. *J Leukoc Biol* **82**, 1382–1389.
136 Julien B, Grenard P, Teixeira-Clerc F, Van Nhieu JT, Li L, Karsak M, Zimmer A, Mallat A & Lotersztajn S (2005) Antifibrogenic role of the cannabinoid receptor CB2 in the liver. *Gastroenterology* **128**, 742–755.
137 Mallat A & Lotersztajn S (2008) Endocannabinoids and liver disease. I. Endocannabinoids and their receptors in the liver. *Am J Physiol Gastrointest Liver Physiol* **294**, G9–G12.

138 Cao Z, Mulvihill MM, Mukhopadhyay P, Xu H, Erdélyi K, Hao E, Holovac E, Hasko G, Cravatt BF, Nomura DK *et al*. (2013) Monoacylglycerol lipase controls endocannabinoid and eicosanoid signaling and hepatic injury in mice. *Gastroenterology* **144**, 808–817.
139 Wang H, Dey SK & Maccarrone M (2006) Jekyll and hyde: two faces of cannabinoid signaling in male and female fertility. *Endocr Rev* **27**, 427–448.
140 Maccarrone M (2009) Endocannabinoids: friends and foes of reproduction. *Prog Lipid Res* **48**, 344–354.
141 Rousseaux C, Thuru X, Gelot A, Barnich N, Neut C, Dubuquoy L, Dubuquoy C, Merour E, Geboes K, Chamaillard M *et al*. (2007) Lactobacillus acidophilus modulates intestinal pain and induces opioid and cannabinoid receptors. *Nat Med* **13**, 35–37.
142 Bab I, Ofek O, Tam J, Rehnelt J & Zimmer A (2008) Endocannabinoids and the regulation of bone metabolism. *J Neuroendocrinol* **20** (Suppl 1), 69–74.
143 Bab I & Zimmer A (2008) Cannabinoid receptors and the regulation of bone mass. *Br J Pharmacol* **153**, 182–188.
144 Bab I, Zimmer A & Melamed E (2009) Cannabinoids and the skeleton: from marijuana to reversal of bone loss. *Ann Med* **41**, 560–567.
145 Michalski CW, Laukert T, Sauliunaite D, Pacher P, Bergmann F, Agarwal N, Su Y, Giese T, Giese NA, Batkai S *et al*. (2007) Cannabinoids ameliorate pain and reduce disease pathology in cerulein-induced acute pancreatitis. *Gastroenterology* **132**, 1968–1978.
146 Michalski CW, Maier M, Erkan M, Sauliunaite D, Bergmann F, Pacher P, Batkai S, Giese NA, Giese T, Friess H *et al*. (2008) Cannabinoids reduce markers of inflammation and fibrosis in pancreatic stellate cells. *PLoS ONE* **3**, e1701.
147 Bermudez-Silva FJ, Suarez J, Baixeras E, Cobo N, Bautista D, Cuesta-Munoz AL, Fuentes E, Juan-Pico P, Castro MJ, Milman G *et al*. (2008) Presence of functional cannabinoid receptors in human endocrine pancreas. *Diabetologia* **51**, 476–487.
148 Petrella C, Agostini S, Alema GS, Casolini P, Carpino F, Giuli C, Improta G, Linari G, Petrozza V & Broccardo M (2010) Cannabinoid agonist WIN55,212 in vitro inhibits interleukin-6 (IL-6) and monocyte chemo-attractant protein-1 (MCP-1) release by rat pancreatic acini and in vivo induces dual effects on the course of acute pancreatitis. *Neurogastroenterol Motil* **22**, 1248–1256.
149 Guzman M (2003) Cannabinoids: potential anticancer agents. *Nat Rev Cancer* **3**, 745–755.
150 Izzo AA & Camilleri M (2009) Cannabinoids in intestinal inflammation and cancer. *Pharmacol Res* **60**, 117–125.
151 Izzo AA & Sharkey KA (2010) Cannabinoids and the gut: new developments and emerging concepts. *Pharmacol Ther* **126**, 21–38.
152 Fernandez-Ruiz J, Romero J, Velasco G, Tolon RM, Ramos JA & Guzman M (2007) Cannabinoid CB2 receptor: a new target for controlling neural cell survival? *Trends Pharmacol Sci* **28**, 39–45.
153 Cabral GA, Raborn ES, Griffin L, Dennis J & Marciano-Cabral F (2008) CB2 receptors in the brain: role in central immune function. *Br J Pharmacol* **153**, 240–251.
154 Fernandez-Ruiz J (2009) The endocannabinoid system as a target for the treatment of motor dysfunction. *Br J Pharmacol* **156**, 1029–1040.
155 Pisanti S & Bifulco M (2009) Endocannabinoid system modulation in cancer biology and therapy. *Pharmacol Res* **60**, 107–116.
156 Stella N (2010) Cannabinoid and cannabinoid-like receptors in microglia, astrocytes, and astrocytomas. *Glia* **58**, 1017–1030.
157 Fowler CJ, Gustafsson SB, Chung SC, Persson E, Jacobsson SO & Bergh A (2010) Targeting the endocannabinoid system for the treatment of cancer – a practical view. *Curr Top Med Chem* **10**, 814–827.
158 Ndong C, O'Donnell D, Ahmad S & Groblewski T (2011) Cloning and pharmacological characterization of the dog cannabinoid CB(2)receptor. *Eur J Pharmacol* **669**, 24–31.
159 Makriyannis A, Mechoulam R & Piomelli D (2005) Therapeutic opportunities through modulation of the endocannabinoid system. *Neuropharmacology* **48**, 1068–1071.
160 Cravatt BF, Demarest K, Patricelli MP, Bracey MH, Giang DK, Martin BR & Lichtman AH (2001) Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. *Proc Natl Acad Sci USA* **98**, 9371–9376.
161 Lichtman AH, Shelton CC, Advani T & Cravatt BF (2004) Mice lacking fatty acid amide hydrolase exhibit a cannabinoid receptor-mediated phenotypic hypoalgesia. *Pain* **109**, 319–327.
162 Kathuria S, Gaetani S, Fegley D, Valino F, Duranti A, Tontini A, Mor M, Tarzia G, La Rana G, Calignano A *et al*. (2003) Modulation of anxiety through blockade of anandamide hydrolysis. *Nat Med* **9**, 76–81.
163 Starowicz K, Makuch W, Osikowicz M, Piscitelli F, Petrosino S, Di Marzo V & Przewlocka B (2012) Spinal anandamide produces analgesia in neuropathic rats: possible CB(1)- and TRPV1-mediated mechanisms. *Neuropharmacology* **62**, 1746–1755.
164 Roques BP, Fournie-Zaluski MC & Wurm M (2012) Inhibiting the breakdown of endogenous opioids and cannabinoids to alleviate pain. *Nat Rev Drug Discovery* **11**, 292–310.
165 Clapper JR, Moreno-Sanz G, Russo R, Guijarro A, Vacondio F, Duranti A, Tontini A, Sanchini S,

Sciolino NR, Spradley JM *et al.* (2010) Anandamide suppresses pain initiation through a peripheral endocannabinoid mechanism. *Nat Neurosci* **13**, 1265–1270.
166 Batkai S, Pacher P, Osei-Hyiaman D, Radaeva S, Liu J, Harvey-White J, Offertaler L, Mackie K, Rudd MA, Bukoski RD *et al.* (2004) Endocannabinoids acting at cannabinoid-1 receptors regulate cardiovascular function in hypertension. *Circulation* **110**, 1996–2002.
167 Godlewski G, Alapafuja SO, Batkai S, Nikas SP, Cinar R, Offertaler L, Osei-Hyiaman D, Liu J, Mukhopadhyay B, Harvey-White J *et al.* (2010) Inhibitor of fatty acid amide hydrolase normalizes cardiovascular function in hypertension without adverse metabolic effects. *Chem Biol* **17**, 1256–1266.
168 Tourino C, Oveisi F, Lockney J, Piomelli D & Maldonado R (2010) FAAH deficiency promotes energy storage and enhances the motivation for food. *Int J Obes (Lond)* **34**, 557–568.
169 Brown WH, Gillum MP, Lee HY, Camporez JP, Zhang XM, Jeong JK, Alves TC, Erion DM, Guigni BA, Kahn M *et al.* (2012) Fatty acid amide hydrolase ablation promotes ectopic lipid storage and insulin resistance due to centrally mediated hypothyroidism. *Proc Natl Acad Sci USA* **109**, 14966–14971.
170 Ahn K, Smith SE, Liimatta MB, Beidler D, Sadagopan N, Dudley DT, Young T, Wren P, Zhang Y, Swaney S *et al.* (2011) Mechanistic and pharmacological characterization of PF-04457845: a highly potent and selective fatty acid amide hydrolase inhibitor that reduces inflammatory and noninflammatory pain. *J Pharmacol Exp Ther* **338**, 114–124.
171 Di Marzo V (2012) Inhibitors of endocannabinoid breakdown for pain: not so FA(AH)cile, after all. *Pain* **153**, 1785–1786.
172 Gunduz-Cinar O, Macpherson KP, Cinar R, Gamble-George J, Sugden K, Williams B, Godlewski G, Ramikie TS, Gorka AX, Alapafuja SO *et al.* (2012) Convergent translational evidence of a role for anandamide in amygdala-mediated fear extinction, threat processing and stress-reactivity. *Mol Psychiatry* doi:10.1038/mp.2012.72.
173 Sticht MA, Long JZ, Rock EM, Limebeer CL, Mechoulam R, Cravatt BF & Parker LA (2012) Inhibition of monoacylglycerol lipase attenuates vomiting in *Suncus murinus* and 2-arachidonoyl glycerol attenuates nausea in rats. *Br J Pharmacol* **165**, 2425–2435.
174 Nomura DK, Long JZ, Niessen S, Hoover HS, Ng SW & Cravatt BF (2010) Monoacylglycerol lipase regulates a fatty acid network that promotes cancer pathogenesis. *Cell* **140**, 49–61.
175 Schlosburg JE, Blankman JL, Long JZ, Nomura DK, Pan B, Kinsey SG, Nguyen PT, Ramesh D, Booker L, Burston JJ *et al.* (2010) Chronic monoacylglycerol lipase blockade causes functional antagonism of the endocannabinoid system. *Nat Neurosci* **13**, 1113–1119.
176 Piro JR, Benjamin DI, Duerr JM, Pi Y, Gonzales C, Wood KM, Schwartz JW, Nomura DK & Samad TA (2012) A dysregulated endocannabinoid-eicosanoid network supports pathogenesis in a mouse model of Alzheimer's disease. *Cell Reports* **1**, 617–623.
177 Carloni S, Alonso-Alconada D, Girelli S, Duranti A, Tontini A, Piomelli D, Hilario E, Alvarez A & Balduini W (2012) Pretreatment with the monoacylglycerol lipase inhibitor URB602 protects from the long-term consequences of neonatal hypoxic-ischemic brain injury in rats. *Pediatr Res* **72**, 400–406.
178 Nomura DK, Morrison BE, Blankman JL, Long JZ, Kinsey SG, Marcondes MC, Ward AM, Hahn YK, Lichtman AH, Conti B *et al.* (2011) Endocannabinoid hydrolysis generates brain prostaglandins that promote neuroinflammation. *Science* **334**, 809–813.
179 Mulvihill MM & Nomura DK (2013) Therapeutic potential of monoacylglycerol lipase inhibitors. *Life Sci* **92**, 492–497.
180 Fowler CJ (2012) Monoacylglycerol lipase – a target for drug development? *Br J Pharmacol* **166**, 1568–1585.
181 Gatta L, Piscitelli F, Giordano C, Boccella S, Lichtman A, Maione S & Di Marzo V (2012) Discovery of prostamide F2alpha and its role in inflammatory pain and dorsal horn nociceptive neuron hyperexcitability. *PLoS ONE* **7**, e31111.
182 Hsu KL, Tsuboi K, Adibekian A, Pugh H, Masuda K & Cravatt BF (2012) DAGLbeta inhibition perturbs a lipid network involved in macrophage inflammatory responses. *Nat Chem Biol* **8**, 999–1007.
183 Di Venere A, Dainese E, Fezza F, Angelucci BC, Rosato N, Cravatt BF, Finazzi-Agro A, Mei G & Maccarrone M (2012) Rat and human fatty acid amide hydrolases: overt similarities and hidden differences. *Biochim Biophys Acta* **1821**, 1425–1433.
184 Di Filippo C, Rossi F, Rossi S & D'Amico M (2004) Cannabinoid CB2 receptor activation reduces mouse myocardial ischemia-reperfusion injury: involvement of cytokine/chemokines and PMN. *J Leukoc Biol* **75**, 453–459.
185 Montecucco F, Lenglet S, Braunersreuther V, Burger F, Pelli G, Bertolotto M, Mach F & Steffens S (2009) CB(2) cannabinoid receptor activation is cardioprotective in a mouse model of ischemia/reperfusion. *J Mol Cell Cardiol* **46**, 612–620.
186 Defer N, Wan J, Souktani R, Escoubet B, Perier M, Caramelle P, Manin S, Deveaux V, Bourin MC, Zimmer A *et al.* (2009) The cannabinoid receptor type 2 promotes cardiac myocyte and fibroblast survival

and protects against ischemia/reperfusion-induced cardiomyopathy. *FASEB J* **23**, 2120–2130.
187 Lamontagne D, Lepicier P, Lagneux C & Bouchard JF (2006) The endogenous cardiac cannabinoid system: a new protective mechanism against myocardial ischemia. *Arch Mal Coeur Vaiss* **99**, 242–246.
188 Weis F, Beiras-Fernandez A, Sodian R, Kaczmarek I, Reichart B, Beiras A, Schelling G & Kreth S (2010) Substantially altered expression pattern of cannabinoid receptor 2 and activated endocannabinoid system in patients with severe heart failure. *J Mol Cell Cardiol* **48**, 1187–1193.
189 Batkai S, Jarai Z, Wagner JA, Goparaju SK, Varga K, Liu J, Wang L, Mirshahi F, Khanolkar AD, Makriyannis A *et al*. (2001) Endocannabinoids acting at vascular CB1 receptors mediate the vasodilated state in advanced liver cirrhosis. *Nat Med* **7**, 827–832.
190 Moezi L, Gaskari SA & Lee SS (2008) Endocannabinoids and liver disease. V. endocannabinoids as mediators of vascular and cardiac abnormalities in cirrhosis. *Am J Physiol Gastrointest Liver Physiol* **295**, G649–G653.
191 Batkai S, Mukhopadhyay P, Harvey-White J, Kechrid R, Pacher P & Kunos G (2007) Endocannabinoids acting at CB1 receptors mediate the cardiac contractile dysfunction in vivo in cirrhotic rats. *Am J Physiol Heart Circ Physiol* **293**, H1689–H1695.
192 Batkai S & Pacher P (2009) Endocannabinoids and cardiac contractile function: pathophysiological implications. *Pharmacol Res* **60**, 99–106.
193 Montecucco F, Matias I, Lenglet S, Petrosino S, Burger F, Pelli G, Braunersreuther V, Mach F, Steffens S & Di Marzo V (2009) Regulation and possible role of endocannabinoids and related mediators in hypercholesterolemic mice with atherosclerosis. *Atherosclerosis* **205**, 433–441.
194 Mach F & Steffens S (2008) The role of the endocannabinoid system in atherosclerosis. *J Neuroendocrinol* **20** (Suppl 1), 53–57.
195 Steffens S, Veillard NR, Arnaud C, Pelli G, Burger F, Staub C, Karsak M, Zimmer A, Frossard JL & Mach F (2005) Low dose oral cannabinoid therapy reduces progression of atherosclerosis in mice. *Nature* **434**, 782–786.
196 Montecucco F, Burger F, Mach F & Steffens S (2008) CB2 cannabinoid receptor agonist JWH-015 modulates human monocyte migration through defined intracellular signaling pathways. *Am J Physiol Heart Circ Physiol* **294**, H1145–H1155.
197 Pacher P & Ungvari Z (2008) Pleiotropic effects of the CB2 cannabinoid receptor activation on human monocyte migration: implications for atherosclerosis and inflammatory diseases. *Am J Physiol Heart Circ Physiol* **294**, H1133–H1134.
198 Montecucco F, Di Marzo V, da Silva RF, Vuilleumier N, Capettini L, Lenglet S, Pagano S, Piscitelli F, Quintao S, Bertolotto M *et al*. (2012) The activation of the cannabinoid receptor type 2 reduces neutrophilic protease-mediated vulnerability in atherosclerotic plaques. *Eur Heart J* **33**, 846–856.
199 Naccarato M, Pizzuti D, Petrosino S, Simonetto M, Ferigo L, Grandi FC, Pizzolato G & Di Marzo V (2010) Possible anandamide and palmitoylethanolamide involvement in human stroke. *Lipids Health Dis* **9**, 47.
200 Hillard CJ (2008) Role of cannabinoids and endocannabinoids in cerebral ischemia. *Curr Pharm Des* **14**, 2347–2361.
201 Muthian S, Rademacher DJ, Roelke CT, Gross GJ & Hillard CJ (2004) Anandamide content is increased and CB1 cannabinoid receptor blockade is protective during transient, focal cerebral ischemia. *Neuroscience* **129**, 743–750.
202 Zhang M, Adler MW, Abood ME, Ganea D, Jallo J & Tuma RF (2009) CB2 receptor activation attenuates microcirculatory dysfunction during cerebral ischemic/reperfusion injury. *Microvasc Res* **78**, 86–94.
203 Zhang M, Martin BR, Adler MW, Razdan RK, Jallo JI & Tuma RF (2007) Cannabinoid CB(2) receptor activation decreases cerebral infarction in a mouse focal ischemia/reperfusion model. *J Cereb Blood Flow Metab* **27**, 1387–1396.
204 Zhang M, Martin BR, Adler MW, Razdan RK, Ganea D & Tuma RF (2008) Modulation of the balance between cannabinoid CB(1) and CB(2) receptor activation during cerebral ischemic/reperfusion injury. *Neuroscience* **152**, 753–760.
205 Baty DE, Zhang M, Li H, Erb CJ, Adler MW, Ganea D, Loftus CM, Jallo JI & Tuma RF (2008) Cannabinoid CB2 receptor activation attenuates motor and autonomic function deficits in a mouse model of spinal cord injury. *Clin Neurosurg* **55**, 172–177.
206 Murikinati S, Juttler E, Keinert T, Ridder DA, Muhammad S, Waibler Z, Ledent C, Zimmer A, Kalinke U & Schwaninger M (2010) Activation of cannabinoid 2 receptors protects against cerebral ischemia by inhibiting neutrophil recruitment. *FASEB J* **24**, 788–798.
207 Kohro S, Imaizumi H, Yamakage M, Masuda Y, Namiki A & Asai Y (2004) Reductions in levels of bacterial superantigens/cannabinoids by plasma exchange in a patient with severe toxic shock syndrome. *Anaesth Intensive Care* **32**, 588–591.
208 Kohro S, Imaizumi H, Yamakage M, Masuda Y, Namiki A, Asai Y & Maruyama I (2006) Anandamide absorption by direct hemoperfusion with polymixin B-immobilized fiber improves the prognosis and organ failure assessment score in patients with sepsis. *J Anesth* **20**, 11–16.

209 Csoka B, Nemeth ZH, Mukhopadhyay P, Spolarics Z, Rajesh M, Federici S, Deitch EA, Batkai S, Pacher P & Hasko G (2009) CB2 cannabinoid receptors contribute to bacterial invasion and mortality in polymicrobial sepsis. *PLoS ONE* **4**, e6409.
210 Tschop J, Kasten KR, Nogueiras R, Goetzman HS, Cave CM, England LG, Dattilo J, Lentsch AB, Tschop MH & Caldwell CC (2009) The cannabinoid receptor 2 is critical for the host response to sepsis. *J Immunol* **183**, 499–505.
211 Kurabayashi M, Takeyoshi I, Yoshinari D, Matsumoto K, Maruyama I & Morishita Y (2005) 2-Arachidonoylglycerol increases in ischemia-reperfusion injury of the rat liver. *J Invest Surg* **18**, 25–31.
212 Batkai S, Osei-Hyiaman D, Pan H, El-Assal O, Rajesh M, Mukhopadhyay P, Hong F, Harvey-White J, Jafri A, Hasko G *et al.* (2007) Cannabinoid-2 receptor mediates protection against hepatic ischemia/reperfusion injury. *FASEB J* **21**, 1788–1800.
213 Ishii Y, Sakamoto T, Ito R & Yanaga K (2010) F2-isoprostanes and 2-arachidonylglycerol as biomarkers of lipid peroxidation in pigs with hepatic ischemia/reperfusion injury. *J Surg Res* **161**, 139–145.
214 Mendez-Sanchez N, Zamora-Valdes D, Pichardo-Bahena R, Barredo-Prieto B, Ponciano-Rodriguez G, Bermejo-Martinez L, Chavez-Tapia NC, Baptista-Gonzalez HA & Uribe M (2007) Endocannabinoid receptor CB2 in nonalcoholic fatty liver disease. *Liver Int* **27**, 215–219.
215 Deveaux V, Cadoudal T, Ichigotani Y, Teixeira-Clerc F, Louvet A, Manin S, Nhieu JT, Belot MP, Zimmer A, Even P *et al.* (2009) Cannabinoid CB2 receptor potentiates obesity-associated inflammation, insulin resistance and hepatic steatosis. *PLoS ONE* **4**, e5844.
216 Agudo J, Martin M, Roca C, Molas M, Bura AS, Zimmer A, Bosch F & Maldonado R (2010) Deficiency of CB2 cannabinoid receptor in mice improves insulin sensitivity but increases food intake and obesity with age. *Diabetologia* **53**, 2629–2640.
217 Rajesh M, Batkai S, Kechrid M, Mukhopadhyay P, Lee WS, Horvath B, Holovac E, Cinar R, Liaudet L, Mackie K *et al.* (2012) Cannabinoid 1 receptor promotes cardiac dysfunction, oxidative stress, inflammation, and fibrosis in diabetic cardiomyopathy. *Diabetes* **61**, 716–727.
218 Cote M, Matias I, Lemieux I, Petrosino S, Almeras N, Despres JP & Di Marzo V (2007) Circulating endocannabinoid levels, abdominal adiposity and related cardiometabolic risk factors in obese men. *Int J Obes (Lond)* **31**, 692–699.
219 Barutta F, Piscitelli F, Pinach S, Bruno G, Gambino R, Rastaldi MP, Salvidio G, Di Marzo V, Cavallo Perin P & Gruden G (2011) Protective role of cannabinoid receptor type 2 in a mouse model of diabetic nephropathy. *Diabetes* **60**, 2386–2396.
220 Barutta F, Corbelli A, Mastrocola R, Gambino R, Di Marzo V, Pinach S, Rastaldi MP, Perin PC & Gruden G (2010) Cannabinoid receptor 1 blockade ameliorates albuminuria in experimental diabetic nephropathy. *Diabetes* **59**, 1046–1054.
221 Annuzzi G, Piscitelli F, Di Marino L, Patti L, Giacco R, Costabile G, Bozzetto L, Riccardi G, Verde R, Petrosino S *et al.* (2010) Differential alterations of the concentrations of endocannabinoids and related lipids in the subcutaneous adipose tissue of obese diabetic patients. *Lipids Health Dis* **9**, 43.
222 Siegmund SV & Schwabe RF (2008) Endocannabinoids and liver disease. II. Endocannabinoids in the pathogenesis and treatment of liver fibrosis. *Am J Physiol Gastrointest Liver Physiol* **294**, G357–G362.
223 Munoz-Luque J, Ros J, Fernandez-Varo G, Tugues S, Morales-Ruiz M, Alvarez CE, Friedman SL, Arroyo V & Jimenez W (2008) Regression of fibrosis after chronic stimulation of cannabinoid CB2 receptor in cirrhotic rats. *J Pharmacol Exp Ther* **324**, 475–483.
224 Zyromski NJ, Mathur A, Pitt HA, Wade TE, Wang S, Swartz-Basile DA, Prather AD & Lillemoe KD (2009) Cannabinoid receptor-1 blockade attenuates acute pancreatitis in obesity by an adiponectin mediated mechanism. *J Gastrointest Surg* **13**, 831–838.
225 Borrelli F & Izzo AA (2009) Role of acylethanolamides in the gastrointestinal tract with special reference to food intake and energy balance. *Best Pract Res Clin Endocrinol Metab* **23**, 33–49.
226 Wright K, Rooney N, Feeney M, Tate J, Robertson D, Welham M & Ward S (2005) Differential expression of cannabinoid receptors in the human colon: cannabinoids promote epithelial wound healing. *Gastroenterology* **129**, 437–453.
227 Kimball ES, Schneider CR, Wallace NH & Hornby PJ (2006) Agonists of cannabinoid receptor 1 and 2 inhibit experimental colitis induced by oil of mustard and by dextran sulfate sodium. *Am J Physiol Gastrointest Liver Physiol* **291**, G364–G371.
228 Storr MA, Keenan CM, Zhang H, Patel KD, Makriyannis A & Sharkey KA (2009) Activation of the cannabinoid 2 receptor (CB2) protects against experimental colitis. *Inflamm Bowel Dis* **15**, 1678–1685.
229 Storr MA, Keenan CM, Emmerdinger D, Zhang H, Yuce B, Sibaev A, Massa F, Buckley NE, Lutz B, Goke B *et al.* (2008) Targeting endocannabinoid degradation protects against experimental colitis in mice: involvement of CB1 and CB2 receptors. *J Mol Med* **86**, 925–936.
230 Mukhopadhyay P, Rajesh M, Pan H, Patel V, Mukhopadhyay B, Batkai S, Gao B, Hasko G & Pacher P (2010) Cannabinoid-2 receptor limits inflammation, oxidative/nitrosative stress, and cell

death in nephropathy. *Free Radic Biol Med* **48**, 457–467.
231 Mukhopadhyay P, Pan H, Rajesh M, Batkai S, Patel V, Harvey-White J, Mukhopadhyay B, Hasko G, Gao B, Mackie K *et al*. (2010) CB1 cannabinoid receptors promote oxidative/nitrosative stress, inflammation and cell death in a murine nephropathy model. *Br J Pharmacol* **160**, 657–668.
232 Horvath B, Mukhopadhyay P, Kechrid M, Patel V, Tanchian G, Wink DA, Gertsch J & Pacher P (2012) beta-Caryophyllene ameliorates cisplatin-induced nephrotoxicity in a cannabinoid 2 receptor-dependent manner. *Free Radical Biol Med* **52**, 1325–1333.
233 Lim JC, Lim SK, Han HJ & Park SH (2010) Cannabinoid receptor 1 mediates palmitic acid-induced apoptosis via endoplasmic reticulum stress in human renal proximal tubular cells. *J Cell Physiol* **225**, 654–663.
234 Benito C, Nunez E, Tolon RM, Carrier EJ, Rabano A, Hillard CJ & Romero J (2003) Cannabinoid CB2 receptors and fatty acid amide hydrolase are selectively overexpressed in neuritic plaque-associated glia in Alzheimer's disease brains. *J Neurosci* **23**, 11136–11141.
235 Benito C, Romero JP, Tolon RM, Clemente D, Docagne F, Hillard CJ, Guaza C & Romero J (2007) Cannabinoid CB1 and CB2 receptors and fatty acid amide hydrolase are specific markers of plaque cell subtypes in human multiple sclerosis. *J Neurosci* **27**, 2396–2402.
236 Ramirez BG, Blazquez C, Gomez del Pulgar T, Guzman M & de Ceballos ML (2005) Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation. *J Neurosci* **25**, 1904–1913.
237 Maresz K, Carrier EJ, Ponomarev ED, Hillard CJ & Dittel BN (2005) Modulation of the cannabinoid CB2 receptor in microglial cells in response to inflammatory stimuli. *J Neurochem* **95**, 437–445.
238 Maresz K, Pryce G, Ponomarev ED, Marsicano G, Croxford JL, Shriver LP, Ledent C, Cheng X, Carrier EJ, Mann MK *et al*. (2007) Direct suppression of CNS autoimmune inflammation via the cannabinoid receptor CB1 on neurons and CB2 on autoreactive T cells. *Nat Med* **13**, 492–497.
239 Kim K, Moore DH, Makriyannis A & Abood ME (2006) AM1241, a cannabinoid CB2 receptor selective compound, delays disease progression in a mouse model of amyotrophic lateral sclerosis. *Eur J Pharmacol* **542**, 100–105.
240 Shoemaker JL, Seely KA, Reed RL, Crow JP & Prather PL (2007) The CB2 cannabinoid agonist AM-1241 prolongs survival in a transgenic mouse model of amyotrophic lateral sclerosis when initiated at symptom onset. *J Neurochem* **101**, 87–98.
241 Price DA, Martinez AA, Seillier A, Koek W, Acosta Y, Fernandez E, Strong R, Lutz B, Marsicano G, Roberts JL *et al*. (2009) WIN55,212–2, a cannabinoid receptor agonist, protects against nigrostriatal cell loss in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. *Eur J Neurosci* **29**, 2177–2186.
242 Benito C, Tolon RM, Pazos MR, Nunez E, Castillo AI & Romero J (2008) Cannabinoid CB2 receptors in human brain inflammation. *Br J Pharmacol* **153**, 277–285.
243 Palazuelos J, Aguado T, Pazos MR, Julien B, Carrasco C, Resel E, Sagredo O, Benito C, Romero J, Azcoitia I *et al*. (2009) Microglial CB2 cannabinoid receptors are neuroprotective in Huntington's disease excitotoxicity. *Brain* **132**, 3152–3164.
244 Palazuelos J, Davoust N, Julien B, Hatterer E, Aguado T, Mechoulam R, Benito C, Romero J, Silva A, Guzman M *et al*. (2008) The CB(2) cannabinoid receptor controls myeloid progenitor trafficking: involvement in the pathogenesis of an animal model of multiple sclerosis. *J Biol Chem* **283**, 13320–13329.
245 Sagredo O, Gonzalez S, Aroyo I, Pazos MR, Benito C, Lastres-Becker I, Romero JP, Tolon RM, Mechoulam R, Brouillet E *et al*. (2009) Cannabinoid CB2 receptor agonists protect the striatum against malonate toxicity: relevance for Huntington's disease. *Glia* **57**, 1154–1167.
246 Tolon RM, Nunez E, Pazos MR, Benito C, Castillo AI, Martinez-Orgado JA & Romero J (2009) The activation of cannabinoid CB2 receptors stimulates in situ and in vitro beta-amyloid removal by human macrophages. *Brain Res* **1283**, 148–154.
247 De March Z, Zuccato C, Giampa C, Patassini S, Bari M, Gasperi V, De Ceballos ML, Bernardi G, Maccarrone M, Cattaneo E *et al*. (2008) Cortical expression of brain derived neurotrophic factor and type-1 cannabinoid receptor after striatal excitotoxic lesions. *Neuroscience* **152**, 734–740.
248 Mestre L, Docagne F, Correa F, Loria F, Hernangomez M, Borrell J & Guaza C (2009) A cannabinoid agonist interferes with the progression of a chronic model of multiple sclerosis by downregulating adhesion molecules. *Mol Cell Neurosci* **40**, 258–266.
249 Loria F, Petrosino S, Hernangomez M, Mestre L, Spagnolo A, Correa F, Di Marzo V, Docagne F & Guaza C (2010) An endocannabinoid tone limits excitotoxicity in vitro and in a model of multiple sclerosis. *Neurobiol Dis* **37**, 166–176.
250 Pertwee RG (2005) The therapeutic potential of drugs that target cannabinoid receptors or modulate the tissue levels or actions of endocannabinoids. *AAPS J* **7**, E625–E654.

251 Calignano A, La Rana G, Giuffrida A & Piomelli D (1998) Control of pain initiation by endogenous cannabinoids. *Nature* **394**, 277–281.
252 Hanus L, Breuer A, Tchilibon S, Shiloah S, Goldenberg D, Horowitz M, Pertwee RG, Ross RA, Mechoulam R & Fride E (1999) HU-308: a specific agonist for CB(2), a peripheral cannabinoid receptor. *Proc Natl Acad Sci USA* **96**, 14228–14233.
253 Malan TP Jr, Ibrahim MM, Deng H, Liu Q, Mata HP, Vanderah T, Porreca F & Makriyannis A (2001) CB2 cannabinoid receptor-mediated peripheral antinociception. *Pain* **93**, 239–245.
254 Clayton N, Marshall FH, Bountra C & O'Shaughnessy CT (2002) CB1 and CB2 cannabinoid receptors are implicated in inflammatory pain. *Pain* **96**, 253–260.
255 Ibrahim MM, Deng H, Zvonok A, Cockayne DA, Kwan J, Mata HP, Vanderah TW, Lai J, Porreca F, Makriyannis A *et al.* (2003) Activation of CB2 cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: pain inhibition by receptors not present in the CNS. *Proc Natl Acad Sci USA* **100**, 10529–10533.
256 Ibrahim MM, Porreca F, Lai J, Albrecht PJ, Rice FL, Khodorova A, Davar G, Makriyannis A, Vanderah TW, Mata HP *et al.* (2005) CB2 cannabinoid receptor activation produces antinociception by stimulating peripheral release of endogenous opioids. *Proc Natl Acad Sci USA* **102**, 3093–3098.
257 Ibrahim MM, Rude ML, Stagg NJ, Mata HP, Lai J, Vanderah TW, Porreca F, Buckley NE, Makriyannis A & Malan TP Jr (2006) CB2 cannabinoid receptor mediation of antinociception. *Pain* **122**, 36–42.
258 Nackley AG, Makriyannis A & Hohmann AG (2003) Selective activation of cannabinoid CB(2) receptors suppresses spinal fos protein expression and pain behavior in a rat model of inflammation. *Neuroscience* **119**, 747–757.
259 Nackley AG, Suplita RL II & Hohmann AG (2003) A peripheral cannabinoid mechanism suppresses spinal fos protein expression and pain behavior in a rat model of inflammation. *Neuroscience* **117**, 659–670.
260 Nackley AG, Zvonok AM, Makriyannis A & Hohmann AG (2004) Activation of cannabinoid CB2 receptors suppresses C-fiber responses and windup in spinal wide dynamic range neurons in the absence and presence of inflammation. *J Neurophysiol* **92**, 3562–3574.
261 Quartilho A, Mata HP, Ibrahim MM, Vanderah TW, Porreca F, Makriyannis A & Malan TP Jr (2003) Inhibition of inflammatory hyperalgesia by activation of peripheral CB2 cannabinoid receptors. *Anesthesiology* **99**, 955–960.
262 Elmes SJ, Jhaveri MD, Smart D, Kendall DA & Chapman V (2004) Cannabinoid CB2 receptor activation inhibits mechanically evoked responses of wide dynamic range dorsal horn neurons in naive rats and in rat models of inflammatory and neuropathic pain. *Eur J Neurosci* **20**, 2311–2320.
263 Hohmann AG, Farthing JN, Zvonok AM & Makriyannis A (2004) Selective activation of cannabinoid CB2 receptors suppresses hyperalgesia evoked by intradermal capsaicin. *J Pharmacol Exp Ther* **308**, 446–453.
264 Scott DA, Wright CE & Angus JA (2004) Evidence that CB-1 and CB-2 cannabinoid receptors mediate antinociception in neuropathic pain in the rat. *Pain* **109**, 124–131.
265 Whiteside GT, Lee GP & Valenzano KJ (2007) The role of the cannabinoid CB2 receptor in pain transmission and therapeutic potential of small molecule CB2 receptor agonists. *Curr Med Chem* **14**, 917–936.
266 Rahn EJ, Zvonok AM, Thakur GA, Khanolkar AD, Makriyannis A & Hohmann AG (2008) Selective activation of cannabinoid CB2 receptors suppresses neuropathic nociception induced by treatment with the chemotherapeutic agent paclitaxel in rats. *J Pharmacol Exp Ther* **327**, 584–591.
267 Muller-Vahl KR & Emrich HM (2008) Cannabis and schizophrenia: towards a cannabinoid hypothesis of schizophrenia. *Expert Rev Neurother* **8**, 1037–1048.
268 Andreasson S, Allebeck P, Engstrom A & Rydberg U (1987) Cannabis and schizophrenia. A longitudinal study of Swedish conscripts. *Lancet* **2**, 1483–1486.
269 De Marchi N, De Petrocellis L, Orlando P, Daniele F, Fezza F & Di Marzo V (2003) Endocannabinoid signalling in the blood of patients with schizophrenia. *Lipids Health Dis* **2**, 5.
270 Ishiguro H, Horiuchi Y, Ishikawa M, Koga M, Imai K, Suzuki Y, Morikawa M, Inada T, Watanabe Y, Takahashi M *et al.* (2010) Brain cannabinoid CB2 receptor in schizophrenia. *Biol Psychiatry* **67**, 974–982.
271 Khan A, Kendall DA & Fone KCF (2010) The effects of the cannabinoid CB2 receptor antagonist, AM630, on isolation rearing-induced behavioural deficits in rats. *Schizophr Res* **117**, 391–392.
272 Grinspoon L & Bakalar JB (1995) Marihuana as medicine. A plea for reconsideration. *JAMA* **273**, 1875–1876.
273 Grinspoon L, Bakalar JB, Zimmer L & Morgan JP (1997) Marijuana addiction. *Science* **277**, 749; author reply 750-2.
274 Onaivi ES, Ishiguro H, Gong JP, Patel S, Meozzi PA, Myers L, Perchuk A, Mora Z, Tagliaferro PA, Gardner E *et al.* (2008) Functional expression of brain neuronal CB2 cannabinoid receptors are involved in the effects of drugs of abuse and in depression. *Ann NY Acad Sci* **1139**, 434–449.

275 Garcia-Gutierrez MS, Perez-Ortiz JM, Gutierrez-Adan A & Manzanares J (2010) Depression-resistant endophenotype in mice overexpressing cannabinoid CB(2) receptors. *Br J Pharmacol* **160**, 1773–1784.
276 Hu B, Doods H, Treede RD & Ceci A (2009) Depression-like behaviour in rats with mononeuropathy is reduced by the CB2-selective agonist GW405833. *Pain* **143**, 206–212.
277 Garci AGMA & Manzanares J (2011) Overexpression of CB2 cannabinoid receptors decreased vulnerability to anxiety and impaired anxiolytic action of alprazolam in mice. *J Psychopharmacol* **25**, 11–20.
278 Richardson D *et al*. (2008) Characterisation of the cannabinoid receptor system in synovial tissue and fluid in patients with osteoarthritis and rheumatoid arthritis. *Arthritis Res Ther* **10**, R43.
279 Blazquez C, Carracedo A, Barrado L, Real PJ, Fernandez-Luna JL, Velasco G, Malumbres M & Guzman M (2006) Cannabinoid receptors as novel targets for the treatment of melanoma. *FASEB J* **20**, 2633–2635.
280 Zheng D, Bode AM, Zhao Q, Cho YY, Zhu F, Ma WY & Dong Z (2008) The cannabinoid receptors are required for ultraviolet-induced inflammation and skin cancer development. *Cancer Res* **68**, 3992–3998.
281 McKallip RJ, Lombard C, Fisher M, Martin BR, Ryu S, Grant S, Nagarkatti PS & Nagarkatti M (2002) Targeting CB2 cannabinoid receptors as a novel therapy to treat malignant lymphoblastic disease. *Blood* **100**, 627–634.
282 Guida M, Ligresti A, De Filippis D, D'Amico A, Petrosino S, Cipriano M, Bifulco G, Simonetti S, Orlando P, Insabato L *et al*. (2010) The levels of the endocannabinoid receptor CB2 and its ligand 2-arachidonoylglycerol are elevated in endometrial carcinoma. *Endocrinology* **151**, 921–928.

the FEBS Journal

MINIREVIEW

Modulating the endocannabinoid system in human health and disease – successes and failures

Pál Pacher and George Kunos

Laboratory of Physiologic Studies, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD, USA

Keywords
cannabinoids; clinical trials; disease; endocannabinoid system; human; pharmacology; therapeutic potential

Correspondence
P. Pacher, Laboratory of Physiological Studies, National Institutes of Health/ NIAAA, 5625 Fishers Lane, MSC-9413, Bethesda, MD 20892-9413, USA
Fax: +1 301 480 0257
Tel: +1 301 443 4830
E-mail: pacher@mail.nih.gov

(Received 14 January 2013, revised 11 March 2013, accepted 19 March 2013)

doi:10.1111/febs.12260

The discovery of the endocannabinoid system, comprising the G-protein coupled cannabinoid 1 and 2 receptors ($CB_{1/2}$), their endogenous lipid ligands or endocannabinoids, and synthetic and metabolizing enzymes, has triggered an avalanche of experimental studies implicating the endocannabinoid system in a growing number of physiological/pathological functions. These studies have also suggested that modulating the activity of the endocannabinoid system holds therapeutic promise for a broad range of diseases, including neurodegenerative, cardiovascular and inflammatory disorders; obesity/metabolic syndrome; cachexia; chemotherapy-induced nausea and vomiting; and tissue injury and pain, amongst others. However, clinical trials with globally acting CB_1 antagonists in obesity/metabolic syndrome, and other studies with peripherally-restricted $CB_{1/2}$ agonists and inhibitors of the endocannabinoid metabolizing enzyme in pain, have introduced unexpected complexities, suggesting that a better understanding of the pathophysiological role of the endocannabinoid system is required to devise clinically successful treatment strategies.

Introduction

Although *Cannabis sativa* (the marijuana plant) is one of the most ancient medicinal plants in the history of medicine [1], the clinical use of synthetic cannabinoids or medicinal plant extracts has been largely empirical and limited to a few specific indications related to pain, wasting disorders, and chemotherapy-induced nausea and vomiting, as a result of their socially undesirable psychoactive properties [2]. The discovery of endocannabinoids (ECs), which mimic some of the effects of synthetic cannabinoids *in vivo*, their G-protein coupled receptors, as well as their synthetic and metabolizing enzymes, has prompted preclinical studies aiming to explore the role of the endocannabinoid system (ECS) in health and disease [2–4]. These studies have been greatly facilitated by the introduction of mice deficient in cannabinoid receptors or EC degrading enzymes, as well as selective cannabinoid receptor ligands and inhibitors of EC metabolism. The results of these studies have implicated the ECS in a variety of physiopathological processes, both in the peripheral and central nervous systems and in various peripheral organs [2]. Such studies have further suggested that modulating ECS activity may have therapeutic potential in almost all diseases affecting humans, including obesity/metabolic syndrome [5]; diabetes and diabetic complications [6]; neurodegenerative [7,8], inflammatory [9], cardiovascular [10–12], liver [13,14], gastrointestinal [15] and skin [16] diseases; pain [17,18]; psychiatric disorders [19,20]; cachexia [2]; cancer [21,22]; and chemotherapy-induced nausea and vomiting [23], amongst many others [2].

Abbreviations
2-AG, 2-arachidonoylglycerol; AEA, anandamide or arachidonoyl ethanolamide; $CB_{1/2}$, cannabinoid receptor 1 or 2; CBD, cannabidiol; CNS, central nervous system; EC, endocannabinoid; ECS, endocannabinoid system; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; MS, multiple sclerosis; THC, Δ9-tetrahydrocannabinol; $TRPV_1$, transient receptor potential cation channel subfamily V member 1.

These investigations have also uncovered the remarkable complexity of the ECS, as exemplified by differences in the therapeutic profile of activating/inhibiting the same receptor in the central nervous system (CNS) or in peripheral tissues, by the intriguing overlap between EC and eicosanoid signalling, or by the often opposite effects mediated by cannabinoid 1 and 2 receptors ($CB_{1/2}$) receptors in disease models [2–4,6,24]. Similar complexities have emerged in clinical trials targeting the ECS. Although globally acting (i.e. brain-penetrant) CB_1 antagonists/inverse agonists were shown to have therapeutic efficacy in obesity/metabolic syndrome, they elicited anxiety/depression in a small proportion of subjects, which has led to their withdrawal from the market worldwide and halted their further therapeutic development [5,25,26]. The first human trial with peripherally-restricted mixed $CB_{1/2}$ agonist(s) for pain failed as a result of cardiovascular and metabolic side effects and hepatotoxicity [27,28]. Amplifying the ECS tone by inhibiting EC metabolism was ineffective in alleviating osteoarthritic pain in human subjects [29,30]. Thus, we need to better understand the pathophysiological function of the ECS in humans, as well as refine the indications and design of clinical trials, so that it is possible to successfully translate recent progress in cannabinoid biology into clinically effective treatment strategies.

The present minireview discusses preclinical evidence implicating the ECS in human disease, and reviews the treatment strategies that target the ECS for therapeutic gain in humans. Because of limitations of space, reference is also made to recent overviews on specific subjects, rather than to original papers.

The ECS

Δ9-Tetrahydrocannabinol (THC), the putative psychoactive ingredient of marijuana, and its endogenous counterparts, anandamide (arachidonoyl ethanolamide) (AEA) and 2-arachidonoylglycerol (2-AG), exert their primary effects through $CB_{1/2}$ receptors; 2-AG favours CB_2, whereas AEA binds with higher affinity to CB_1 [2], although, at higher concentrations, it may also modulate transient receptor potential cation channel subfamily V member 1 ($TRPV_1$) and other receptors. Signalling by cannabinoid receptors is complex because it may involve both G protein-dependent pathways, such as inhibition of adenyl cyclase or the modulation of ion channel function, and G protein-independent mechanisms, including the activation of various mitogen-activated protein kinases (p44/42 mitogen-activated protein kinases, p38, extracellular signal-regulated kinase and c-Jun N-terminal kinase) or ceramide signalling [2,31,32].

CB_1 receptors, the most abundant G-protein coupled receptor in the mammalian brain, mediate the socially undesirable psychoactive effects of cannabis. Although their expression was initially considered to be restricted to the brain, more recent studies have identified CB_1 receptors in almost all peripheral tissues and cell types, albeit at much lower densities than in the brain, and documented their important regulatory functions [2,3,5]. CB_2 receptors are largely restricted to immune and haematopoetic cells, although functionally relevant expression has been found in specific regions of the brain and in the myocardium, gut, endothelial, vascular smooth muscle and Kupffer cells, exocrine and endocrine pancreas, bone, and reproductive organs/cells, as well as in various tumours [4]. Both cannabinoid receptors may undergo rapid internalization and intracellular trafficking upon agonist exposure [33,34].

In the CNS, AEA and 2-AG are synthesized 'on demand' and released to act as retrograde transmitters on CB_1 receptors [35–37]. They are not stored and are rapidly degraded after exerting a transient and localized effect [38]. The synthesis of ECs largely depends on the intracellular Ca^{2+}-concentration. AEA is mainly formed via a two step-pathway, involving a Ca^{2+}-dependent *N*-acyltranferase and *N*-acylphosphatidylethanolamine-hydrolyzing phospholipase D, whereas diacylglycerol lipase and phospholipase Cβ are mainly responsible for the biosynthesis of 2-AG [3,37]. The existence of additional, parallel biosynthetic pathways for AEA has also been proposed [39,40].

AEA and 2-AG are removed from the extracellular space by a process of cellular uptake and metabolism; however, the putative transporter(s) involved have not yet been cloned, and are the subject of much controversy [41–43]. AEA is degraded primarily by fatty acid amide hydrolase (FAAH) and 2-AG is degraded by monoacylglycerol lipase (MAGL) [3,44], although additional enzymes have also been implicated in the degradation of both AEA and 2-AG [45,46]. Endocannabinoids may also be metabolized by cyclooxygenases, lipooxygenases and cytochrome P450, leading to the formation of bioactive metabolites that may activate CB receptor-independent mechanisms [24,47]. It is also important to note that FAAH and MAGL are also responsible for the degradation of numerous potentially bioactive lipids. Thus, the biological consequences of the inhibition of these enzymes are not necessarily a result of enhanced EC levels. Some of the enzymes involved in EC synthesis/degradation may exist in several forms and their activity may vary in

different tissues or even in different regions of the same tissue [3,37,48–52].

In addition to AEA and 2-AG, several other EC-like molecules have been discovered, although their activities have not been studied in sufficient detail [53,54]. Interestingly, recent studies have identified novel peptide allosteric negative modulators of CB_1 receptors [55], the biological significance of which is yet to be determined. Additionally, the anti-inflammatory lipid lipoxin A4 may be an endogenous allosteric enhancer of CB_1 receptors [56]. A comprehensive overview of the ECS is beyond the scope of the present minireview; instead, several detailed reviews are available on this subject [3,24,37,57].

The ECS in health and disease

Despite the ubiquitous expression of the various components of the ECS, their genetic ablation or pharmacological blockade in normal, healthy animals has minimal functional consequences, which suggests that the ECS has minimal or no tonic activity under normal physiological conditions [2,4]. On the other hand, an increase or decrease in ECS tone is associated with various pathological states, as a result of the altered expression of CB receptors, endocannabinoid metabolizing enzymes and/or synthetic pathways, in a tissue-specific and time-dependent manner. Examples of selected pathologies in which dysregulation of the ECS was reported (in most cases, up-regulation of $CB_{1/2}$ and/or an increase in tissue levels of ECs) are shown in Table 1, and have been summarized in more detail elsewhere [2–4,58,59]. In some cases, altered ECS activity is transient and forms part of the body's compensatory response to a particular insult, thus reducing symptoms and/or slowing progression of the disease (e.g. in neuropathic pain); in other cases, activation of the ECS may be pathogenic (e.g. in various forms of shock or diabetic complications) or may reflect a deficiency (e.g. in various tumours) of unknown significance [2].

From a therapeutic standpoint, the identification of regional or tissue-specific changes in CB receptors is important because their possible selective targeting may mitigate unwanted side effects [59,60]. However, these changes can serve as a basis for successful drug development only as long as they are determined using appropriate tools (e.g. specific antibodies), the specificity of which needs to be carefully validated [4,61]. It is also very important to understand the underlying mechanisms of these alterations; for example, is the increase in the tissue level of an EC the result of its increased biosynthesis or a decrease in its enzymatic degradation?

Cardiovascular consequences of targeting the ECS in health and disease

Because many promising drugs fail in clinical development as a result of cardiovascular side effects, it is important to briefly overview the cardiovascular consequences of modulating the ECS. ECs exert complex cardiovascular effects that are dominated by a decrease in blood pressure and myocardial contractility, mediated primarily by CB_1 receptors located in the myocardium, vasculature and neurones in the central and autonomic nervous systems [2,62]. In cultured human coronary artery endothelial cells [63] and cardiomyocytes [64], CB_1 activation promotes stress signalling and cell death, and decreases contractility [10,12]. By contrast, activation of cardiovascular CB_2 receptors does not have adverse haemodynamic consequences [11]. CB_1, CB_2 or FAAH knockout mice have normal blood pressure, myocardial contractility and/or baroreflex sensitivity, indicating the minimal role of the ECS in normal cardiovascular regulation [2]. However, in several pathological conditions (e.g. shock, heart failure, cardiomyopathies, advanced liver cirrhosis), the ECS may become activated to promote hypotension/cardiodepression through cardiovascular CB_1 receptors [2,10]. CB_1 receptor signalling may also promote disease progression in preclinical models of heart failure [64–66] and atherosclerosis [67,68], and contributes to increased cardiovascular risk (e.g. plasma lipid alterations, abdominal obesity, hepatic steatosis, insulin and leptin resistance) in obesity/metabolic syndrome and diabetes, both in rodents and humans [5,69–71]. By contrast, CB_2 signalling in the heart and vasculature may activate cardioprotective mechanisms and limit inflammation [11].

Acute or chronic use of marijuana may decrease or increase the heart rate and decrease blood pressure depending on the duration of the use, dose and route of administration [2,10]. An elevated resting heart rate is a known independent risk factor for cardiovascular disease in healthy men and women [72]. A recent controlled study at the National Institute on Drug Abuse evaluated the development of tolerance to the effects of oral synthetic THC in 13 healthy male daily cannabis smokers who were residing on a secure research unit over a period of 6 days [73]. Despite the development of tolerance to the subjective intoxicating effect of THC, no tolerance was observed to its hypotensive and tachycardic effects [73]. Another recent study of 72 young male cannabis users and 72 matched controls reported an increased heart rate variability in cannabis users [74]. Surinabant, a selective CB_1 antagonist, has

Table 1. Examples of the dysregulation of the ECS in disease. C, canine; H, human; P, pig; R, rodent. ND, not determined.

Disease, sample	Expression/changes in $CB_{1/2}$	Changes in endocannabinoid levels	Proposed role of CB receptors in disease	Reference
Myocardial infarction (ischaemia-reperfusion injury) (R, P, H)	Myocardium, in human epicardial adipose tissues of ischaemic hearts, up-regulation of CB_1 and protein kinase A, accompanied by CB_2 and FAAH down-regulation, increased inducible NOS/endothelial NOS ration and reduced cell survival signalling	Increase in circulating immune cells or in serum of obese patients with adverse cardiovascular events; Elevated endocannabinoid plasma levels are strongly associated with coronary dysfunction in obese human subjects	CB_2: decrease in leukocyte infiltration and enhancement of pro-survival pathways; CB_1: contribution to cardiovascular dysfunction, cell death/dysfunction in human endothelial cells and cardiomyocytes; central hypothermia (the latter is only in rodents and can be protective)	11,12,76, 85–87,90, 184–187
Heart failure, cardiomyopathies (R, H)	Myocardium, cardiomyocytes, endothelial cells	Myocardium, cardiomyocytes, circulating immune cells and platelets	CB_2: attenuation of inflammation/injury; CB_1: promotion of cardiac dysfunction and cell death in cardiomyocytes and endothelial cells	64,65,186, 188–192
Atherosclerosis, restenosis (R, H)	Infiltrating and other immune cells, vascular smooth muscle and endothelium	Serum, atherosclerotic plaques	CB_2: context-dependent attenuation or promotion of vascular inflammation (monocyte chemotaxis, infiltration and activation) and factors of plaque stability; attenuation of vascular smooth muscle proliferation; CB_1: increase of vascular inflammation and/or plaque vulnerability	67,84,133,134, 193–198
Stroke, spinal cord injury (R, H)	Brain, microglia, infiltrating immune cells, endothelium	Serum, brain	CB_2: attenuation of inflammation (endothelial activation, leukocyte infiltration), and tissue injury, attenuation of motor and autonomic deficits in a mouse model of spinal cord injury; CB_1: promotes hypothermia-dependent protection but, if hypothermia is compensated, ineffective or enhances injury	90,199–206
Cirrhotic cardiomyopathy (R, H)	ND	Myocardium, circulating immune cells and platelets	CB_2: attenuation of hypotension by decreasing liver inflammation; CB_1: contribution to cardiovascular dysfunction	189–192
Septic shock by live bacteria (R, H)	ND	Serum	CB_2: decrease or increase in inflammation and tissue injury most likely by affecting bacterial load; CB_1: contribution to cardiovascular collapse	10,207–210
Hepatic ischaemia-reperfusion injury (R, P, H)	Inflammatory immune cells, activated endothelium	Liver, serum, hepatocytes, Kupffer and endothelial cells	CB_2: attenuation of inflammation (endothelial activation, leukocyte chemotaxis, infiltration and activation), oxidative stress, and tissue injury; CB_1: promotion of liver injury	135,138,211–213
Obesity, non-alcoholic fatty liver disease, diabetic complications (R, H)	Hepatocytes, inflammatory cells, adipocytes, certain neurones, sites of diabetic complications (kidneys, retina and myocardium)	Liver, adipose tissue, brain, skeletal muscle, diabetic kidneys, hearts, retinas, serum	CB_2: enhancement of high fat diet-induced steatosis and inflammation or attenuation of obesity associated one with age; CB_1: increase in fat storage, decrease in metabolism, promotion of insulin and leptin resistance and inflammation in adipose tissue and in the liver	5,6,70,101,108, 214–221

Table 1. (Continued).

Disease, sample	Expression/changes in $CB_{1/2}$	Changes in endocannabinoid levels	Proposed role of CB receptors in disease	Reference
Liver fibrosis, cirrhosis, alcohol-induced liver injury (R, H)	Activated stellate cells, inflammatory cells, hepatocytes, Kupffer cells	Liver, serum, inflammatory cells	CB_2: attenuation of fibrosis and injury/inflammation; CB_1: increase in fibrosis/injury	14,136,137,191, 222,223
Pancreatitis (R, H)	Pancreas	Inflamed pancreas	CB_2: attenuation of inflammation; CB_1: context-dependent effect	145,146,148,224
Inflammatory bowel disease, colitis, diverticulitis (R, H)	Epithelial cells, infiltrating inflammatory cells, enteric nerves	Inflamed gut	Attenuation of inflammation and visceral sensitivity	130,151,225–229
Nephropathy (R, H)	Kidney, human proximal tubular cells, podocytes	Kidney	CB_2: attenuation of inflammation (chemokine signalling and chemotaxis, inflammatory cell infiltration and endothelial activation) and oxidative stress; CB_1: promotion of inflammation/injury	105,219,220, 230–233
Neurodegenerative/ neuroinflammatory disorders (multiple sclerosis, Alzheimer's, Parkinson's and Huntington's disease, spinal cord injury) (R, H)	Microglia, inflammatory cells, brain lesions, neurones?	Brain, spinal fluid	CB_2: attenuation of inflammation (microglia activation, secondary immune cell infiltration), facilitation of neurogenesis; CB_1: attenuation of excitotoxicity, hypothermia; context-dependent effect on injury/inflammation	2,7,91,92,152, 205,234–250
Pain (R)	Inflammatory cells, certain neurones	Site of induced chronic inflammatory pain	CB_2: attenuation of inflammatory pain via unknown mechanism(s); CB_1: attenuation of various forms of pain by inhibiting neurotransmission	17,95,96,251–266
Psychiatric disorders (anxiety and depression, schizophrenia) (R, H)	Glial, inflammatory cells, neurones?	Blood, cerebrospinal fluid, brain (increased in schizophrenia, but decreased in brain in depression)	CB_2: largely unexplored, in rodent models of depression/anxiety, it may modulate CNS inflammation and either attenuate or promote anxiety like behaviour; CB_1: context-dependent effect on anxiety, improved sleep	19,267–277
Rheumatoid arthritis (H)	ND	Synovial fluid, synovia	CB_2: attenuation of the autoimmune inflammatory response; CB_1: attenuation of pain	278
Cancer (R, H)	In various tumours or cancer cells	Various tumours	$CB_{1/2}$: context-dependent attenuation or promotion of tumour growth (apoptosis, angiogenesis, proliferation, etc.)	279–282, 2,22,149, 155,157

recently been reported to inhibit THC-induced central nervous system and heart rate effects in humans, providing proof of principle that those effects were indeed mediated by CB_1 receptor activation [75]. At the 20th International Cannabinoid Research Society meeting in Sweden, AstraZeneca presented data from the first clinical studies investigating two novel, peripherally-restricted, orally active mixed $CB_{1/2}$ agonists (AZD1940 and AZD1704). The study was terminated as a result of adverse cardiovascular effects, weight gain and mild hepatotoxicity [27,28].

An increasing number of case reports associates marijuana smoking with the precipitation of acute coronary syndrome [76]. Alarmingly, this occurs mostly in young healthy subjects without any previous cardiovascular disease [77,78]. A retrospective study assessed the risk of acute coronary syndrome after exposure to marijuana smoke. It was found that the risk of myocardial infarction was highest during the first hour of exposure [79]. The effect of marijuana use on mortality after acute myocardial infarction was assessed in a prospective study involving 1913 adults who were hospitalized with myocardial infarction at 45 US hospitals between 1989 and 1994, with a median follow-up of 3.8 years. The results indicated that marijuana use may pose an increased risk of infarction in susceptible individuals with coronary heart disease [80]. A more recent study evaluated the consequences of marijuana use and long-term mortality among survivors of acute myocardial infarction, and found that habitual marijuana use among patients presenting with acute myocardial infarction was associated with an apparent increase in mortality rate (29% higher) over the subsequent 18 years, although this did not reach statistical significance because of the limited sample size [81]. In the absence of large-scale, long-term controlled studies with repeated measures of marijuana use, a firm conclusion on the long-term impact of cannabis use on cardiovascular mortality cannot be drawn. Nevertheless, the above findings are of concern. Because THC is a relatively weak CB_1 agonist compared to many synthetic ligands, and also activates cardioprotective CB_2 receptors and is a potent antioxidant, it may be predicted that the uncontrolled spread and use of mixtures of potent synthetic CB_1 agonists (spice, K2, etc.) employed as recreational drugs would lead to significantly greater cardiovascular morbidity. Indeed, in a recent case series in healthy children, myocardial infarction was precipitated by synthetic cannabinoid use [82], and another study reported tachycardia, loss of consciousness and diffuse pain in two adolescents [83].

What is the situation regarding the ECS and cardiovascular pathology? As noted previously, EC/CB_1 receptor signalling has been implicated as a pathogenic factor in rodent models of cardiovascular diseases, including atherosclerosis, shock and various forms of cardiomyopathy. However, ECs were also reported to exert protective effects, based mostly on *ex vivo* and indirect studies, via CB_2 and CB-receptor independent mechanisms. Clearly, selective CB_2 agonists exert beneficial effects in rodent models of myocardial infarction by limiting inflammatory cell infiltration (in cardiomyocytes, the expression of CB_2 is very low, if any) [11]. To analyze the role of the ECS more directly, a recent study employed FAAH knockout mice with a 2.5- to three-fold increase in myocardial AEA content. When such mice were used to induce various experimental models of cardiomyopathy, they displayed increased mortality, tissue injury and neutrophil infiltration in the heart, which could be partially rescued by CB_1 antagonists [66]. Consistent with this report, a recent study showed that FAAH deficiency enhanced intraplaque neutrophil recruitment in atherosclerotic mice and increased a pro-inflammatory immune response [84]. These findings indicate that the primary cardiovascular effects of elevated EC tone are deletorious and are mediated by CB_1 receptors.

In obese human subjects, increased plasma levels of AEA and 2-AG were strongly associated with coronary circulatory dysfunction, suggesting that plasma EC levels may be used as biomarkers of cardiovascular risk in obesity [85]. In another study, increased plasma AEA and 2-AG levels positively correlated with impaired coronary endothelial function in obese subjects [86]. In samples of epicardial fat from ischaemic human hearts, the up-regulation of CB_1 was accompanied by down-regulation of CB_2 and FAAH compared to non-ischaemic hearts [87]. CB_1 receptor density was significantly higher in atherosclerotic coronary artery sections from patients with unstable angina compared to those with stable angina [67]. A G1359A polymorphism in the CB_1 receptor gene was also associated with coronary artery disease in the Chinese Han population, although the effect of this polymorphism on receptor function is unknown [88]. Both ECs were reported to inhibit human cardiac Kv4.3 channels at fairly low concentrations in ovary cells expressing Kv4.3 or in human cardiomyocytes in a receptor-independent manner [89], a harbinger of pro-arrhythmic risk.

Thus, it is clear that the activation of CB_1 receptors by synthetic ligands or ECs is associated with adverse cardiovascular consequences, which must be given very careful consideration during the preclinical/clinical development of new drugs targeting the ECS.

Activation of $CB_{1/2}$ receptors: THC, synthetic agonists and cannabinoid extracts

THC (dronabinol; Marinol; Solvay Pharmaceuticals, Brussels, Belgium) and its synthetic analogue nabilone (Cesamet; Valeant Pharmaceuticals, Irvine, CA, USA) have been approved by the Food and Drug Administration for treatment of chemotherapy-induced nausea and vomiting and for stimulating appetite in wasting disorders (e.g. AIDS, tumour cachexia, etc). Sativex (GW Pharmaceuticals, Salisbury, Wiltshire, UK), an oromucosal spray containing THC and the nonpsychoactive plant cannabinoid, cannabidiol (CBD), has recently been approved in Canada, the UK and several other European countries for the symptomatic relief of neuropathic pain and spasticity associated with multiple sclerosis, and as an adjunctive analgesic treatment for adults with advanced cancer. However, the therapeutic utility of THC and its synthetic analogues are limited because of their unwanted psychotropic effects mediated by central CB_1 receptors. The present minireview summarizes only the clinically most relevant indications.

Earlier preclinical studies suggested that ECs or plant-derived cannabinoids exert neuroprotective effects in the CNS by: (a) modulating excitability and calcium homeostasis via effects on various ion channels (Ca^{2+}, Na^+, K^+), intracellular Ca^{2+} stores and gap junctions and *N*-methyl-D-aspartate receptors; (b) attenuating excitatory glutamatergic transmissions and modulating synaptic plasticity via presynaptic CB_1 receptors; (c) inducing CB_1 receptor-mediated hypothermia; (d) exerting antioxidant effects; and (e) modulating immune responses and the release of proinflammatory mediators by CB_1, CB_2 and non-CB_1/CB_2 receptors on microglia, astrocytes, macrophages, neutrophils, lymphocytes and neurones [2]. Numerous recent studies have suggested that many of the previously described protective effects of synthetic CB_1 ligands were attributable to centrally-mediated hypothermia and/or receptor-independent antioxidant/anti-inflammatory effects of the compounds, and that ECs through the activation of CB_1 receptors may also promote tissue injury and neurodegeneration (e.g. in stroke and other forms of I/R injury) [6,90–92].

Historical documents reveal that one of the earliest uses of cannabis was to treat pain [93]. Studies in modern times initially focused on CB_1 receptors and demonstrated beneficial effects of cannabinoids in rodent models of acute and chronic pain. The results suggested that the observed antinociceptive effects have complex mechanisms involving actions in the CNS, spinal cord and peripheral sensory nerves [2,94]. Recent evidence also implicates CB_2 receptors in the antihyperalgesic activity of cannabinoids [95,96]; however, the exact mechanisms and cellular targets are elusive because of a lack of reliable antibodies for CB_2 [4].

In humans, the analgesic activity of THC and other cannabinoids is less clear-cut because cannabinoids are relatively weak analgesics compared to opiates, even when they do show efficacy [2]. The clinical data on THC, CBD and their combinations have been comprehensively reviewed elsewhere [97,98]. The primary focus of these studies has been the safety/efficacy and symptom relief (e.g. bladder incontinence, limb spasticity, pain and sleep quality) in multiple sclerosis (MS) or other pain-related conditions. Three studies have demonstrated that cannabis extract in MS patients improved urinary incontinence [98]. A number of controlled and blinded trials evaluating the efficacy of oral or sublingual cannabis/Sativex on spasticity in MS found that, at doses lacking overt psychoactivity, these drugs show no or minimal efficacy, as assessed by the objective outcomes using the Ashworth scale. However, the treatment consistently improved subjective, patient-assessed endpoints (spasms, pain, spasticity, sleep quality). Follow-up studies using a patient assessed numeric rating scale for spasticity showed significant benefits of Sativex compared to placebo [98]. It could be argued that some of the benefits observed were a result of mood improvement (patients feel subjective improvement) but, because only some of the symptoms were improved (spasticity, pain and sleep quality), this may not be the case. In patients treated with THC for 1 year, improvements using the Ashworth scale were reported [98]. Zhornitsky and Potvin [97] performed a meta-analyses of the data from 33 studies with CBD alone or in various combinations with THC, with the rationale for combining THC and CBD being to attenuate the psychoactive effects of THC by CBD, based on empirical evidence obtained in some studies. Among these studies, 16 had been conducted in healthy subjects and 17 in clinical populations, including four in MS, three in neuropathic and cancer pain, four in schizophrenia and bipolar mania, two in social anxiety disorder, and one each in cancer-related anorexia, Huntington's disease, insomnia and epilepsy [97]. It was concluded that, depending on the study and on the THC/CBD ratio, CBD may prolong/intensify or inhibit THC-induced effects. In some of these studies, THC or CBD+THC was more effective at reducing pain, although, in other studies, CBD alone also exerted (or completely lacked) analgesic properties. Notably, several of these studies used

multiple pain assessment scores, and the treatments were effective when evaluated by some but not by other scales [97]. In one of the studies in which the oral administration of CBD+THC in MS was not effective in improving symptoms, immunological analysis unexpectedly revealed a certain pro-inflammatory effect of the drug [97]. The preliminary clinical evidence was concluded to suggest that high-dose oral CBD may have therapeutic benefits in social anxiety disorder, insomnia and epilepsy, although it may also cause mental sedation [97].

Taken together, the above studies in MS show consistent improvements in subjective rather than quantitative symptomatic outcome measures (including pain), which supports the beneficial effects of cannabinoid-based medicines in neuropathic pain associated with MS. The relatively poor efficacy observed in some clinical studies may be attributable to pharmacokinetic problems such as first-pass effects via the liver and slow absorption via the oral route of administration, which may also limit the success of self-titration [98]. In most of these studies, formulations containing THC frequently caused generally mild to moderate side effects. However, with individual dose-titration, which can be better achieved by using the oromucosal Sativex spray, side effects can be further attenuated. Initial dose-titration may also help in the early selection of responders and exclusion of nonresponders. Future clinical studies should explore how cannabinoid-based medicines affect MS progression. In light of the preclinical data, the combination of THC with CBD appears to be the most promising, given the neuroprotective effects of CBD observed in numerous preclinical studies [99].

There is considerable interest in developing THC-based medicines for other forms of pain, such as pain associated with cancer or diabetic neuropathy. However, under these conditions, we should also carefully weigh the potential effect of the treatment on cancer and/or diabetes progression. Regarding cancer, although numerous studies suggest that THC may slow down the growth/progression of certain types of cancers in preclinical models, others suggest that THC may in fact promote cancer growth, and cannabinoid receptor deletion or inhibition is beneficial [2,4,22]. In addition, the results of a clinical study evaluating the association between ECS activity and survival and pain in pancreatic cancer indicate that, although patients with high CB_1 receptor expression in enlarged nerves in pancreatic ductal adenocarcinoma had a lower combined pain score (intensity, frequency, duration), they had significantly shorter survival [100]. For CBD, the evidence more clearly suggests potential benefits in multiple preclinical tumour models [99]. In the case of diabetes and diabetic complications, there is strong evidence (both preclinical and clinical) indicating that CB_1 activation promotes primary diabetes and also contributes to all diabetic complications (including neuropathy), and that CB_1 antagonists can prevent or reverse these changes, as well as insulin resistance [6,69,101].

Interestingly, analysis of cross-sectional data from the National Health and Nutrition Examination Survey (NHANES III, 1988–1994) indicated that marijuana use was independently associated with a lower prevalence of diabetes mellitus [102], and glucose tolerance and insulin sensitivity were found to be unchaged in chronic marijuana smokers [103]. In view of the demonstrated ability of acute marijuana smoking to induce insulin resistance [104], these findings may reflect desensitization of peripheral CB_1 receptors in chronic users. Further clinical studies are needed to analyze the differential mechanisms involved in the acute and chronic effects of marijuana use on glycaemic control.

Nevertheless, in light of the overwhelming preclinical and clinical evidence suggesting that CB_1 receptor activation contributes to diabetes development and its complications (cardiovascular, neuropathy, retinopathy, and nephropathy) [6], and a recent study by the Centers for Disease Control and Prevention associating cases of acute kidney injury with synthetic cannabinoid use [105], the use of THC would be risky from a clinical point of view in patients with established diabetes. Diabetic patients also have impaired immune functions and wound healing, which could be adversely affected by immunosuppressive/immunomodulatory drugs such as THC. By contrast, CBD demonstrated beneficial effects as a result of its anti-inflammatory and antioxidant properties both in preclinical models of primary diabetes and in models of all major diabetic complications, which is encouraging for its potential testing in diabetic patients [6].

As noted above, THC and its synthetic analogue Nabilone are used to treat chemotherapy-induced nausea and vomiting, as well as to stimulate appetite in cachexia associated with AIDS or terminal tumours [2]. In the case of AIDS, recent controlled studies in nonhuman primates showed unexpectedly that chronic THC administration before and during simian immunodeficiency virus infection ameliorates disease progression, and also attenuates viral load and tissue inflammation, significantly reducing the morbidity and mortality of virus-infected macaques [106], which is very encouraging.

There is considerable preclinical and clinical evidence showing that the combination of THC with

opioids or nonsteroidal anti-inflammatory drugs may enhance their efficacy in pain and also limit their side effects [2,95,96]. It has become clear that cannabinoid analgesia is predominantly mediated via peripheral CB_1 receptors in nociceptors [107], providing the rationale for selectively targeting peripheral CB_1 receptors by peripherally-restricted (brain impermeable) agonists, thereby eliminating the undesirable CNS consequences of CB_1 stimulation [71]. Astra Zeneca (London, UK) has developed two novel peripherally-restricted, orally bioavailable $CB_{1/2}$ agonists (AZD1940 and AZD1704). Despite their mixed agonist activity at CB_1 and CB_2 receptors, the analgesic efficacy in rodent models was mainly driven by CB_1 receptors, as validated through the use of CB_1 selective antagonist and knockout mice [27]. The clinical efficacy of AZD1940 as a pain reliever was tested in two single-dose, phase II studies (human capsaicin and third molar extraction models) and in a multiple ascending doses study performed in subjects with chronic low-back pain. The two single-dose, phase II studies showed no efficacy at the primary endpoints (pain intensity and heat pain threshold for capsaicin study) [28]. In the multiple ascending dose study where AZD1940 was administered for 12 days, repeated dosing led to slow compound accumulation, significant weight gain and elevation of hepatic transaminases. AZD1704 also induced profound hypotensive effects [28]. Thus, the analgesic efficacy of peripherally-restricted CB_1 agonists remains to be established in humans. Although their cardiovascular and metabolic side effects confirm the role of CB_1 receptors in these functions in humans, they further limit their usefulness as therapeutic agents. The above studies of Astra Zeneca with novel, peripherally-restricted, orally bioavailable $CB_{1/2}$ agonists did not indicate CB_2 involvement in preclinical models of analgesia, whereas other studies suggest that CB_2 activation may attenuate certain types of pain [95,96]. CB_2-selective peripherally-restricted agonists (instead of mixed $CB_{1/2}$ agonists) may offer the better optimization of dosing in humans because metabolic and cardiovascular side effects are less likely to occur.

Inhibition of the CB_1 receptors: global and peripherally-restricted CB_1 antagonists

Recent preclinical studies have provided compelling evidence that ECs modulate food intake, energy balance, glucose and lipid metabolism through CB_1 receptors expressed in the brain and various peripheral tissues, such as fat, liver and skeletal muscle [5,70,108,109]. Treatment with brain-penetrant CB_1 receptor antagonists/inverse agonists resulted in improvements of multiple cardiovascular risk factors both in preclinical studies and in clinical trials in obese/overweight subjects [110–116]. Parallel preclinical studies clearly demonstrated that reduced food intake was not the primary mechanism responsible for the weight-reducing effect of CB_1 antagonists, and suggested that peripheral energy metabolism might be directly under EC control [5]. These studies demonstrated that ECs promote lipogenesis in adipose tissue and liver but inhibit fatty acid oxidation and mitochondrial biogenesis, whereas CB_1 antagonists exert the opposite effects [5]. Meanwhile, clinical trials have revealed that a small but statistically significant fraction of subjects treated with the CB_1 inverse agonist rimonabant exhibited anxiety, depression and/or suicidal ideations, eventually leading to the withdrawal of rimonabant from the market in over 50 countries and discontinuation of the therapeutic development of this class of compounds [117].

By that time, there were several lines of evidence strongly suggesting that selective inhibition of peripheral CB_1 receptors may preserve much of the metabolic benefit of global CB_1 blockade at the same time as minimizing side effects as a result of the blockade of CB_1 receptors in the CNS [5]. A proof of principle study by Tam *et al.* [118] demonstrated that chronic treatment of DIO mice with AM6545 (the first high-affinity, selective, peripherally-restricted neutral CB_1 antagonist) improved glucose tolerance, insulin sensitivity and the plasma lipid profile, and also reversed fatty liver, although it was less effective than its parent compound rimonabant in reducing body weight because it did not affect caloric intake. The same study also provided evidence for the importance of CB_1 receptors in hepatocytes in the development of diet-induced insulin resistance. A subsequent study provided additional mechanistic insight by demonstrating that CB_1-mediated hepatic insulin resistance involves ER stress-dependent impairment of insulin signalling, as well as reduced insulin clearance [119]. In a follow-up study, a highly potent, selective and brain impermeable CB_1 receptor inverse agonist, JD5037, was even more effective in improving metabolic parameters in mouse models of obesity, and it not only improved cardiometabolic risk, but also had antiobesity and hypophagic effects by reversing leptin resistance [101]. This compound is currently undergoing toxicology screening as a prelude to its clinical testing.

As discussed above, we have learned important lessons from the first clinical trials aiming to attenuate pain with the peripherally-restricted mixed $CB_{1/2}$ agonists, which were terminated because of excessive weight

gain, hepatotoxicity and cardiovascular adverse effects. Interestingly, this side-effect profile strongly supports the rationale for the development and therapeutic use of peripherally-restricted CB_1 antagonists in humans [27,28].

Activation of CB_2 receptors by selective agonists

Overwhelming evidence for the therapeutic potential of EC/CB_2 receptor signalling in some of the major pathologies affecting humans has been reviewed recently [4]. An important consideration for the therapeutic development of selective CB_2 receptor agonists is the absence of psychoactive effects, coupled with the anti-inflammatory and tissue protective activity of these ligands in numerous preclinical disease models [4].

CB_2 receptors are predominantly expressed in peripheral blood immune cells where the level of their expression is strongly modulated by pro-inflammatory and other stimuli, largely depending on the experimental conditions [120]. Initial studies focusing on the immunomodulatory effects of THC and other cannabinoid ligands *in vivo* in rodents and *in vitro* in human immune cell cultures demonstrated immunosuppressive effects in T and B lymphocytes, natural killer cells and macrophages, which most likely involved both CB_1 and CB_2 receptors, as well as CB receptor-independent mechanisms [9,120,121]. ECs were also found to modulate T and B cell proliferation and apoptosis, immune cell activation and inflammatory cytokine production, chemotaxis and inflammatory cell migration, and macrophage-mediated killing of sensitized cells [9,120,122]. These generally inhibitory effects were ligand- and cell type-dependent and were also influenced by the experimental conditions used [9,120,123,124]. A complicating factor is the agonist-induced rapid internalization and trafficking of CB_2 receptors *in vitro*, which can confound any interpretation of the results [33,34]. The effects of ECs or synthetic analogues on microglia activation/migration also appear to be largely experimental condition-dependent [123].

One important recent development has been the identification of low levels of CB_2 receptor expression in tissues previously considered to be devoid of these receptors. These include specific regions of the brain [125–127], spinal cord and dorsal root ganglia [17,95,128], neurones in the myenteric and submucosal plexus of the enteric nervous system [129–131], myocardium or cardiomyocytes [64,65,132], human vascular smooth muscle and endothelium [25,133–135], activated hepatic stellate cells [136,137], Kupffer cells [138], reproductive organs/cells [139,140], colonic epithelial cells [141], bone [142–144], mouse and human exocrine and endocrine pancreas [145–148], and various human tumours [149]. Further studies are needed to fully explore the function of CB_2 receptors at these sites.

More importantly, disease-induced changes (usually increases) in CB_2 receptor expression have been reported (Table 1), and synthetic CB_2 receptor agonists exerted protective effects in a variety of preclinical disease models and pathological conditions [4], ranging from cardiovascular disorders [11], various forms of ischaemic-reperfusion injury [90], gastrointestinal and liver inflammation [13,150,151], autoimmune and neurodegenerative disorders [7,152–154], kidney disorders [4], bone disorders [143,144], cancer [149,155–157], and pain [17,95].

As for the therapeutic potential of CB_2 agonists, it is important to note that, although, under conditions of a sterile inflammatory response, CB_2 agonists may limit injury, in pathogen-induced inflammation, the immunosuppressive effects of the CB_2 receptor activation may enhance or even inflict tissue damage, and may also lead to accelerated cancer growth in certain types of tumours [4]. To successfully target CB_2 in selected human diseases, it is imperative to identify the exact cellular location and disease-induced, time-dependent changes in the expression of CB_2 receptors. This will necessitate the development of improved research tools, such as more reliable and specific antibodies. This is particularly important because, in many injury models, CB_2 agonists appear to be most effective when given before the initiation of the insult, and may lose their efficacy or even promote inflammation when given at later time [4]. Thus, a better understanding of the underlying pathology and its effects on CB_2 expression is required for the development of meaningful therapeutic approaches. Before going to clinical development for a particular indication, it is also important to confirm previous preclinical findings with novel and more selective CB_2 agonists, because currently available ligands may not be entirely specific. Better knowledge of the pharmacokinetics and metabolism of ligands is also essential, particularly given the bell-shaped dose–response often seen with recently available CB_2 agonists in various disease models [4]. The reason for the latter may be that, when used at higher doses, currently used CB_2 agonists may also activate CB_1 receptors, particularly when the relative expression of CB_1 over CB_2 is high. Our understanding of the complexities of CB_2 receptor signalling is still limited, and important interspecies differences in CB_2 receptor signalling and in the pharmacology of CB_2 ligands must also be considered [158].

Problems with the use of peripherally-restricted $CB_{1/2}$ agonists for pain relief as a result of cardiovascular and metabolic side effects have been discussed above. A plausible alternative could be the testing of peripherally-restricted selective CB_2 agonists for analgesia in humans because such compounds would be expected to be devoid of cardiometabolic liabilities. However, the preclinical data with AZD1940 and AZD1704 indicate that the analgesic efficacy of this class of compounds was mainly driven by the CB_1 receptor [27] which, if confirmed in humans, would limit the promise of this approach. Nevertheless, the therapeutic development of selective CB_2 receptor ligands (agonists or inverse agonists/antagonists depending on the pathology and its stage) is still a promising strategy for a number of disease conditions, provided that the issues discussed above are successfully resolved [4].

Inhibition of EC metabolism, cellular uptake or biosyntheses

The hypothesis behind the therapeutic inhibition of EC degradation was that increasing EC tissue levels would be less likely to cause psychoactive effects than would the use of synthetic CB_1 ligands (endocannabinoids are biosynthesized and degraded in a site and time-dependent manner), whereas the beneficial effects of $CB_{1/2}$ activation, such as analgesia, would be maintained [159]. In support of this, FAAH knockout mice or mice treated with a FAAH inhibitor have elevated AEA levels in the brain and other tissues, are supersensitive to exogenous AEA, and exhibit CB_1 receptor-mediated hypoalgesia [160,161] and reduced anxiety, although they do not display catalepsy, an indicator of psychoactivity in humans [162]. The antinociceptive effect of FAAH inhibitors, likely mediated through increases in AEA and PEA levels that activate $CB_{1/2}$, peroxisome proliferator-activated receptor α and/or TRPV1 [163], was investigated in acute and chronic rodent models of pain [164]. Most of the initial results were based on using URB597, which irreversibly inhibits FAAH both in the CNS and periphery [164]. Recent studies with a peripherally-restricted FAAH inhibitor, URB937, showed efficacy in neuropathic and inflammatory pain [165], confirming that the analgesic effects of AEA are initiated at the peripheral sites [107]. However, similar to direct-acting peripheral $CB_{1/2}$ agonists, URB597 has both hypotensive [166] and diabetogenic effects [167] mediated by CB_1 receptors, and FAAH knockout mice are also prone to diet-induced obesity and diabetes [168]. The diabetogenic effect of URB597 has been attributed to blocking FAAH in the liver, and the novel FAAH inhibitor AM3506, which does not block FAAH in the liver as a result of its rapid uptake and metabolism by hepatocytes, was found to be devoid of glycaemic side effects in rodents [167]. FAAH antagonism may also promote fat accumulation and insulin resistance through centrally-mediated hypothyroidism [169].

The analgesic effects of FAAH inhibition in preclinical models prompted the development of PF-04457845, an irreversible FAAH inhibitor with excellent analgesic efficacy in animal models [29,170], which was selected for clinical development. In a randomized, placebo-controlled, phase II clinical trial PF-04457845 was recently evaluated in patients with osteoarthritic pain of the knee [30]. The results clearly demonstrated that PF-04457845 inhibited FAAH activity in white blood cells and raised the concentrations of various fatty acid amides 3.5-10 fold, which persisted for up to 2 weeks after discontinuation of the drug, and did not affect cognitive function in test subjects. However, the study failed to show any analgesic efficacy of PF-04457845, whereas the nonsteroidal anti-inflammatory drug naproxen, used as a positive control, was effective [30]. These results were also highlighted and discussed in a recent editorial [171].

A promising alternative indication for the therapeutic use of FAAH antagonists is post-traumatic stress syndrome. The FAAH inhibitor AM3506 was recently found to be effective in increasing fear extinction in a CB_1 receptor-dependent manner in a mouse model of post-traumatic stress syndrome, and human carriers of a low-expressing FAAH variant displayed quicker habituation of amygdala reactivity to threat, as detected by brain imaging [172].

The main rationale for the development of MAGL inhibitors, which metabolize 2-AG, is similar to the rationale for FAAH inhibitors. Numerous recent studies have demonstrated that MAGL inhibition or genetic deletion exerts anti-emetic [173], antineoplastic [174], and anxiolytic and antinociceptive effects in rodents [175], and also protects against brain injury [176,177], acute liver injury/inflammation [138] and colitis either via enhancing $CB_{1/2}$ signalling or by attenuating eicosanoid synthesis in specific tissues, such as the brain and liver [178], or by a combination of both. In the case of cancer, MAGL inhibition modulates fatty acid release for the synthesis of protumourigenic signalling lipids [174], as reviewed recently [179,180].

Although the above preclinical findings are indeed exciting, they also highlight important limitations. (a) Raising the tissue levels of ECs may promote the formation of cyclooxygenase-, lipoxygenase- and

cytochrome P450-derived pro-inflammatory metabolites [47,181]. (b) Some of the prostaglandins that were attenuated by MAGL inhibitors have well documented tissue protective functions. (c) Although the dual effect of MAGL inhibition on attenuating eicosanoid and enhancing EC signalling can be beneficial in certain tissues (e.g. the brain and liver) where MAGL links the EC and eicosanoid systems through the hydrolysis of 2-AG, in other tissues, it can promote inflammation and injury (e.g. in the myocardium) through the non-CB mechanisms described above (the cardiotoxicity of COX-2 inhibitors is well documented in humans). (d) Chronic MAGL inhibition leads to functional antagonism of the ECS [175]. (e) As previously discussed, very strong preclinical and clinical evidence suggests that, in cardiovascular disease and diabetes/diabetic complications, endocannabinoids (through CB_1 and most likely through the first two mechanisms described above) promote cardiovascular injury. (f) There is growing evidence that ECs exert pro-inflammatory effects in various disease models through both CB_1-dependent and -independent mechanisms [6]. This is supported by a recent study demonstrating that the inhibition of EC synthesis is anti-inflammatory in macrophages [182]. (g) Various isoforms of metabolizing enzymes (e.g. FAAH) may have distinct functions [52], and the functional properties of rodent and human FAAH may also be different [183]. (h) Most of the benefits observed with inhibitors of FAAH or MAGL were reported in acute models; the safety of chronic inhibition of these enzymes has not yet been determined, particularly in pathological situations. (i) The use of irreversible inhibitors of FAAH and MAGL could be a disadvantage for accurate dose titration and would make it difficult to treat toxicity [164].

Conclusions and future directions

Recent clinical studies show that cannabinoid-based medicines with controlled doses of plant-derived cannabinoids can provide symptomatic relief in a subset of patients suffering from pain and spasticity associated with MS and certain other types of pain, and there is hope (based on preclinical studies) that these medications would also positively modulate disease progression. Synthetic cannabinoids are also useful in subset of patients with wasting disorders and chemotherapy-induced nausea and vomiting. There are numerous promising new targets (plant-derived cannabinoids, peripherally-restricted CB_1 antagonists, selective CB_2 agonists, inhibitors of endocannabinoid metabolism/transport) 'in waiting', as discussed in the present

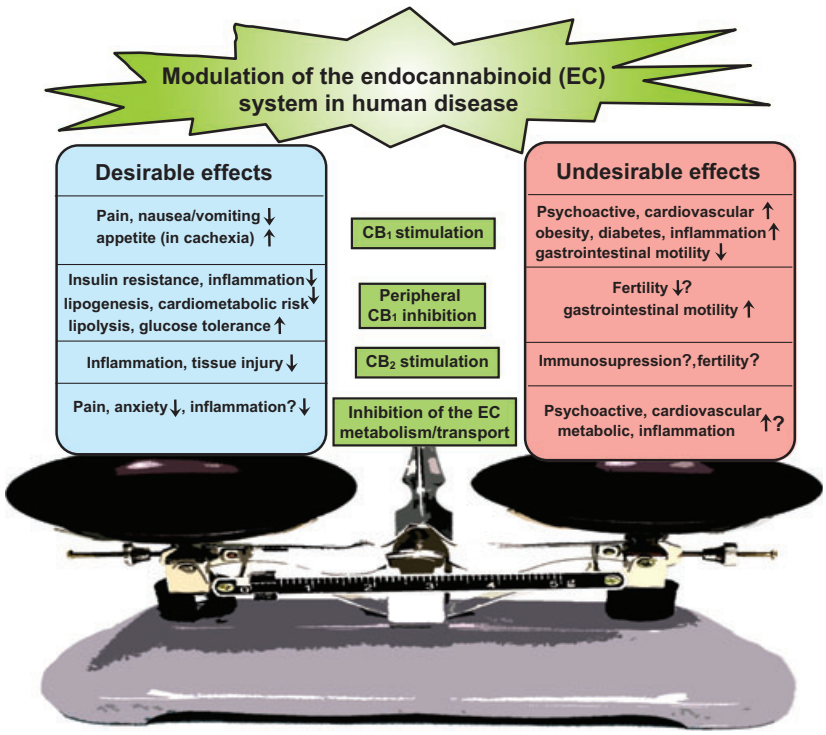


Fig. 1. Cannabinoid therapeutics: finding the right balance.

Table 2. Potential approaches/directions for future success.

Therapeutic approach (target)	Possible directions/approaches for success	Possibly therapeutic indications in humans (realistic)	Potential/expected adverse effects
THC based medicines, cannabinoid based extracts (CB_1, CB_2 and unrelated antioxidant anti-inflammatory mechanisms)	Optimization of route of administration, dosing and indication Better selection criteria for trials, identification of potential positive responders by initial titration Placebo-controlled trials to establish short- and long-term efficacy in given indications Long-term controlled studies to determine possible disease-modifying effects (e.g. in multiple sclerosis) and adverse consequences (e.g. immune and/or cardiovascular effects, etc.) Combination approaches in pain to achieve better efficacy and fewer side effects (e.g. with opioids, nonsteroid anti-inflammatory drugs, etc.) Optimization of the extract composition for improved benefit/risk profile	Symptomatic relief in certain forms of pain and spasticity (as in neurodegenerative disorders such as multiple sclerosis) Stimulation of appetite in patients with wasting disorders Attenuation of chemotherapy-induced nausea and vomiting Topical administration in certain skin disorders? Nonpsychoactive constituents of marijuana, such as CBD or their analogues, may have therapeutic utility in certain forms of acute tissue injury, inflammatory disorders, diabetes and diabetic complications	In the case of THC-containing formulations, effects related to CB_1 stimulation at higher doses (e.g. psychoactive, cardiovascular, metabolic side effects) and potential modulation of immune responses
Peripherally restricted CB_1 agonists (peripheral CB_1)	Evaluation of the feasibility of the topical/local use of peripherally restricted CB_1 agonists in certain forms of pain and skin conditions (e.g. pruritus)	Topical/local use in certain forms of pain and skin conditions/ diseases? (the systematic administration/use is not likely because of the established adverse cardiovascular and metabolic consequences of this approach)	Cardiovascular Metabolic Kidney Gastrointestinal (decreased motility) Pro-inflammatory?
Peripherally restricted or global CB_2 agonists (peripheral CB_2)	Re-evaluation of human indications based on previous failures of trials with mixed peripherally restricted $CB_{1/2}$ agonists Search for new indications More preclinical and clinical research to understand the significance of tissue and time specific changes in CB_2 receptor expression in pathological conditions Development of novel, specific and orally available ligands for proof of the principle studies; evaluation of toxicology and pharmacokinetics	Various forms of acute tissue injuries associated with inflammation (stroke, myocardial infarction, traumatic injury, organ transplantation, etc.) Various forms of inflammatory diseases if the anti-inflammatory effects are confirmed in humans	Most likely related to effects on immune and haematopoietic system Effects on fertility?
Peripherally restricted CB_1 antagonists, inverse agonists (peripheral CB_1)	Development and testing of new ligands, toxicology and safety studies in rodents, large animals, and humans Proof of the principle studies in large animals and humans	Diabetes and diabetic complications, Cardiometabolic syndrome Kidney disease?	Gastrointestinal (increased motility) Effects on fertility?
Inhibition of EC metabolism, cellular uptake or biosynthesis ($CB_{1/2}$, $TRPV_1$ and nuclear receptors, prostaglandin and leukotriene signalling)	Preclinical research to identify the putative endocannabinoid transporter(s), and to better understand the tissue, time, and disease-specific metabolism of endocannabinoids to various other bioactive mediators (e.g. prostaglandins, leukotriens, etc.) Re-evaluation of human indications based on previous failures of trials with FAAH inhibitors in pain Search for new indications, better and more selective ligands	Pain? Certain disorders associated with anxiety? Certain forms of acute tissue injury?	Similar, but acutely less pronounced than with CB_1 agonists. However, long-term use may be associated with adverse effects similar to cyclooxygenase 2 inhibitors (e.g. cardiovascular). Pro-inflammatory effects in certain cases?

minireview. However, it is clear that, for the successful translation of preclinical findings to clinical practice, a better understanding of the pathological role of the ECS in various diseases, of the potential side effects of targeting this system, and of endocannabinoid pharmacology is required, coupled with the development of improved research tools to dissect these processes (Fig. 1 and Table 2).

Future studies should focus on a rigorous evaluation of the CB receptor dependent/independent and hypothermia-independent effects of THC in preclinical models (e.g. in tissue injury, cancer, inflammation, etc.) using global and tissue/cell specific knockout mice and also aim to identify potential novel targets/mechanisms of action of THC and other plant-derived cannabinoids, coupled with the identification of nonpsychoactive constituents in cannabis extracts with potential therapeutic effects. Novel highly selective, orally available nontoxic cannabinoid ligands should be developed and evaluated in preclinical disease models. Large animal studies (e.g. canine, pig, primate) should confirm the efficacy of cannabinoid ligands obtained in rodent disease models before initiating human trials. The development of specific novel antibodies for $CB_{1/2}$ receptors and endocannabinoid metabolic enzymes (FAAH, MAGL, diacylglycerol lipase α/β) validated by using positive and negative controls is essential for accurately assessing the time-dependent changes in $CB_{1/2}$ receptors and metabolic enzyme expression in diseased animal and human tissues, with the aim of understanding the human relevance of these changes. Our limited knowledge should be expanded to enable an understanding of $CB_{1/2}$ receptor trafficking, signalling and their interspecies differences. The development of reliable radioligands suitable for human imaging studies and research could contribute to a better understanding of the role of ECS in human health and disease.

Acknowledgements

This study was supported by funds from the Intramural Research Program of NIAAA to P.P. and G.K. The authors apologize to colleagues whose important work could not be cited because of space limitations.

References

1 Hanus LO (2009) Pharmacological and therapeutic secrets of plant and brain (endo)cannabinoids. *Med Res Rev* **29**, 213–271.
2 Pacher P, Batkai S & Kunos G (2006) The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol Rev* **58**, 389–462.
3 Di Marzo V (2008) Targeting the endocannabinoid system: to enhance or reduce? *Nat Rev Drug Discov* **7**, 438–455.
4 Pacher P & Mechoulam R (2011) Is lipid signaling through cannabinoid 2 receptors part of a protective system? *Prog Lipid Res* **50**, 193–211.
5 Kunos G & Tam J (2011) The case for peripheral CB (1) receptor blockade in the treatment of visceral obesity and its cardiometabolic complications. *Br J Pharmacol* **163**, 1423–1431.
6 Horvath B, Mukhopadhyay P, Hasko G & Pacher P (2012) The endocannabinoid system and plant-derived cannabinoids in diabetes and diabetic complications. *Am J Pathol* **180**, 432–442.
7 Centonze D, Finazzi-Agro A, Bernardi G & Maccarrone M (2007) The endocannabinoid system in targeting inflammatory neurodegenerative diseases. *Trends Pharmacol Sci* **28**, 180–187.
8 Skaper SD & Di Marzo V (2012) Endocannabinoids in nervous system health and disease: the big picture in a nutshell. Philosophical transactions of the Royal Society of London. *Series B, Biol Sci* **367**, 3193–3200.
9 Klein TW (2005) Cannabinoid-based drugs as anti-inflammatory therapeutics. *Nat Rev Immunol* **5**, 400–411.
10 Pacher P, Mukhopadhyay P, Mohanraj R, Godlewski G, Batkai S & Kunos G (2008) Modulation of the endocannabinoid system in cardiovascular disease: therapeutic potential and limitations. *Hypertension* **52**, 601–607.
11 Steffens S & Pacher P (2012) Targeting cannabinoid receptor CB(2) in cardiovascular disorders: promises and controversies. *Br J Pharmacol* **167**, 313–323.
12 Montecucco F & Di Marzo V (2012) At the heart of the matter: the endocannabinoid system in cardiovascular function and dysfunction. *Trends Pharmacol Sci* **33**, 331–340.
13 Lotersztajn S, Teixeira-Clerc F, Julien B, Deveaux V, Ichigotani Y, Manin S, Tran-Van-Nhieu J, Karsak M, Zimmer A & Mallat A (2008) CB2 receptors as new therapeutic targets for liver diseases. *Br J Pharmacol* **153**, 286–289.
14 Tam J, Liu J, Mukhopadhyay B, Cinar R, Godlewski G & Kunos G (2011) Endocannabinoids in liver disease. *Hepatology* **53**, 346–355.
15 Izzo AA & Camilleri M (2008) Emerging role of cannabinoids in gastrointestinal and liver diseases: basic and clinical aspects. *Gut* **57**, 1140–1155.
16 Biro T, Toth BI, Hasko G, Paus R & Pacher P (2009) The endocannabinoid system of the skin in health and disease: novel perspectives and therapeutic opportunities. *Trends Pharmacol Sci* **30**, 411–420.
17 Guindon J & Hohmann AG (2008) Cannabinoid CB2 receptors: a therapeutic target for the treatment of

inflammatory and neuropathic pain. *Br J Pharmacol* **153**, 319–334.
18 Guindon J & Hohmann AG (2009) The endocannabinoid system and pain. *CNS Neurol Disord Drug Targets* **8**, 403–421.
19 Mechoulam R & Parker LA (2013) The Endocannabinoid System and the Brain. *Annu Rev Psychol* **64**, 21–47.
20 Hillard CJ, Weinlander KM & Stuhr KL (2012) Contributions of endocannabinoid signaling to psychiatric disorders in humans: genetic and biochemical evidence. *Neuroscience* **204**, 207–229.
21 Guindon J & Hohmann AG (2011) The endocannabinoid system and cancer: therapeutic implication. *Br J Pharmacol* **163**, 1447–1463.
22 Velasco G, Sanchez C & Guzman M (2012) Towards the use of cannabinoids as antitumour agents. *Nat Rev Cancer* **12**, 436–444.
23 Parker LA, Rock EM & Limebeer CL (2011) Regulation of nausea and vomiting by cannabinoids. *Br J Pharmacol* **163**, 1411–1422.
24 Piscitelli F & Di Marzo V (2012) 'Redundancy' of endocannabinoid inactivation: new challenges and opportunities for pain control. *ACS Chem Neurosci* **3**, 356–363.
25 Pacher P & Steffens S (2009) The emerging role of the endocannabinoid system in cardiovascular disease. *Semin Immunopathol* **31**, 63–77.
26 Di Marzo V (2008) Play an adagio with a Stradivarius: the right patient for CB1 receptor antagonists? *Nat Clin Pract Cardiovasc Med* **5**, 610–612.
27 Groblewski T, Hong X, Lessard E, St-Onge S, Yang H, Panetta R, Cao CQ, Swedberg MD, Cebers G, Nyberg S *et al*. (2010) Pre-clinical pharmacological properties of novel peripherally-acting CB1-CB2 agonists. Proccedings of 20th Annual Symposium of the International Cannabinoid Research Society, Lund, Sweden, 2010.
28 Groblewski T, Karlsten R, Segerdhal M, Kalliomäki J, Jonzon B, Bielenstein M, Cebers G, Swedberg M, Annas A, Christoph G *et al*. (2010) Peripherally-acting CB1-CB2 agonists for pain: do they still hold promise? Proceedings of the 20th Annual Symposium of the International Cannabinoid Research Society, Lund, Sweden, 2010.
29 Li GL, Winter H, Arends R, Jay GW, Le V, Young T & Huggins JP (2012) Assessment of the pharmacology and tolerability of PF-04457845, an irreversible inhibitor of fatty acid amide hydrolase-1, in healthy subjects. *Br J Clin Pharmacol* **73**, 706–716.
30 Huggins JP, Smart TS, Langman S, Taylor L & Young T (2012) An efficient randomised, placebo-controlled clinical trial with the irreversible fatty acid amide hydrolase-1 inhibitor PF-04457845, which modulates endocannabinoids but fails to induce effective analgesia in patients with pain due to osteoarthritis of the knee. *Pain* **153**, 1837–1846.
31 Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, Felder CC, Herkenham M, Mackie K, Martin BR *et al*. (2002) International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol Rev* **54**, 161–202.
32 Pertwee RG, Howlett AC, Abood ME, Alexander SP, Di Marzo V, Elphick MR, Greasley PJ, Hansen HS, Kunos G, Mackie K *et al*. (2010) International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB1 and CB2. *Pharmacol Rev* **62**, 588–631.
33 Atwood BK, Wager-Miller J, Haskins C, Straiker A & Mackie K (2012) Functional selectivity in CB2 cannabinoid receptor signaling and regulation: implications for the therapeutic potential of CB2 ligands. *Mol Pharmacol* **81**, 250–263.
34 Kleyer J, Nicolussi S, Taylor P, Simonelli D, Furger E, Anderle P & Gertsch J (2012) Cannabinoid receptor trafficking in peripheral cells is dynamically regulated by a binary biochemical switch. *Biochem Pharmacol* **83**, 1393–1412.
35 Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A & Mechoulam R (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**, 1946–1949.
36 Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martin BR, Compton DR *et al*. (1995) Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol* **50**, 83–90.
37 Wang J & Ueda N (2009) Biology of endocannabinoid synthesis system. *Prostaglandins Other Lipid Mediat* **89**, 112–119.
38 Di Marzo V, Bifulco M & De Petrocellis L (2004) The endocannabinoid system and its therapeutic exploitation. *Nat Rev Drug Discov* **3**, 771–784.
39 Liu J, Wang L, Harvey-White J, Huang BX, Kim HY, Luquet S, Palmiter RD, Krystal G, Rai R, Mahadevan A *et al*. (2008) Multiple pathways involved in the biosynthesis of anandamide. *Neuropharmacology* **54**, 1–7.
40 Simon GM & Cravatt BF (2008) Anandamide biosynthesis catalyzed by the phosphodiesterase GDE1 and detection of glycerophospho-N-acyl ethanolamine precursors in mouse brain. *J Biol Chem* **283**, 9341–9349.
41 Fowler CJ (2012) Anandamide uptake explained? *Trends Pharmacol Sci* **33**, 181–185.
42 Fowler CJ (2013) Transport of endocannabinoids across the plasma membrane and within the cell. *FEBS J* **280**, 1895–1904.

43 Kaczocha M, Vivieca S, Sun J, Glaser ST & Deutsch DG (2012) Fatty acid-binding proteins transport N-acylethanolamines to nuclear receptors and are targets of endocannabinoid transport inhibitors. *J Biol Chem* **287**, 3415–3424.
44 Cravatt BF & Lichtman AH (2003) Fatty acid amide hydrolase: an emerging therapeutic target in the endocannabinoid system. *Curr Opin Chem Biol* **7**, 469–475.
45 Ueda N, Tsuboi K & Uyama T (2010) N-acylethanolamine metabolism with special reference to N-acylethanolamine-hydrolyzing acid amidase (NAAA). *Prog Lipid Res* **49**, 299–315.
46 Ueda N, Tsuboi K, Uyama T & Ohnishi T (2011) Biosynthesis and degradation of the endocannabinoid 2-arachidonoylglycerol. *BioFactors* **37**, 1–7.
47 Rouzer CA & Marnett LJ (2011) Endocannabinoid oxygenation by cyclooxygenases, lipoxygenases, and cytochromes P450: cross-talk between the eicosanoid and endocannabinoid signaling pathways. *Chem Rev* **111**, 5899–5921.
48 Cravatt BF & Lichtman AH (2002) The enzymatic inactivation of the fatty acid amide class of signaling lipids. *Chem Phys Lipids* **121**, 135–148.
49 McKinney MK & Cravatt BF (2005) Structure and function of fatty acid amide hydrolase. *Annu Rev Biochem* **74**, 411–432.
50 Fezza F, De Simone C, Amadio D & Maccarrone M (2008) Fatty acid amide hydrolase: a gate-keeper of the endocannabinoid system. *Subcell Biochem* **49**, 101–132.
51 Palkovits M, Harvey-White J, Liu J, Kovacs ZS, Bobest M, Lovas G, Bago AG & Kunos G (2008) Regional distribution and effects of postmortal delay on endocannabinoid content of the human brain. *Neuroscience* **152**, 1032–1039.
52 Fu J, Bottegoni G, Sasso O, Bertorelli R, Rocchia W, Masetti M, Guijarro A, Lodola A, Armirotti A, Garau G *et al*. (2012) A catalytically silent FAAH-1 variant drives anandamide transport in neurons. *Nat Neurosci* **15**, 64–69.
53 Di Marzo V & De Petrocellis L (2010) Endocannabinoids as regulators of transient receptor potential (TRP) channels: A further opportunity to develop new endocannabinoid-based therapeutic drugs. *Curr Med Chem* **17**, 1430–1449.
54 Hanus LO & Mechoulam R (2010) Novel natural and synthetic ligands of the endocannabinoid system. *Curr Med Chem* **17**, 1341–1359.
55 Bauer M, Chicca A, Tamborrini M, Eisen D, Lerner R, Lutz B, Poetz O, Pluschke G & Gertsch J (2012) Identification and quantification of a new family of peptide endocannabinoids (Pepcans) showing negative allosteric modulation at CB1 receptors. *J Biol Chem* **287**, 36944–36967.
56 Pamplona FA, Ferreira J, Menezes de Lima O Jr, Duarte FS, Bento AF, Forner S, Villarinho JG, Bellochio L, Wotjak CT, Lerner R *et al*. (2012) Anti-inflammatory lipoxin A4 is an endogenous allosteric enhancer of CB1 cannabinoid receptor. *Proc Natl Acad Sci USA* **109**, 21134–21139.
57 Ueda N, Tsuboi K & Uyama T (2013) Metabolism of endocannabinoids and related N-acylethanolamines: Canonical and alternative pathways. *FEBS J* **280**, 1874–1894.
58 Di Marzo V (2008) Endocannabinoids: synthesis and degradation. *Rev Physiol Biochem Pharmacol* **160**, 1–24.
59 Miller LK & Devi LA (2011) The highs and lows of cannabinoid receptor expression in disease: mechanisms and their therapeutic implications. *Pharmacol Rev* **63**, 461–470.
60 Pertwee RG (2012) Targeting the endocannabinoid system with cannabinoid receptor agonists: pharmacological strategies and therapeutic possibilities. Philosophical Transactions of the Royal Society of London. *Series B, Biol Sci* **367**, 3353–3363.
61 Atwood BK & Mackie K (2010) CB2: a cannabinoid receptor with an identity crisis. *Br J Pharmacol* **160**, 467–479.
62 Pacher P, Batkai S & Kunos G (2005) Cardiovascular pharmacology of cannabinoids. *Handb Exp Pharmacol* **168**, 599–625.
63 Rajesh M, Mukhopadhyay P, Hasko G, Liaudet L, Mackie K & Pacher P (2010) Cannabinoid-1 receptor activation induces reactive oxygen species-dependent and -independent mitogen-activated protein kinase activation and cell death in human coronary artery endothelial cells. *Br J Pharmacol* **160**, 688–700.
64 Mukhopadhyay P, Rajesh M, Batkai S, Patel V, Kashiwaya Y, Liaudet L, Evgenov OV, Mackie K, Hasko G & Pacher P (2010) CB1 cannabinoid receptors promote oxidative stress and cell death in murine models of doxorubicin-induced cardiomyopathy and in human cardiomyocytes. *Cardiovasc Res* **85**, 773–784.
65 Mukhopadhyay P, Batkai S, Rajesh M, Czifra N, Harvey-White J, Hasko G, Zsengeller Z, Gerard NP, Liaudet L, Kunos G *et al*. (2007) Pharmacological inhibition of CB1 cannabinoid receptor protects against doxorubicin-induced cardiotoxicity. *J Am Coll Cardiol* **50**, 528–536.
66 Mukhopadhyay P, Horvath B, Rajesh M, Matsumoto S, Saito K, Batkai S, Patel V, Tanchian G, Gao RY, Cravatt BF *et al*. (2011) Fatty acid amide hydrolase is a key regulator of endocannabinoid-induced myocardial tissue injury. *Free Radic Biol Med* **50**, 179–195.
67 Sugamura K, Sugiyama S, Nozaki T, Matsuzawa Y, Izumiya Y, Miyata K, Nakayama M, Kaikita K,

Obata T, Takeya M *et al*. (2009) Activated endocannabinoid system in coronary artery disease and antiinflammatory effects of cannabinoid 1 receptor blockade on macrophages. *Circulation* **119**, 28–36.
68 Dol-Gleizes F, Paumelle R, Visentin V, Mares AM, Desitter P, Hennuyer N, Gilde A, Staels B, Schaeffer P & Bono F (2009) Rimonabant, a selective cannabinoid CB1 receptor antagonist, inhibits atherosclerosis in LDL receptor-deficient mice. *Arterioscler Thromb Vasc Biol* **29**, 12–18.
69 Kunos G, Osei-Hyiaman D, Liu J, Godlewski G & Batkai S (2008) Endocannabinoids and the control of energy homeostasis. *J Biol Chem* **283**, 33021–33025.
70 Di Marzo V (2008) The endocannabinoid system in obesity and type 2 diabetes. *Diabetologia* **51**, 1356–1367.
71 Kunos G, Osei-Hyiaman D, Batkai S, Sharkey KA & Makriyannis A (2009) Should peripheral CB(1) cannabinoid receptors be selectively targeted for therapeutic gain? *Trends Pharmacol Sci* **30**, 1–7.
72 Cooney MT, Vartiainen E, Laatikainen T, Juolevi A, Dudina A & Graham IM (2010) Elevated resting heart rate is an independent risk factor for cardiovascular disease in healthy men and women. *Am Heart J* **159**, 612–619e3.
73 Gorelick DA, Goodwin RS, Schwilke E, Schwope DM, Darwin WD, Kelly DL, McMahon RP, Liu F, Ortemann-Renon C, Bonnet D *et al*. (2013) Tolerance to effects of high-dose oral {Delta}9-tetrahydrocannabinol and plasma cannabinoid concentrations in male daily cannabis smokers. *J Anal Toxicol* **37**, 11–16.
74 Schmid K, Schonlebe J, Drexler H & Mueck-Weymann M (2010) The effects of cannabis on heart rate variability and well-being in young men. *Pharmacopsychiatry* **43**, 147–150.
75 Klumpers LE, Roy C, Ferron G, Turpault S, Poitiers F, Pinquier JL, van Hasselt JG, Zuurman L, Erwich FA & van Gerven JM (2012) Surinabant, a selective CB(1) antagonist, inhibits THC-induced central nervous system and heart rate effects in humans. *Br J Clin Pharmacol* doi:10.1111/bcp.12071.
76 Singla S, Sachdeva R & Mehta JL (2012) Cannabinoids and atherosclerotic coronary heart disease. *Clin Cardiol* **35**, 329–335.
77 Pratap B & Korniyenko A (2012) Toxic effects of marijuana on the cardiovascular system. *Cardiovasc Toxicol* **12**, 143–148.
78 Leblanc A, Tirel-Badets A, Paleiron N, Castellant P, Cornily JC, Andre M, Grassin F, Feuvrier Y, Blanchard C, Zagnoli F *et al*. (2011) Cannabis and myocardial infarction without angiographic stenosis in young patient: guilty or not guilty? A case report. *Annales de Cardiologie et d'Angeiologie* **60**, 154–158.
79 Mittleman MA, Lewis RA, Maclure M, Sherwood JB & Muller JE (2001) Triggering myocardial infarction by marijuana. *Circulation* **103**, 2805–2809.
80 Mukamal KJ, Maclure M, Muller JE & Mittleman MA (2008) An exploratory prospective study of marijuana use and mortality following acute myocardial infarction. *Am Heart J* **155**, 465–470.
81 Frost L, Mostofsky E, Rosenbloom JI, Mukamal KJ & Mittleman MA (2013) Marijuana use and long-term mortality among survivors of acute myocardial infarction. *Am Heart J* **165**, 170–175.
82 Mir A, Obafemi A, Young A & Kane C (2011) Myocardial infarction associated with use of the synthetic cannabinoid K2. *Pediatrics* **128**, e1622–e1627.
83 Heath TS, Burroughs Z, Thompson AJ & Tecklenburg FW (2012) Acute intoxication caused by a synthetic cannabinoid in two adolescents. *J Pediatr Pharmacol Ther* **17**, 177–181.
84 Lenglet S, Thomas A, Soehnlein O, Montecucco F, Burger F, Pelli G, Galan K, Cravatt B, Staub C & Steffens S (2013) Fatty acid amide hydrolase deficiency enhances intraplaque neutrophil recruitment in atherosclerotic mice. *Arterioscler Thromb Vasc Biol* **33**, 215–223.
85 Quercioli A, Pataky Z, Vincenti G, Makoundou V, Di Marzo V, Montecucco F, Carballo S, Thomas A, Staub C, Steffens S *et al*. (2011) Elevated endocannabinoid plasma levels are associated with coronary circulatory dysfunction in obesity. *Eur Heart J* **32**, 1369–1378.
86 Quercioli A, Pataky Z, Montecucco F, Carballo S, Thomas A, Staub C, Di Marzo V, Vincenti G, Ambrosio G, Ratib O *et al*. (2012) Coronary vasomotor control in obesity and morbid obesity: contrasting flow responses with endocannabinoids, leptin, and inflammation. *JACC Cardiovasc Imaging* **5**, 805–815.
87 Cappellano G, Uberti F, Caimmi PP, Pietronave S, Mary DA, Dianzani C, Micalizzi E, Melensi M, Boldorini R, Nicosia G *et al*. (2013) Different expression and function of the endocannabinoid system in human epicardial adipose tissue in relation to heart disease. *Can J Cardiol* **29**, 499–509.
88 Liu R & Zhang Y (2011) G1359A polymorphism in the cannabinoid receptor-1 gene is associated with coronary artery disease in the Chinese Han population. *Clin Lab* **57**, 689–693.
89 Amoros I, Barana A, Caballero R, Gomez R, Osuna L, Lillo MP, Tamargo J & Delpon E (2010) Endocannabinoids and cannabinoid analogues block human cardiac Kv4.3 channels in a receptor-independent manner. *J Mol Cell Cardiol* **48**, 201–210.
90 Pacher P & Hasko G (2008) Endocannabinoids and cannabinoid receptors in ischaemia-reperfusion

injury and preconditioning. *Br J Pharmacol* **153**, 252–262.
91 Bisogno T & Di Marzo V (2010) Cannabinoid receptors and endocannabinoids: role in neuroinflammatory and neurodegenerative disorders. *CNS Neurol Disord Drug Targets* **9**, 564–573.
92 Fowler CJ, Rojo ML & Rodriguez-Gaztelumendi A (2010) Modulation of the endocannabinoid system: neuroprotection or neurotoxicity? *Exp Neurol* **224**, 37–47.
93 Mechoulam R & Hanus L (2000) A historical overview of chemical research on cannabinoids. *Chem Phys Lipids* **108**, 1–13.
94 Hohmann AG & Suplita RL II (2006) Endocannabinoid mechanisms of pain modulation. *AAPS J* **8**, E693–E708.
95 Anand P, Whiteside G, Fowler CJ & Hohmann AG (2009) Targeting CB2 receptors and the endocannabinoid system for the treatment of pain. *Brain Res Rev* **60**, 255–266.
96 Rahn EJ & Hohmann AG (2009) Cannabinoids as pharmacotherapies for neuropathic pain: from the bench to the bedside. *Neurotherapeutics* **6**, 713–737.
97 Zhornitsky S & Potvin S (2012) Cannabidiol in humans-the quest for therapeutic targets. *Pharmaceuticals* **5**, 529–552.
98 Baker AL, Thornton LK, Hides L & Dunlop A (2012) Treatment of cannabis use among people with psychotic disorders: a critical review of randomised controlled trials. *Curr Pharm Des* **18**, 4923–4937.
99 Izzo AA, Borrelli F, Capasso R, Di Marzo V & Mechoulam R (2009) Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. *Trends Pharmacol Sci* **30**, 515–527.
100 Michalski CW, Oti FE, Erkan M, Sauliunaite D, Bergmann F, Pacher P, Batkai S, Muller MW, Giese NA, Friess H *et al.* (2008) Cannabinoids in pancreatic cancer: correlation with survival and pain. *Int J Cancer* **122**, 742–750.
101 Tam J, Cinar R, Liu J, Godlewski G, Wesley D, Jourdan T, Szanda G, Mukhopadhyay B, Chedester L, Liow JS *et al.* (2012) Peripheral cannabinoid-1 receptor inverse agonism reduces obesity by reversing leptin resistance. *Cell Metab* **16**, 167–179.
102 Rajavashisth TB, Shaheen M, Norris KC, Pan D, Sinha SK, Ortega J & Friedman TC (2012) Decreased prevalence of diabetes in marijuana users: cross-sectional data from the National Health and Nutrition Examination Survey (NHANES) III. *BMJ Open* **2**, e000494.
103 Muniyappa R, Sable S, Ouwerkerk R, Mari A, Gharib AM, Walter M, Courville A, Hall G, Chen KY, Volkow ND *et al.* (2013) Metabolic effects of chronic cannabis smoking. *Diabetes Care* **36**, 1–8.
104 Hollister LE & Reaven GM (1974) Delta-9-tetrahydrocannabinol and glucose tolerance. *Clin Pharmacol Ther* **16**, 297–302.
105 Murphy TD, Weidenbach KN, Houten CV, Gerona RR, Moran JH, Kirschner RI, Maraffa JM, Stork CM, Birkhead GS, Newman A *et al.* (2013) Acute kidney injury associated with synthetic cannabinoid use – multiple States, 2012. *MMWR Morb Mortal Wkly Rep* **62**, 93–98.
106 Molina PE, Winsauer P, Zhang P, Walker E, Birke L, Amedee A, Stouwe CV, Troxclair D, McGoey R, Varner K *et al.* (2011) Cannabinoid administration attenuates the progression of simian immunodeficiency virus. *AIDS Res Hum Retroviruses* **27**, 585–592.
107 Agarwal N, Pacher P, Tegeder I, Amaya F, Constantin CE, Brenner GJ, Rubino T, Michalski CW, Marsicano G, Monory K *et al.* (2007) Cannabinoids mediate analgesia largely via peripheral type 1 cannabinoid receptors in nociceptors. *Nat Neurosci* **10**, 870–879.
108 Osei-Hyiaman D, DePetrillo M, Pacher P, Liu J, Radaeva S, Batkai S, Harvey-White J, Mackie K, Offertaler L, Wang L *et al.* (2005) Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. *J Clin Invest* **115**, 1298–1305.
109 Pagotto U, Marsicano G, Cota D, Lutz B & Pasquali R (2006) The emerging role of the endocannabinoid system in endocrine regulation and energy balance. *Endocr Rev* **27**, 73–100.
110 Despres JP, Golay A & Sjostrom L (2005) Effects of rimonabant on metabolic risk factors in overweight patients with dyslipidemia. *N Engl J Med* **353**, 2121–2134.
111 Van Gaal LF, Rissanen AM, Scheen AJ, Ziegler O & Rossner S (2005) Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. *Lancet* **365**, 1389–1397.
112 Scheen AJ, Van Gaal LG, Despres JP, Pi-Sunyer X, Golay A & Hanotin C (2006) Rimonabant improves cardiometabolic risk profile in obese or overweight subjects: overview of RIO studies. *Rev Med Suisse* **2**, 1916–1923.
113 Despres JP, Ross R, Boka G, Almeras N & Lemieux I (2009) Effect of rimonabant on the high-triglyceride/low-HDL-cholesterol dyslipidemia, intraabdominal adiposity, and liver fat: the ADAGIO-Lipids trial. *Arterioscler Thromb Vasc Biol* **29**, 416–423.
114 Nissen SE, Nicholls SJ, Wolski K, Rodes-Cabau J, Cannon CP, Deanfield JE, Despres JP, Kastelein JJ, Steinhubl SR, Kapadia S *et al.* (2008) Effect of rimonabant on progression of atherosclerosis in

patients with abdominal obesity and coronary artery disease: the STRADIVARIUS randomized controlled trial. *JAMA* **299**, 1547–1560.
115 Rosenstock J, Hollander P, Chevalier S & Iranmanesh A (2008) SERENADE: the Study Evaluating Rimonabant Efficacy in Drug-naive Diabetic Patients: effects of monotherapy with rimonabant, the first selective CB1 receptor antagonist, on glycemic control, body weight, and lipid profile in drug-naive type 2 diabetes. *Diabetes Care* **31**, 2169–2176.
116 Hollander PA, Amod A, Litwak LE & Chaudhari U (2010) Effect of rimonabant on glycemic control in insulin-treated type 2 diabetes: the ARPEGGIO trial. *Diabetes Care* **33**, 605–607.
117 Di Marzo V & Despres JP (2009) CB1 antagonists for obesity–what lessons have we learned from rimonabant? *Nat Rev Endocrinol* **5**, 633–638.
118 Tam J, Vemuri VK, Liu J, Batkai S, Mukhopadhyay B, Godlewski G, Osei-Hyiaman D, Ohnuma S, Ambudkar SV, Pickel J *et al*. (2010) Peripheral CB1 cannabinoid receptor blockade improves cardiometabolic risk in mouse models of obesity. *J Clin Investig* **120**, 2953–2966.
119 Liu J, Zhou L, Xiong K, Godlewski G, Mukhopadhyay B, Tam J, Yin S, Gao P, Shan X, Pickel J *et al*. (2012) Hepatic cannabinoid receptor-1 mediates diet-induced insulin resistance via inhibition of insulin signaling and clearance in mice. *Gastroenterology* **142**, 1218–1228.
120 Klein TW, Newton C, Larsen K, Lu L, Perkins I, Nong L & Friedman H (2003) The cannabinoid system and immune modulation. *J Leukoc Biol* **74**, 486–496.
121 Cabral GA & Staab A (2005) Effects on the immune system. *Handb Exp Pharmacol* **168**, 385–423.
122 Cencioni MT, Chiurchiu V, Catanzaro G, Borsellino G, Bernardi G, Battistini L & Maccarrone M (2010) Anandamide suppresses proliferation and cytokine release from primary human T-lymphocytes mainly via CB2 receptors. *PLoS ONE* **5**, e8688.
123 Miller AM & Stella N (2008) CB2 receptor-mediated migration of immune cells: it can go either way. *Br J Pharmacol* **153**, 299–308.
124 Buckley NE (2008) The peripheral cannabinoid receptor knockout mice: an update. *Br J Pharmacol* **153**, 309–318.
125 Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K, Stella N, Makriyannis A, Piomelli D, Davison JS *et al*. (2005) Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science* **310**, 329–332.
126 Onaivi ES (2006) Neuropsychobiological evidence for the functional presence and expression of cannabinoid CB2 receptors in the brain. *Neuropsychobiology* **54**, 231–246.
127 Viscomi MT, Oddi S, Latini L, Pasquariello N, Florenzano F, Bernardi G, Molinari M & Maccarrone M (2009) Selective CB2 receptor agonism protects central neurons from remote axotomy-induced apoptosis through the PI3K/Akt pathway. *J Neurosci* **29**, 4564–4570.
128 Beltramo M (2009) Cannabinoid type 2 receptor as a target for chronic – pain. *Mini Rev Med Chem* **9**, 11–25.
129 Wright KL, Duncan M & Sharkey KA (2008) Cannabinoid CB2 receptors in the gastrointestinal tract: a regulatory system in states of inflammation. *Br J Pharmacol* **153**, 263–270.
130 Marquez L, Suarez J, Iglesias M, Bermudez-Silva FJ, Rodriguez de Fonseca F & Andreu M (2009) Ulcerative colitis induces changes on the expression of the endocannabinoid system in the human colonic tissue. *PLoS ONE* **4**, e6893.
131 Duncan M, Mouihate A, Mackie K, Keenan CM, Buckley NE, Davison JS, Patel KD, Pittman QJ & Sharkey KA (2008) Cannabinoid CB2 receptors in the enteric nervous system modulate gastrointestinal contractility in lipopolysaccharide-treated rats. *Am J Physiol Gastrointest Liver Physiol* **295**, G78–G87.
132 Bouchard JF, Lepicier P & Lamontagne D (2003) Contribution of endocannabinoids in the endothelial protection afforded by ischemic preconditioning in the isolated rat heart. *Life Sci* **72**, 1859–1870.
133 Rajesh M, Mukhopadhyay P, Hasko G, Huffman JW, Mackie K & Pacher P (2008) CB2 cannabinoid receptor agonists attenuate TNF-alpha-induced human vascular smooth muscle cell proliferation and migration. *Br J Pharmacol* **153**, 347–357.
134 Rajesh M, Mukhopadhyay P, Batkai S, Hasko G, Liaudet L, Huffman JW, Csiszar A, Ungvari Z, Mackie K, Chatterjee S *et al*. (2007) CB2-receptor stimulation attenuates TNF-alpha-induced human endothelial cell activation, transendothelial migration of monocytes, and monocyte-endothelial adhesion. *Am J Physiol Heart Circ Physiol* **293**, H2210–H2218.
135 Rajesh M, Pan H, Mukhopadhyay P, Batkai S, Osei-Hyiaman D, Hasko G, Liaudet L, Gao B & Pacher P (2007) Cannabinoid-2 receptor agonist HU-308 protects against hepatic ischemia/reperfusion injury by attenuating oxidative stress, inflammatory response, and apoptosis. *J Leukoc Biol* **82**, 1382–1389.
136 Julien B, Grenard P, Teixeira-Clerc F, Van Nhieu JT, Li L, Karsak M, Zimmer A, Mallat A & Lotersztajn S (2005) Antifibrogenic role of the cannabinoid receptor CB2 in the liver. *Gastroenterology* **128**, 742–755.
137 Mallat A & Lotersztajn S (2008) Endocannabinoids and liver disease. I. Endocannabinoids and their receptors in the liver. *Am J Physiol Gastrointest Liver Physiol* **294**, G9–G12.

138 Cao Z, Mulvihill MM, Mukhopadhyay P, Xu H, Erdélyi K, Hao E, Holovac E, Hasko G, Cravatt BF, Nomura DK *et al*. (2013) Monoacylglycerol lipase controls endocannabinoid and eicosanoid signaling and hepatic injury in mice. *Gastroenterology* **144**, 808–817.
139 Wang H, Dey SK & Maccarrone M (2006) Jekyll and hyde: two faces of cannabinoid signaling in male and female fertility. *Endocr Rev* **27**, 427–448.
140 Maccarrone M (2009) Endocannabinoids: friends and foes of reproduction. *Prog Lipid Res* **48**, 344–354.
141 Rousseaux C, Thuru X, Gelot A, Barnich N, Neut C, Dubuquoy L, Dubuquoy C, Merour E, Geboes K, Chamaillard M *et al*. (2007) Lactobacillus acidophilus modulates intestinal pain and induces opioid and cannabinoid receptors. *Nat Med* **13**, 35–37.
142 Bab I, Ofek O, Tam J, Rehnelt J & Zimmer A (2008) Endocannabinoids and the regulation of bone metabolism. *J Neuroendocrinol* **20** (Suppl 1), 69–74.
143 Bab I & Zimmer A (2008) Cannabinoid receptors and the regulation of bone mass. *Br J Pharmacol* **153**, 182–188.
144 Bab I, Zimmer A & Melamed E (2009) Cannabinoids and the skeleton: from marijuana to reversal of bone loss. *Ann Med* **41**, 560–567.
145 Michalski CW, Laukert T, Sauliunaite D, Pacher P, Bergmann F, Agarwal N, Su Y, Giese T, Giese NA, Batkai S *et al*. (2007) Cannabinoids ameliorate pain and reduce disease pathology in cerulein-induced acute pancreatitis. *Gastroenterology* **132**, 1968–1978.
146 Michalski CW, Maier M, Erkan M, Sauliunaite D, Bergmann F, Pacher P, Batkai S, Giese NA, Giese T, Friess H *et al*. (2008) Cannabinoids reduce markers of inflammation and fibrosis in pancreatic stellate cells. *PLoS ONE* **3**, e1701.
147 Bermudez-Silva FJ, Suarez J, Baixeras E, Cobo N, Bautista D, Cuesta-Munoz AL, Fuentes E, Juan-Pico P, Castro MJ, Milman G *et al*. (2008) Presence of functional cannabinoid receptors in human endocrine pancreas. *Diabetologia* **51**, 476–487.
148 Petrella C, Agostini S, Alema GS, Casolini P, Carpino F, Giuli C, Improta G, Linari G, Petrozza V & Broccardo M (2010) Cannabinoid agonist WIN55,212 in vitro inhibits interleukin-6 (IL-6) and monocyte chemo-attractant protein-1 (MCP-1) release by rat pancreatic acini and in vivo induces dual effects on the course of acute pancreatitis. *Neurogastroenterol Motil* **22**, 1248–1256.
149 Guzman M (2003) Cannabinoids: potential anticancer agents. *Nat Rev Cancer* **3**, 745–755.
150 Izzo AA & Camilleri M (2009) Cannabinoids in intestinal inflammation and cancer. *Pharmacol Res* **60**, 117–125.
151 Izzo AA & Sharkey KA (2010) Cannabinoids and the gut: new developments and emerging concepts. *Pharmacol Ther* **126**, 21–38.
152 Fernandez-Ruiz J, Romero J, Velasco G, Tolon RM, Ramos JA & Guzman M (2007) Cannabinoid CB2 receptor: a new target for controlling neural cell survival? *Trends Pharmacol Sci* **28**, 39–45.
153 Cabral GA, Raborn ES, Griffin L, Dennis J & Marciano-Cabral F (2008) CB2 receptors in the brain: role in central immune function. *Br J Pharmacol* **153**, 240–251.
154 Fernandez-Ruiz J (2009) The endocannabinoid system as a target for the treatment of motor dysfunction. *Br J Pharmacol* **156**, 1029–1040.
155 Pisanti S & Bifulco M (2009) Endocannabinoid system modulation in cancer biology and therapy. *Pharmacol Res* **60**, 107–116.
156 Stella N (2010) Cannabinoid and cannabinoid-like receptors in microglia, astrocytes, and astrocytomas. *Glia* **58**, 1017–1030.
157 Fowler CJ, Gustafsson SB, Chung SC, Persson E, Jacobsson SO & Bergh A (2010) Targeting the endocannabinoid system for the treatment of cancer – a practical view. *Curr Top Med Chem* **10**, 814–827.
158 Ndong C, O'Donnell D, Ahmad S & Groblewski T (2011) Cloning and pharmacological characterization of the dog cannabinoid CB(2)receptor. *Eur J Pharmacol* **669**, 24–31.
159 Makriyannis A, Mechoulam R & Piomelli D (2005) Therapeutic opportunities through modulation of the endocannabinoid system. *Neuropharmacology* **48**, 1068–1071.
160 Cravatt BF, Demarest K, Patricelli MP, Bracey MH, Giang DK, Martin BR & Lichtman AH (2001) Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. *Proc Natl Acad Sci USA* **98**, 9371–9376.
161 Lichtman AH, Shelton CC, Advani T & Cravatt BF (2004) Mice lacking fatty acid amide hydrolase exhibit a cannabinoid receptor-mediated phenotypic hypoalgesia. *Pain* **109**, 319–327.
162 Kathuria S, Gaetani S, Fegley D, Valino F, Duranti A, Tontini A, Mor M, Tarzia G, La Rana G, Calignano A *et al*. (2003) Modulation of anxiety through blockade of anandamide hydrolysis. *Nat Med* **9**, 76–81.
163 Starowicz K, Makuch W, Osikowicz M, Piscitelli F, Petrosino S, Di Marzo V & Przewlocka B (2012) Spinal anandamide produces analgesia in neuropathic rats: possible CB(1)- and TRPV1-mediated mechanisms. *Neuropharmacology* **62**, 1746–1755.
164 Roques BP, Fournie-Zaluski MC & Wurm M (2012) Inhibiting the breakdown of endogenous opioids and cannabinoids to alleviate pain. *Nat Rev Drug Discovery* **11**, 292–310.
165 Clapper JR, Moreno-Sanz G, Russo R, Guijarro A, Vacondio F, Duranti A, Tontini A, Sanchini S,

Sciolino NR, Spradley JM *et al.* (2010) Anandamide suppresses pain initiation through a peripheral endocannabinoid mechanism. *Nat Neurosci* **13**, 1265–1270.
166 Batkai S, Pacher P, Osei-Hyiaman D, Radaeva S, Liu J, Harvey-White J, Offertaler L, Mackie K, Rudd MA, Bukoski RD *et al.* (2004) Endocannabinoids acting at cannabinoid-1 receptors regulate cardiovascular function in hypertension. *Circulation* **110**, 1996–2002.
167 Godlewski G, Alapafuja SO, Batkai S, Nikas SP, Cinar R, Offertaler L, Osei-Hyiaman D, Liu J, Mukhopadhyay B, Harvey-White J *et al.* (2010) Inhibitor of fatty acid amide hydrolase normalizes cardiovascular function in hypertension without adverse metabolic effects. *Chem Biol* **17**, 1256–1266.
168 Tourino C, Oveisi F, Lockney J, Piomelli D & Maldonado R (2010) FAAH deficiency promotes energy storage and enhances the motivation for food. *Int J Obes (Lond)* **34**, 557–568.
169 Brown WH, Gillum MP, Lee HY, Camporez JP, Zhang XM, Jeong JK, Alves TC, Erion DM, Guigni BA, Kahn M *et al.* (2012) Fatty acid amide hydrolase ablation promotes ectopic lipid storage and insulin resistance due to centrally mediated hypothyroidism. *Proc Natl Acad Sci USA* **109**, 14966–14971.
170 Ahn K, Smith SE, Liimatta MB, Beidler D, Sadagopan N, Dudley DT, Young T, Wren P, Zhang Y, Swaney S *et al.* (2011) Mechanistic and pharmacological characterization of PF-04457845: a highly potent and selective fatty acid amide hydrolase inhibitor that reduces inflammatory and noninflammatory pain. *J Pharmacol Exp Ther* **338**, 114–124.
171 Di Marzo V (2012) Inhibitors of endocannabinoid breakdown for pain: not so FA(AH)cile, after all. *Pain* **153**, 1785–1786.
172 Gunduz-Cinar O, Macpherson KP, Cinar R, Gamble-George J, Sugden K, Williams B, Godlewski G, Ramikie TS, Gorka AX, Alapafuja SO *et al.* (2012) Convergent translational evidence of a role for anandamide in amygdala-mediated fear extinction, threat processing and stress-reactivity. *Mol Psychiatry* doi:10.1038/mp.2012.72.
173 Sticht MA, Long JZ, Rock EM, Limebeer CL, Mechoulam R, Cravatt BF & Parker LA (2012) Inhibition of monoacylglycerol lipase attenuates vomiting in *Suncus murinus* and 2-arachidonoyl glycerol attenuates nausea in rats. *Br J Pharmacol* **165**, 2425–2435.
174 Nomura DK, Long JZ, Niessen S, Hoover HS, Ng SW & Cravatt BF (2010) Monoacylglycerol lipase regulates a fatty acid network that promotes cancer pathogenesis. *Cell* **140**, 49–61.
175 Schlosburg JE, Blankman JL, Long JZ, Nomura DK, Pan B, Kinsey SG, Nguyen PT, Ramesh D, Booker L, Burston JJ *et al.* (2010) Chronic monoacylglycerol lipase blockade causes functional antagonism of the endocannabinoid system. *Nat Neurosci* **13**, 1113–1119.
176 Piro JR, Benjamin DI, Duerr JM, Pi Y, Gonzales C, Wood KM, Schwartz JW, Nomura DK & Samad TA (2012) A dysregulated endocannabinoid-eicosanoid network supports pathogenesis in a mouse model of Alzheimer's disease. *Cell Reports* **1**, 617–623.
177 Carloni S, Alonso-Alconada D, Girelli S, Duranti A, Tontini A, Piomelli D, Hilario E, Alvarez A & Balduini W (2012) Pretreatment with the monoacylglycerol lipase inhibitor URB602 protects from the long-term consequences of neonatal hypoxic-ischemic brain injury in rats. *Pediatr Res* **72**, 400–406.
178 Nomura DK, Morrison BE, Blankman JL, Long JZ, Kinsey SG, Marcondes MC, Ward AM, Hahn YK, Lichtman AH, Conti B *et al.* (2011) Endocannabinoid hydrolysis generates brain prostaglandins that promote neuroinflammation. *Science* **334**, 809–813.
179 Mulvihill MM & Nomura DK (2013) Therapeutic potential of monoacylglycerol lipase inhibitors. *Life Sci* **92**, 492–497.
180 Fowler CJ (2012) Monoacylglycerol lipase – a target for drug development? *Br J Pharmacol* **166**, 1568–1585.
181 Gatta L, Piscitelli F, Giordano C, Boccella S, Lichtman A, Maione S & Di Marzo V (2012) Discovery of prostamide F2alpha and its role in inflammatory pain and dorsal horn nociceptive neuron hyperexcitability. *PLoS ONE* **7**, e31111.
182 Hsu KL, Tsuboi K, Adibekian A, Pugh H, Masuda K & Cravatt BF (2012) DAGLbeta inhibition perturbs a lipid network involved in macrophage inflammatory responses. *Nat Chem Biol* **8**, 999–1007.
183 Di Venere A, Dainese E, Fezza F, Angelucci BC, Rosato N, Cravatt BF, Finazzi-Agro A, Mei G & Maccarrone M (2012) Rat and human fatty acid amide hydrolases: overt similarities and hidden differences. *Biochim Biophys Acta* **1821**, 1425–1433.
184 Di Filippo C, Rossi F, Rossi S & D'Amico M (2004) Cannabinoid CB2 receptor activation reduces mouse myocardial ischemia-reperfusion injury: involvement of cytokine/chemokines and PMN. *J Leukoc Biol* **75**, 453–459.
185 Montecucco F, Lenglet S, Braunersreuther V, Burger F, Pelli G, Bertolotto M, Mach F & Steffens S (2009) CB(2) cannabinoid receptor activation is cardioprotective in a mouse model of ischemia/reperfusion. *J Mol Cell Cardiol* **46**, 612–620.
186 Defer N, Wan J, Souktani R, Escoubet B, Perier M, Caramelle P, Manin S, Deveaux V, Bourin MC, Zimmer A *et al.* (2009) The cannabinoid receptor type 2 promotes cardiac myocyte and fibroblast survival

and protects against ischemia/reperfusion-induced cardiomyopathy. *FASEB J* **23**, 2120–2130.
187 Lamontagne D, Lepicier P, Lagneux C & Bouchard JF (2006) The endogenous cardiac cannabinoid system: a new protective mechanism against myocardial ischemia. *Arch Mal Coeur Vaiss* **99**, 242–246.
188 Weis F, Beiras-Fernandez A, Sodian R, Kaczmarek I, Reichart B, Beiras A, Schelling G & Kreth S (2010) Substantially altered expression pattern of cannabinoid receptor 2 and activated endocannabinoid system in patients with severe heart failure. *J Mol Cell Cardiol* **48**, 1187–1193.
189 Batkai S, Jarai Z, Wagner JA, Goparaju SK, Varga K, Liu J, Wang L, Mirshahi F, Khanolkar AD, Makriyannis A *et al*. (2001) Endocannabinoids acting at vascular CB1 receptors mediate the vasodilated state in advanced liver cirrhosis. *Nat Med* **7**, 827–832.
190 Moezi L, Gaskari SA & Lee SS (2008) Endocannabinoids and liver disease. V. endocannabinoids as mediators of vascular and cardiac abnormalities in cirrhosis. *Am J Physiol Gastrointest Liver Physiol* **295**, G649–G653.
191 Batkai S, Mukhopadhyay P, Harvey-White J, Kechrid R, Pacher P & Kunos G (2007) Endocannabinoids acting at CB1 receptors mediate the cardiac contractile dysfunction in vivo in cirrhotic rats. *Am J Physiol Heart Circ Physiol* **293**, H1689–H1695.
192 Batkai S & Pacher P (2009) Endocannabinoids and cardiac contractile function: pathophysiological implications. *Pharmacol Res* **60**, 99–106.
193 Montecucco F, Matias I, Lenglet S, Petrosino S, Burger F, Pelli G, Braunersreuther V, Mach F, Steffens S & Di Marzo V (2009) Regulation and possible role of endocannabinoids and related mediators in hypercholesterolemic mice with atherosclerosis. *Atherosclerosis* **205**, 433–441.
194 Mach F & Steffens S (2008) The role of the endocannabinoid system in atherosclerosis. *J Neuroendocrinol* **20** (Suppl 1), 53–57.
195 Steffens S, Veillard NR, Arnaud C, Pelli G, Burger F, Staub C, Karsak M, Zimmer A, Frossard JL & Mach F (2005) Low dose oral cannabinoid therapy reduces progression of atherosclerosis in mice. *Nature* **434**, 782–786.
196 Montecucco F, Burger F, Mach F & Steffens S (2008) CB2 cannabinoid receptor agonist JWH-015 modulates human monocyte migration through defined intracellular signaling pathways. *Am J Physiol Heart Circ Physiol* **294**, H1145–H1155.
197 Pacher P & Ungvari Z (2008) Pleiotropic effects of the CB2 cannabinoid receptor activation on human monocyte migration: implications for atherosclerosis and inflammatory diseases. *Am J Physiol Heart Circ Physiol* **294**, H1133–H1134.
198 Montecucco F, Di Marzo V, da Silva RF, Vuilleumier N, Capettini L, Lenglet S, Pagano S, Piscitelli F, Quintao S, Bertolotto M *et al*. (2012) The activation of the cannabinoid receptor type 2 reduces neutrophilic protease-mediated vulnerability in atherosclerotic plaques. *Eur Heart J* **33**, 846–856.
199 Naccarato M, Pizzuti D, Petrosino S, Simonetto M, Ferigo L, Grandi FC, Pizzolato G & Di Marzo V (2010) Possible anandamide and palmitoylethanolamide involvement in human stroke. *Lipids Health Dis* **9**, 47.
200 Hillard CJ (2008) Role of cannabinoids and endocannabinoids in cerebral ischemia. *Curr Pharm Des* **14**, 2347–2361.
201 Muthian S, Rademacher DJ, Roelke CT, Gross GJ & Hillard CJ (2004) Anandamide content is increased and CB1 cannabinoid receptor blockade is protective during transient, focal cerebral ischemia. *Neuroscience* **129**, 743–750.
202 Zhang M, Adler MW, Abood ME, Ganea D, Jallo J & Tuma RF (2009) CB2 receptor activation attenuates microcirculatory dysfunction during cerebral ischemic/reperfusion injury. *Microvasc Res* **78**, 86–94.
203 Zhang M, Martin BR, Adler MW, Razdan RK, Jallo JI & Tuma RF (2007) Cannabinoid CB(2) receptor activation decreases cerebral infarction in a mouse focal ischemia/reperfusion model. *J Cereb Blood Flow Metab* **27**, 1387–1396.
204 Zhang M, Martin BR, Adler MW, Razdan RK, Ganea D & Tuma RF (2008) Modulation of the balance between cannabinoid CB(1) and CB(2) receptor activation during cerebral ischemic/reperfusion injury. *Neuroscience* **152**, 753–760.
205 Baty DE, Zhang M, Li H, Erb CJ, Adler MW, Ganea D, Loftus CM, Jallo JI & Tuma RF (2008) Cannabinoid CB2 receptor activation attenuates motor and autonomic function deficits in a mouse model of spinal cord injury. *Clin Neurosurg* **55**, 172–177.
206 Murikinati S, Juttler E, Keinert T, Ridder DA, Muhammad S, Waibler Z, Ledent C, Zimmer A, Kalinke U & Schwaninger M (2010) Activation of cannabinoid 2 receptors protects against cerebral ischemia by inhibiting neutrophil recruitment. *FASEB J* **24**, 788–798.
207 Kohro S, Imaizumi H, Yamakage M, Masuda Y, Namiki A & Asai Y (2004) Reductions in levels of bacterial superantigens/cannabinoids by plasma exchange in a patient with severe toxic shock syndrome. *Anaesth Intensive Care* **32**, 588–591.
208 Kohro S, Imaizumi H, Yamakage M, Masuda Y, Namiki A, Asai Y & Maruyama I (2006) Anandamide absorption by direct hemoperfusion with polymixin B-immobilized fiber improves the prognosis and organ failure assessment score in patients with sepsis. *J Anesth* **20**, 11–16.

209 Csoka B, Nemeth ZH, Mukhopadhyay P, Spolarics Z, Rajesh M, Federici S, Deitch EA, Batkai S, Pacher P & Hasko G (2009) CB2 cannabinoid receptors contribute to bacterial invasion and mortality in polymicrobial sepsis. *PLoS ONE* **4**, e6409.
210 Tschop J, Kasten KR, Nogueiras R, Goetzman HS, Cave CM, England LG, Dattilo J, Lentsch AB, Tschop MH & Caldwell CC (2009) The cannabinoid receptor 2 is critical for the host response to sepsis. *J Immunol* **183**, 499–505.
211 Kurabayashi M, Takeyoshi I, Yoshinari D, Matsumoto K, Maruyama I & Morishita Y (2005) 2-Arachidonoylglycerol increases in ischemia-reperfusion injury of the rat liver. *J Invest Surg* **18**, 25–31.
212 Batkai S, Osei-Hyiaman D, Pan H, El-Assal O, Rajesh M, Mukhopadhyay P, Hong F, Harvey-White J, Jafri A, Hasko G *et al.* (2007) Cannabinoid-2 receptor mediates protection against hepatic ischemia/reperfusion injury. *FASEB J* **21**, 1788–1800.
213 Ishii Y, Sakamoto T, Ito R & Yanaga K (2010) F2-isoprostanes and 2-arachidonylglycerol as biomarkers of lipid peroxidation in pigs with hepatic ischemia/reperfusion injury. *J Surg Res* **161**, 139–145.
214 Mendez-Sanchez N, Zamora-Valdes D, Pichardo-Bahena R, Barredo-Prieto B, Ponciano-Rodriguez G, Bermejo-Martinez L, Chavez-Tapia NC, Baptista-Gonzalez HA & Uribe M (2007) Endocannabinoid receptor CB2 in nonalcoholic fatty liver disease. *Liver Int* **27**, 215–219.
215 Deveaux V, Cadoudal T, Ichigotani Y, Teixeira-Clerc F, Louvet A, Manin S, Nhieu JT, Belot MP, Zimmer A, Even P *et al.* (2009) Cannabinoid CB2 receptor potentiates obesity-associated inflammation, insulin resistance and hepatic steatosis. *PLoS ONE* **4**, e5844.
216 Agudo J, Martin M, Roca C, Molas M, Bura AS, Zimmer A, Bosch F & Maldonado R (2010) Deficiency of CB2 cannabinoid receptor in mice improves insulin sensitivity but increases food intake and obesity with age. *Diabetologia* **53**, 2629–2640.
217 Rajesh M, Batkai S, Kechrid M, Mukhopadhyay P, Lee WS, Horvath B, Holovac E, Cinar R, Liaudet L, Mackie K *et al.* (2012) Cannabinoid 1 receptor promotes cardiac dysfunction, oxidative stress, inflammation, and fibrosis in diabetic cardiomyopathy. *Diabetes* **61**, 716–727.
218 Cote M, Matias I, Lemieux I, Petrosino S, Almeras N, Despres JP & Di Marzo V (2007) Circulating endocannabinoid levels, abdominal adiposity and related cardiometabolic risk factors in obese men. *Int J Obes (Lond)* **31**, 692–699.
219 Barutta F, Piscitelli F, Pinach S, Bruno G, Gambino R, Rastaldi MP, Salvidio G, Di Marzo V, Cavallo Perin P & Gruden G (2011) Protective role of cannabinoid receptor type 2 in a mouse model of diabetic nephropathy. *Diabetes* **60**, 2386–2396.
220 Barutta F, Corbelli A, Mastrocola R, Gambino R, Di Marzo V, Pinach S, Rastaldi MP, Perin PC & Gruden G (2010) Cannabinoid receptor 1 blockade ameliorates albuminuria in experimental diabetic nephropathy. *Diabetes* **59**, 1046–1054.
221 Annuzzi G, Piscitelli F, Di Marino L, Patti L, Giacco R, Costabile G, Bozzetto L, Riccardi G, Verde R, Petrosino S *et al.* (2010) Differential alterations of the concentrations of endocannabinoids and related lipids in the subcutaneous adipose tissue of obese diabetic patients. *Lipids Health Dis* **9**, 43.
222 Siegmund SV & Schwabe RF (2008) Endocannabinoids and liver disease. II. Endocannabinoids in the pathogenesis and treatment of liver fibrosis. *Am J Physiol Gastrointest Liver Physiol* **294**, G357–G362.
223 Munoz-Luque J, Ros J, Fernandez-Varo G, Tugues S, Morales-Ruiz M, Alvarez CE, Friedman SL, Arroyo V & Jimenez W (2008) Regression of fibrosis after chronic stimulation of cannabinoid CB2 receptor in cirrhotic rats. *J Pharmacol Exp Ther* **324**, 475–483.
224 Zyromski NJ, Mathur A, Pitt HA, Wade TE, Wang S, Swartz-Basile DA, Prather AD & Lillemoe KD (2009) Cannabinoid receptor-1 blockade attenuates acute pancreatitis in obesity by an adiponectin mediated mechanism. *J Gastrointest Surg* **13**, 831–838.
225 Borrelli F & Izzo AA (2009) Role of acylethanolamides in the gastrointestinal tract with special reference to food intake and energy balance. *Best Pract Res Clin Endocrinol Metab* **23**, 33–49.
226 Wright K, Rooney N, Feeney M, Tate J, Robertson D, Welham M & Ward S (2005) Differential expression of cannabinoid receptors in the human colon: cannabinoids promote epithelial wound healing. *Gastroenterology* **129**, 437–453.
227 Kimball ES, Schneider CR, Wallace NH & Hornby PJ (2006) Agonists of cannabinoid receptor 1 and 2 inhibit experimental colitis induced by oil of mustard and by dextran sulfate sodium. *Am J Physiol Gastrointest Liver Physiol* **291**, G364–G371.
228 Storr MA, Keenan CM, Zhang H, Patel KD, Makriyannis A & Sharkey KA (2009) Activation of the cannabinoid 2 receptor (CB2) protects against experimental colitis. *Inflamm Bowel Dis* **15**, 1678–1685.
229 Storr MA, Keenan CM, Emmerdinger D, Zhang H, Yuce B, Sibaev A, Massa F, Buckley NE, Lutz B, Goke B *et al.* (2008) Targeting endocannabinoid degradation protects against experimental colitis in mice: involvement of CB1 and CB2 receptors. *J Mol Med* **86**, 925–936.
230 Mukhopadhyay P, Rajesh M, Pan H, Patel V, Mukhopadhyay B, Batkai S, Gao B, Hasko G & Pacher P (2010) Cannabinoid-2 receptor limits inflammation, oxidative/nitrosative stress, and cell

death in nephropathy. *Free Radic Biol Med* **48**, 457–467.
231 Mukhopadhyay P, Pan H, Rajesh M, Batkai S, Patel V, Harvey-White J, Mukhopadhyay B, Hasko G, Gao B, Mackie K *et al*. (2010) CB1 cannabinoid receptors promote oxidative/nitrosative stress, inflammation and cell death in a murine nephropathy model. *Br J Pharmacol* **160**, 657–668.
232 Horvath B, Mukhopadhyay P, Kechrid M, Patel V, Tanchian G, Wink DA, Gertsch J & Pacher P (2012) beta-Caryophyllene ameliorates cisplatin-induced nephrotoxicity in a cannabinoid 2 receptor-dependent manner. *Free Radical Biol Med* **52**, 1325–1333.
233 Lim JC, Lim SK, Han HJ & Park SH (2010) Cannabinoid receptor 1 mediates palmitic acid-induced apoptosis via endoplasmic reticulum stress in human renal proximal tubular cells. *J Cell Physiol* **225**, 654–663.
234 Benito C, Nunez E, Tolon RM, Carrier EJ, Rabano A, Hillard CJ & Romero J (2003) Cannabinoid CB2 receptors and fatty acid amide hydrolase are selectively overexpressed in neuritic plaque-associated glia in Alzheimer's disease brains. *J Neurosci* **23**, 11136–11141.
235 Benito C, Romero JP, Tolon RM, Clemente D, Docagne F, Hillard CJ, Guaza C & Romero J (2007) Cannabinoid CB1 and CB2 receptors and fatty acid amide hydrolase are specific markers of plaque cell subtypes in human multiple sclerosis. *J Neurosci* **27**, 2396–2402.
236 Ramirez BG, Blazquez C, Gomez del Pulgar T, Guzman M & de Ceballos ML (2005) Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation. *J Neurosci* **25**, 1904–1913.
237 Maresz K, Carrier EJ, Ponomarev ED, Hillard CJ & Dittel BN (2005) Modulation of the cannabinoid CB2 receptor in microglial cells in response to inflammatory stimuli. *J Neurochem* **95**, 437–445.
238 Maresz K, Pryce G, Ponomarev ED, Marsicano G, Croxford JL, Shriver LP, Ledent C, Cheng X, Carrier EJ, Mann MK *et al*. (2007) Direct suppression of CNS autoimmune inflammation via the cannabinoid receptor CB1 on neurons and CB2 on autoreactive T cells. *Nat Med* **13**, 492–497.
239 Kim K, Moore DH, Makriyannis A & Abood ME (2006) AM1241, a cannabinoid CB2 receptor selective compound, delays disease progression in a mouse model of amyotrophic lateral sclerosis. *Eur J Pharmacol* **542**, 100–105.
240 Shoemaker JL, Seely KA, Reed RL, Crow JP & Prather PL (2007) The CB2 cannabinoid agonist AM-1241 prolongs survival in a transgenic mouse model of amyotrophic lateral sclerosis when initiated at symptom onset. *J Neurochem* **101**, 87–98.
241 Price DA, Martinez AA, Seillier A, Koek W, Acosta Y, Fernandez E, Strong R, Lutz B, Marsicano G, Roberts JL *et al*. (2009) WIN55,212–2, a cannabinoid receptor agonist, protects against nigrostriatal cell loss in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. *Eur J Neurosci* **29**, 2177–2186.
242 Benito C, Tolon RM, Pazos MR, Nunez E, Castillo AI & Romero J (2008) Cannabinoid CB2 receptors in human brain inflammation. *Br J Pharmacol* **153**, 277–285.
243 Palazuelos J, Aguado T, Pazos MR, Julien B, Carrasco C, Resel E, Sagredo O, Benito C, Romero J, Azcoitia I *et al*. (2009) Microglial CB2 cannabinoid receptors are neuroprotective in Huntington's disease excitotoxicity. *Brain* **132**, 3152–3164.
244 Palazuelos J, Davoust N, Julien B, Hatterer E, Aguado T, Mechoulam R, Benito C, Romero J, Silva A, Guzman M *et al*. (2008) The CB(2) cannabinoid receptor controls myeloid progenitor trafficking: involvement in the pathogenesis of an animal model of multiple sclerosis. *J Biol Chem* **283**, 13320–13329.
245 Sagredo O, Gonzalez S, Aroyo I, Pazos MR, Benito C, Lastres-Becker I, Romero JP, Tolon RM, Mechoulam R, Brouillet E *et al*. (2009) Cannabinoid CB2 receptor agonists protect the striatum against malonate toxicity: relevance for Huntington's disease. *Glia* **57**, 1154–1167.
246 Tolon RM, Nunez E, Pazos MR, Benito C, Castillo AI, Martinez-Orgado JA & Romero J (2009) The activation of cannabinoid CB2 receptors stimulates in situ and in vitro beta-amyloid removal by human macrophages. *Brain Res* **1283**, 148–154.
247 De March Z, Zuccato C, Giampa C, Patassini S, Bari M, Gasperi V, De Ceballos ML, Bernardi G, Maccarrone M, Cattaneo E *et al*. (2008) Cortical expression of brain derived neurotrophic factor and type-1 cannabinoid receptor after striatal excitotoxic lesions. *Neuroscience* **152**, 734–740.
248 Mestre L, Docagne F, Correa F, Loria F, Hernangomez M, Borrell J & Guaza C (2009) A cannabinoid agonist interferes with the progression of a chronic model of multiple sclerosis by downregulating adhesion molecules. *Mol Cell Neurosci* **40**, 258–266.
249 Loria F, Petrosino S, Hernangomez M, Mestre L, Spagnolo A, Correa F, Di Marzo V, Docagne F & Guaza C (2010) An endocannabinoid tone limits excitotoxicity in vitro and in a model of multiple sclerosis. *Neurobiol Dis* **37**, 166–176.
250 Pertwee RG (2005) The therapeutic potential of drugs that target cannabinoid receptors or modulate the tissue levels or actions of endocannabinoids. *AAPS J* **7**, E625–E654.

251 Calignano A, La Rana G, Giuffrida A & Piomelli D (1998) Control of pain initiation by endogenous cannabinoids. *Nature* **394**, 277–281.
252 Hanus L, Breuer A, Tchilibon S, Shiloah S, Goldenberg D, Horowitz M, Pertwee RG, Ross RA, Mechoulam R & Fride E (1999) HU-308: a specific agonist for CB(2), a peripheral cannabinoid receptor. *Proc Natl Acad Sci USA* **96**, 14228–14233.
253 Malan TP Jr, Ibrahim MM, Deng H, Liu Q, Mata HP, Vanderah T, Porreca F & Makriyannis A (2001) CB2 cannabinoid receptor-mediated peripheral antinociception. *Pain* **93**, 239–245.
254 Clayton N, Marshall FH, Bountra C & O'Shaughnessy CT (2002) CB1 and CB2 cannabinoid receptors are implicated in inflammatory pain. *Pain* **96**, 253–260.
255 Ibrahim MM, Deng H, Zvonok A, Cockayne DA, Kwan J, Mata HP, Vanderah TW, Lai J, Porreca F, Makriyannis A *et al.* (2003) Activation of CB2 cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: pain inhibition by receptors not present in the CNS. *Proc Natl Acad Sci USA* **100**, 10529–10533.
256 Ibrahim MM, Porreca F, Lai J, Albrecht PJ, Rice FL, Khodorova A, Davar G, Makriyannis A, Vanderah TW, Mata HP *et al.* (2005) CB2 cannabinoid receptor activation produces antinociception by stimulating peripheral release of endogenous opioids. *Proc Natl Acad Sci USA* **102**, 3093–3098.
257 Ibrahim MM, Rude ML, Stagg NJ, Mata HP, Lai J, Vanderah TW, Porreca F, Buckley NE, Makriyannis A & Malan TP Jr (2006) CB2 cannabinoid receptor mediation of antinociception. *Pain* **122**, 36–42.
258 Nackley AG, Makriyannis A & Hohmann AG (2003) Selective activation of cannabinoid CB(2) receptors suppresses spinal fos protein expression and pain behavior in a rat model of inflammation. *Neuroscience* **119**, 747–757.
259 Nackley AG, Suplita RL II & Hohmann AG (2003) A peripheral cannabinoid mechanism suppresses spinal fos protein expression and pain behavior in a rat model of inflammation. *Neuroscience* **117**, 659–670.
260 Nackley AG, Zvonok AM, Makriyannis A & Hohmann AG (2004) Activation of cannabinoid CB2 receptors suppresses C-fiber responses and windup in spinal wide dynamic range neurons in the absence and presence of inflammation. *J Neurophysiol* **92**, 3562–3574.
261 Quartilho A, Mata HP, Ibrahim MM, Vanderah TW, Porreca F, Makriyannis A & Malan TP Jr (2003) Inhibition of inflammatory hyperalgesia by activation of peripheral CB2 cannabinoid receptors. *Anesthesiology* **99**, 955–960.
262 Elmes SJ, Jhaveri MD, Smart D, Kendall DA & Chapman V (2004) Cannabinoid CB2 receptor activation inhibits mechanically evoked responses of wide dynamic range dorsal horn neurons in naive rats and in rat models of inflammatory and neuropathic pain. *Eur J Neurosci* **20**, 2311–2320.
263 Hohmann AG, Farthing JN, Zvonok AM & Makriyannis A (2004) Selective activation of cannabinoid CB2 receptors suppresses hyperalgesia evoked by intradermal capsaicin. *J Pharmacol Exp Ther* **308**, 446–453.
264 Scott DA, Wright CE & Angus JA (2004) Evidence that CB-1 and CB-2 cannabinoid receptors mediate antinociception in neuropathic pain in the rat. *Pain* **109**, 124–131.
265 Whiteside GT, Lee GP & Valenzano KJ (2007) The role of the cannabinoid CB2 receptor in pain transmission and therapeutic potential of small molecule CB2 receptor agonists. *Curr Med Chem* **14**, 917–936.
266 Rahn EJ, Zvonok AM, Thakur GA, Khanolkar AD, Makriyannis A & Hohmann AG (2008) Selective activation of cannabinoid CB2 receptors suppresses neuropathic nociception induced by treatment with the chemotherapeutic agent paclitaxel in rats. *J Pharmacol Exp Ther* **327**, 584–591.
267 Muller-Vahl KR & Emrich HM (2008) Cannabis and schizophrenia: towards a cannabinoid hypothesis of schizophrenia. *Expert Rev Neurother* **8**, 1037–1048.
268 Andreasson S, Allebeck P, Engstrom A & Rydberg U (1987) Cannabis and schizophrenia. A longitudinal study of Swedish conscripts. *Lancet* **2**, 1483–1486.
269 De Marchi N, De Petrocellis L, Orlando P, Daniele F, Fezza F & Di Marzo V (2003) Endocannabinoid signalling in the blood of patients with schizophrenia. *Lipids Health Dis* **2**, 5.
270 Ishiguro H, Horiuchi Y, Ishikawa M, Koga M, Imai K, Suzuki Y, Morikawa M, Inada T, Watanabe Y, Takahashi M *et al.* (2010) Brain cannabinoid CB2 receptor in schizophrenia. *Biol Psychiatry* **67**, 974–982.
271 Khan A, Kendall DA & Fone KCF (2010) The effects of the cannabinoid CB2 receptor antagonist, AM630, on isolation rearing-induced behavioural deficits in rats. *Schizophr Res* **117**, 391–392.
272 Grinspoon L & Bakalar JB (1995) Marihuana as medicine. A plea for reconsideration. *JAMA* **273**, 1875–1876.
273 Grinspoon L, Bakalar JB, Zimmer L & Morgan JP (1997) Marijuana addiction. *Science* **277**, 749; author reply 750-2.
274 Onaivi ES, Ishiguro H, Gong JP, Patel S, Meozzi PA, Myers L, Perchuk A, Mora Z, Tagliaferro PA, Gardner E *et al.* (2008) Functional expression of brain neuronal CB2 cannabinoid receptors are involved in the effects of drugs of abuse and in depression. *Ann NY Acad Sci* **1139**, 434–449.

275 Garcia-Gutierrez MS, Perez-Ortiz JM, Gutierrez-Adan A & Manzanares J (2010) Depression-resistant endophenotype in mice overexpressing cannabinoid CB(2) receptors. *Br J Pharmacol* **160**, 1773–1784.
276 Hu B, Doods H, Treede RD & Ceci A (2009) Depression-like behaviour in rats with mononeuropathy is reduced by the CB2-selective agonist GW405833. *Pain* **143**, 206–212.
277 Garci AGMA & Manzanares J (2011) Overexpression of CB2 cannabinoid receptors decreased vulnerability to anxiety and impaired anxiolytic action of alprazolam in mice. *J Psychopharmacol* **25**, 11–20.
278 Richardson D *et al*. (2008) Characterisation of the cannabinoid receptor system in synovial tissue and fluid in patients with osteoarthritis and rheumatoid arthritis. *Arthritis Res Ther* **10**, R43.
279 Blazquez C, Carracedo A, Barrado L, Real PJ, Fernandez-Luna JL, Velasco G, Malumbres M & Guzman M (2006) Cannabinoid receptors as novel targets for the treatment of melanoma. *FASEB J* **20**, 2633–2635.
280 Zheng D, Bode AM, Zhao Q, Cho YY, Zhu F, Ma WY & Dong Z (2008) The cannabinoid receptors are required for ultraviolet-induced inflammation and skin cancer development. *Cancer Res* **68**, 3992–3998.
281 McKallip RJ, Lombard C, Fisher M, Martin BR, Ryu S, Grant S, Nagarkatti PS & Nagarkatti M (2002) Targeting CB2 cannabinoid receptors as a novel therapy to treat malignant lymphoblastic disease. *Blood* **100**, 627–634.
282 Guida M, Ligresti A, De Filippis D, D'Amico A, Petrosino S, Cipriano M, Bifulco G, Simonetti S, Orlando P, Insabato L *et al*. (2010) The levels of the endocannabinoid receptor CB2 and its ligand 2-arachidonoylglycerol are elevated in endometrial carcinoma. *Endocrinology* **151**, 921–928.

Munro S, Thomas KL, Abu-Shaar M.. Molecular characterization of a peripheral receptor for cannabinoids. Nature 365: 61-65

Article *in* Nature · October 1993

DOI: 10.1038/365061a0 · Source: PubMed

3 authors, including:

Sean Munro
MRC Laboratory of Molecular Biology, Cambridge UK
164 PUBLICATIONS **19,589** CITATIONS
SEE PROFILE

Kerrie Thomas
Cardiff University
68 PUBLICATIONS **6,988** CITATIONS
SEE PROFILE

Molecular characterization of a peripheral receptor for cannabinoids

Sean Munro, Kerrie L. Thomas & Muna Abu-Shaar

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

THE major active ingredient of marijuana, Δ^9-tetrahydrocannabinol (Δ^9-THC), has been used as a psychoactive agent for thousands of years. Marijuana, and Δ^9-THC, also exert a wide range of other effects including analgesia, anti-inflammation, immunosuppression, anticonvulsion, alleviation of intraocular pressure in glaucoma, and attenuation of vomiting[1]. The clinical application of cannabinoids has, however, been limited by their psychoactive effects, and this has led to interest in the biochemical bases of their action. Progress stemmed initially from the synthesis of potent derivatives of Δ^9-THC[4,5], and more recently from the cloning of a gene encoding a G-protein-coupled receptor for cannabinoids[6]. This receptor is expressed in the brain but not in the periphery, except for a low level in testes. It has been proposed that the non-psychoactive effects of cannabinoids are either mediated centrally or through direct interaction with other, non-receptor proteins[1,7,8]. Here we report the cloning of a receptor for cannabinoids that is not expressed in the brain but rather in macrophages in the marginal zone of spleen.

To identify novel G-protein-coupled receptors expressed in myeloid cells, polymerase chain reaction (PCR) using degenerate primers was done on complementary DNA prepared from the human promyelocytic leukaemic line HL60. Treatment of HL60 cells with dimethylformamide (DMF) induces granulocyte differentiation, whereas tetradecanoylphorbol acetate (TPA) induces macrophage differentiation[9]. Amplification products from DMF-treated cells were cloned and sequenced, and six classes of clone showed homology to the G-protein-coupled receptor family. Two of these classes corresponded to previously identified receptors; the interleukin-8 receptor-B (ref. 10) and the adenosine A3 receptor[11] (S.M., manuscript in preparation). Of the remaining four sequences, only one showed particular homology to a published receptor. This clone, CX5, was related to a cannabinoid receptor cloned originally from rat brain[6]. To investigate the functional significance of this homology, the CX5 insert was used to screen an HL60 cDNA library. Two cDNA clones were obtained, hCX5.1 and hCX5.36, the latter extending the furthest 5′ and the complete nucleotide sequence of this clone is shown in Fig. 1*a*. The protein encoded by hCX5.36 shows 44% identity with the human cannabinoid receptor and the degree of identity rises to 68% for those transmembrane residues proposed to confer ligand specificity[12].

To determine if the CX5 receptor binds cannabinoids, the hCX5.36 cDNA was inserted into an expression vector and transfected into tissue culture cells. Figure 2*a* shows a binding curve of the cannabinoid receptor ligand Win 55212-2 (ref. 13) to membranes prepared from the transfected cells. The control cells do not express receptors for cannabinoids, but expression of hCX5.36 causes the appearance of a saturable number of high-affinity binding sites for WIN 5512-2 and also for a second high-affinity cannabinoid CP55,940 (ref. 5; Fig. 2*b*, and data not shown). The affinities of the receptor for these structurally unrelated ligands (Win 55212-2: dissociation constant K_d 3.7 nM (+/−0.4 nM); CP55,940: K_d 1.6 nM (+/−0.5 nM)), are comparable to the analogous figures (24 nM and 2–15 nM) reported for the brain receptor[13–15]. Furthermore, competition binding analysis showed that the CX5 receptor can distinguish between closely related derivatives of the archetypal cannabinoid Δ^9-THC, showing a lower affinity for the relatively inactive cannabidiol, than for the biologically active Δ^9-THC, cannabinol and 11-OH-Δ^9-THC (Fig. 2*b*). Thus it appears that hCX5.36 encodes a selective, high-affinity receptor for cannabinoids. Note that although cannabinol is only weakly cannabimimetic and binds the brain receptor with an affinity about 10-fold less than that of Δ^9-THC[5,14], this ligand binds to the CX5 receptor with an affinity comparable to that of Δ^9-THC (250 nM versus 320 nM, Fig. 2*b*). This suggests that cannabinol may have a preference for the CX5 receptor over the brain receptor. Recently, a novel compound isolated from brain, arachidonylethanolamide (anandamide), has been identified as a candidate ligand for the brain receptor[16] and Fig. 2*b* shows that this compound can also bind to the CX5 receptor. The binding affinity (K_i 1.6 μM (+/−0.4)) is lower than that reported for brain membranes (K_i 52 nM), but it should be noted that the apparent receptor affinities of cannabinoids can vary depending on the assay system used[5,14,15]. In the following text we shall refer to the original receptor as CB-R and this new receptor as CX5.

When CX5 was used to probe northern blots of RNA from HL60 cells it hybridized to two transcripts of about 2.5 and 5.0 kilobases (kb) (Fig. 3*a*). The two transcripts probably arise from the use of alternative poly(A)$^+$ addition sites. Clone hCX5.1 does not have a poly(A)$^+$ tail at the position of that in hCX5.36, but instead extends further in the 3′ direction (Fig. 1*a*). The putative polyadenylation sequence of hCX5.36 (GAUAAA) is a variant of the AAUAAA consensus that is found in a small fraction of messages and which can be used, albeit inefficiently, *in vitro*[17]. CX5 is expressed in uninduced HL60 cells, but transcript levels are elevated further on myeloid, or granulocyte, differentiation, although the gene does not appear to be expressed in mature neutrophils isolated from blood (data not shown).

To investigate the tissue distribution of CX5, a portion of a rat homologue was isolated by PCR. This rat probe (rCX5) detects an mRNA of about 2.5 kb in spleen, but not in a variety

FIG. 1 Nucleotide and protein sequences of the cDNAs hCX5.36 and hCX5.1. *a*, Nucleotide sequence of the hCX5.36 cDNA and the protein sequence encoded by the longest open ready frame, the in-frame stop codon upstream of the most 5′ ATG is underlined. Also shown is the nucleotide sequence of hCX5.1 where it diverges from that of hCX5.36 after the putative poly(A) addition site (underlined). *b*, Comparison of the protein encoded by hCX5.36 with the previously reported human cannabinoid receptor[28]. Identities are boxed and the seven putative transmembrane segments are underlined.

METHODS. Oligo-dT primed cDNA was synthesized from poly(A)$^+$ RNA prepared from HL60 cells induced with 0.5% DMF for 3 days. cDNA (5 ng in 20 μl) was amplified with *Taq* polymerase using degenerate primers encoding regions conserved between many G-protein-coupled receptors. Those that produced CX5 were GAGGGCCCATYISNNTNGAYMGNTA and TGAAGCTTSHRTANANSANNGGRTT (encoded regions in bold in sequence alignment). 40 cycles of 94 °C, 1 min, 50 °C, 2 min and 72 °C 2 min, in 10 mM Tris–HC1 pH 8.3, 3 mM $MgCl_2$, 100 mM tetramethylammonium chloride, 0.05% Tween 20, 0.05% NP–40, 250 μM dNTPs, 20 μM each primer. Gel-purified amplification products were digested with *Apa*I and *Hind*III and cloned into Bluescript. After classification of the products by sequencing, the insert from clone CX5 was used to screen 2×10^5 colonies of a cDNA library from TPA-treated HL60 cells[29] (from D. Simmons). Both hCX5.1 and hCX5.36 were isolated several times and after restriction mapping and partial sequencing of both clones, the complete nucleotide sequence of hCX5.36 was determined by primer walking using double-stranded dideoxy-sequencing. The GenBank accession number for hCX5.36 (CB2) is X74328.

a

HCX5.36

```
CAGGTCCTGGGAGAGGACAGAAAACAACTGGACTCCTCAGCCCCCGGCAGCTCCCAGTGCCCAGCCACCCACAACACAACCCAAAGCCTT   90

                                                    MetGluGluCysTrpValThrGluIleAlaAsnGlySerLysAspGlyLeuAsp
CTAGACAAGCTCAGTGGAATCTGAAGGGCCCACCCCATGGAGGAATGCTGGGTGACAGAGATAGCCAATGGCTCCAAGGATGGCTTGGAT   180

SerAsnProMetLysAspTyrMetIleLeuSerGlyProGlnLysThrAlaValAlaValLeuCysThrLeuGluGlyLeuLeuSerAla
TCCAACCCTATGAAGGATTACATGATCCTGAGTGGTCCCCAGAAGACAGCTGTTGCTGTGTTGTGCACTCTTCTGGGCCTGCTAAGTGCC   270

LeuGluAsnValAlaValLeuTyrLeuIleLeuSerSerHisGlnLeuArgArgLysProSerTyrLeuPheIleGlySerLeuAlaGly
CTGGAGAACGTGGCTGTGCTCTATCTGATCCTGTCCTCCCACCAACTCCGCCGGAAGCCCTCATACCTGTTCATTGGCAGCTTGGCTGGG   360

AlaAspPheLeuAlaSerValValPheAlaCysSerPheValAsnPheHisValPheHisGlyValAspSerLysAlaValPheLeuLeu
GCTGACTTCCTGGCCAGTGTGGTCTTTGCATGCAGCTTTGTGAATTTCCATGTTTTCCATGGTGTGGATTCCAAGGCTGTCTTCCTGCTG   450

LysIleGlySerValThrMetThrPheThrAlaSerValGlySerLeuLeuLeuThrAlaIleAspArgTyrLeuCysLeuArgTyrPro
AAGATTGGCAGCGTGACTATGACCTTCACAGCCTCTGTGGGTAGCCTCCTGCTGACCGCCATTGACCGATACCTCTGCCTGCGCTATCCA   540

ProSerTyrLysAlaLeuLeuThrArgGlyArgGlyLeuValThrLeuGlyIleMetTrpValLeuSerAlaLeuValSerTyrLeuPro
CCTTCCTACAAAGCTCTGCTCACCCGTGGAAGGGGACTGGTGACCCTGGGCATCATGTGGGTCCTCTCAGCACTAGTCTCCTACCTGCCC   630

LeuMetGlyTrpThrCysCysProArgProCysSerGluLeuProLeuIleProAsnAspTyrLeuLeuSerTrpLeuLeuPheIle
CTCATGGGATGGACTTGCTGTCCCAGGCCCTGCTCTGAGCTTTTCCCACTGATCCCCAATGACTACCTGCTGAGCTGGCTCCTGTTCATC   720

AlaPheLeuPheSerGlyIleIleTyrThrTyrGlyHisValLeuTrpLysAlaHisGlnHisValAlaSerLeuSerGlyHisGlnAsp
GCCTTCCTCTTTTCCGGAATCATCTACACCTATGGGCATGTTCTCTGGAAGGCCCATCAGCATGTGGCCAGCTTGTCTGGCCACCAGGAC   810

ArgGlnValProGlyMetAlaArgMetArgLeuAspValArgLeuAlaLysThrLeuGlyLeuValLeuAlaValLeuLeuIleCysTrp
AGGCAGGTGCCAGGAATGGCCCGAATGAGGCTGGATGTGAGGTTGGCCAAGACCCTAGGGCTAGTGTTGGCTGTGCTCCTCATCTGTTGG   900

ProProValLeuAlaLeuMetAlaHisSerLeuAlaThrThrLeuSerAspGlnValLysLysAlaPheAlaPheCysSerMetLeuCys
TTCCCAGTGCTGGCCCTCATGGCCCACAGCCTGGCCACTACGCTCAGTGACCAGGTCAAGAAGGCCTTTGCTTTCTGCTCCATGCTGTGC   990

LeuIleAsnSerMetValAsnProValIleTyrAlaLeuArgSerGlyGluIleArgSerSerAlaHisHisCysLeuAlaHisTrpLys
CTCATCAACTCCATGGTCAACCCTGTCATCTATGCTCTACGGAGTGGAGAGATCCGCTCCTCTGCCCATCACTGCCTGGCTCACTGGAAG  1080

LysCysValArgGlyLeuGlySerGluAlaLysGluGluAlaProArgSerSerValThrGluThrGluAlaAspGlyLysIleThrPro
AAGTGTGTGAGGGGCCTTGGGTCAGAGGCAAAAGAAGAAGCCCCGAGATCCTCAGTCACCGAGACAGAGGCTGATGGGAAAATCACTCCG  1170

TrpProAspSerArgAspLeuAspLeuSerAspCys***
TGGCCAGATTCCAGAGATCTAGACCTCTCTGATTGCTGATGAGGCCTCTTCCCAATTTAAACAACTCAAGTCAGAAATCAGTTCACTCCC  1260
TGGAAGAGAGAGAGGGGTCTTGGCACTCTCTTCTTACTTAAACCAGTCCCAGACACCTAGACACGGACCCCTTTTTGCTGATGAGTGTTG  1350
GGACTGACTCCTGGAAGACAGCCTGGCCTTGCCCACCTGCACACAGTCTGTTGGATAGGTAGGGCCACGAGGAGTAGCCAGGTAGGCGAG  1440
ACACAAAAAGGCCTGGGACAGGGTCAGTACAAGTCAGGACAGGCTTCATGCCTGCATCCTCCAGAGACCACCAGGAGCCAAAGCGAGCCT  1530
CCAGGCCCAGCAATGAGGGACTTGGGAGAAATCTGAGAAGAATGGGTTGTTCTCTTGGGAAGTCAGGGTATCAGATGGGATGGACATCCA  1620
GGTCTTCTCTCTGCCTAATTGTCAAGGCCTCCTTGGCTCTGGAGCTATGAAAGGCCCCACTTTCAAGTCACCCTTGCCACTGAGGACCGA  1710
GGACTATGCTATGATGAGGATTAAGGTGTTGACTTGCCTCTTTCAGAGATAAATGACAAGCCTTCAAAAAAAAAAAAAAA            1790
```

HCX5.1:

```
....TATGCTATGATGAGGATTAAGGTGTTGACTTGCCTCTTTCAGAGATAAATGACAAGCCTTCAGTTTGGGGCATCCTGTTGTTTG..
```

b

```
hCB-R      MKSILDGLADTTFRTITTDLLYVGSNDIQYEDIKGDMASKLGYFPQ

hCX5                                  MEECWVTEIANGSKDGLDSN----  20
hCB-R KFPLTSFRGSPFQEKMTAGDNPQLVPADQVNITEFYNKSLSSFKENEENI

       --------PMKDYMIILSGPQKTAMAVLCTLLGLLSALENVAVLYLILSSHQ  63
       QCGENFMDIECFMVLNPSQQLAIAVLSLTLGTFTVLENLLVLCVILHSRS
                                                  I

       LRRKPSYLFIGSLAGADFLASVVFACSFVNFHVFHGVDSKAVFLLKIGSV  113
       LRCRPSYHFIGSLAMADLLGSVIFVYSFIDFHVFHRKDSRNVFLFKLGGV
                                 II                  III

       TMTFTASVGSLLTAIDRYLCLRYPPSYKALLTRGRGLVTLGIMWVLSAL  163
       TASFTASVGSLFLTAIDRYISIHRPLAYKRIVTRPKAVVAFCLMWTIAIV
       III                                          IV

       VSYLPLMGWTCCPRP--CSELFPLIPNDYLLSWLLFIAFLFSGIIYTYGH  211
       IAVLPLLGWNCEKLQSVCSDIFPHIDETYLMFWIGVTSVLLLFIMVAYMY
                                                V

       VLWKAHQHVASL------------SGHQDRQV--PGMARMRLDVRLAKT  246
       ILWKAHSHAVRMIQRGTQKSIIIHTSEDGKVQVTRPDQARM--DIRLAKT

       LGLVLAVLLICWFPVLALMAHSLATTLSDQVKKAFAFCSMLCLINSMVNP  296
       LVLILVVLIICWGPLLAIMVYDVFGKMNKLIKTVFAFCSMLCLLNSTVNP
                  VI                              VII

       VIYALRSGEIRSS------------------AHHCLAHWKKCVRGLGS  326
       IIYALRSKDLRHAFRSMFPSCEGTAQPLDNSMGDSDCLHKHANNAASVHR

       EAKEEAPRSSVTETEADGKITPWPDSRDLDLSDC   360
       -AAESCIKSTV-------KIAKVTMSVSTDTSAEAL
```

of other tissues (Fig. 3*b*). In particular, the rCX5 transcript is not detected in brain, even though the 6 kb mRNA encoding the rat CB-R can be readily detected in the same sample. The expression pattern of the CB-R gene corresponds well to the distribution of binding sites for cannabinoids in the brain[6,18]. But it is possible that CX5 is expressed in a subset of these sites and its expression level is too low to be detected in total brain RNA. To investigate this possibility horizontal sections of rat brain were probed by *in situ* hybridization with labelled oligonucleotides corresponding to rat CB-R and to rCX5 (Fig. 4). As previously reported, the brain receptor has a widespread distribution with high levels of expression in the cortex, hippocampus, striatum and cerebellum. When adjacent sections were probed for rCX5, no expression could be detected in these, or any other, regions. The rCX5 oligonucleotide does, however, hybridize to localized regions of the spleen (Fig. 4*b*, *c*). The expression appears concentrated in the marginal zones found around the periarteriolar lymphoid sheaths. The expression of hCX5 in HL60 cells differentiated along the myeloid lineage implies that this expression is likely to be in macrophages. To confirm this, splenic macrophages/monocytes were purified using cell sorting

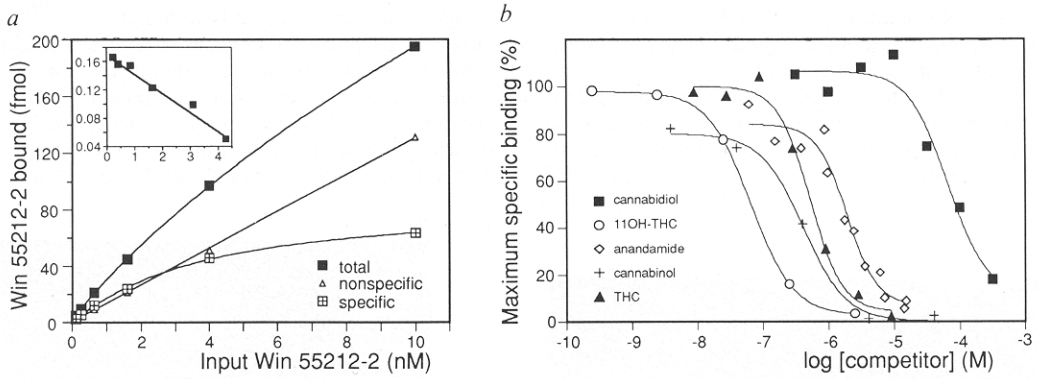


FIG. 2 Binding of cannabinoids to the receptor encoded by hCX5.36. *a*, Binding of [^{3}H]Win 55212-2 to membranes from COS cells transfected with an expression plasmid SC36, that contains the hCX5.36 cDNA. The inset plot show the specific binding presented as bound/free against [bound] ($Mx10^{-10}$). *b*, Displacement by cold cannabinoids of [^{3}H]CP55,940, or of [^{3}H]Win 55212-2, bound to membranes from COS cells transfected with SC36.
METHODS. Plasmid SC36 is hCX5.36 inserted into the vector CDM8[30]. 72 h after transfection, cells were Dounce homogenized and the membranes pelleted from the post-nuclear supernatant at 90,000g for 20 min, washed and then resuspended in 50 mM Tris–HC1 pH 7.4, 3 mM $MgCl_2$, 1 mM EDTA and stored in liquid N_2. Binding of [^{3}H]Win 55212-2 (49.6 Ci $mmol^{-1}$; New England Nuclear) to membranes (40 μg membrane protein per 150 μl reaction), was determined essentially as described, except that siliconized 1.5 ml polypropylene tubes were used for the binding reactions and 5% ethanol, 5% Triton X-100 was used to solubilize the membrane pellets[5]. Nonspecific binding was measured in the presence of 10 μM Δ^9-THC, and data points shown are means of duplicates (average duplicate's difference 4.3%). Displacement by cold cannabinoids (Sigma) was determined using 1.0 nM [^{3}H]CP55,940 (107 Ci mol^{-1}; New England Nuclear), or for anandamide (provided by R. Mechoulam) and cannabidiol, 1.0 nM [^{3}H]Win55212-2, although similar results were obtained for all competitors with both hot ligands (not shown). The anandamide displacement curve comprises data from two separate experiments. All data points are means of duplicates and all experiments were repeated at least twice. Inhibition constants (K_i) in nM: 11-OH-Δ^9-THC, 40 +/− 1.5; Δ^9-THC, 320 +/− 80; cannabinol, 250 +/− 80; cannabidol, 38,000 +/−18,000.

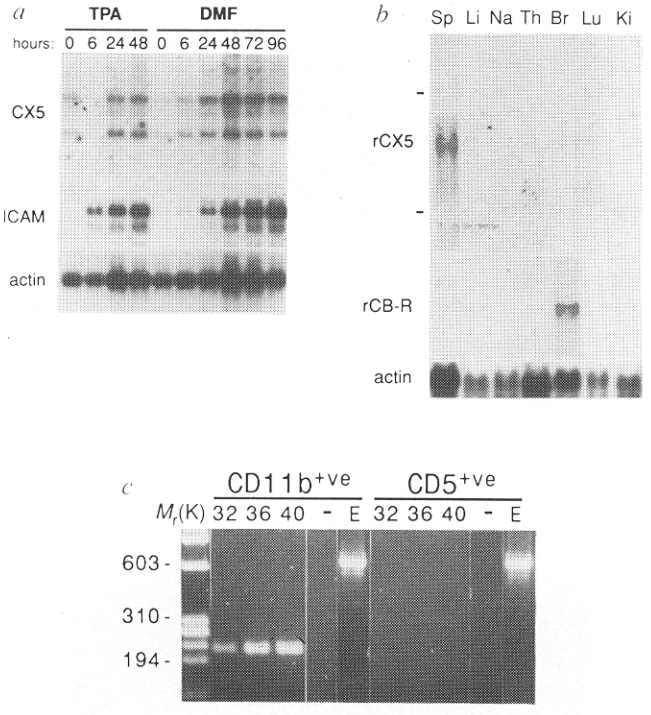


FIG. 3 Expression of CX5 transcripts in HL60 cells and in rat tissues. *a*, Northern blots of RNA from HL60 cells induced with either 20 ng ml^{-1} TPA or with 0.7% DMF, probed with either hCX5, or with ICAM-1 to follow induction. The hCX5 blot was then reprobed for γ-actin. *b*, Northern blot of RNA from various rat tissues probed with a rat homologue of CX5 (SP, spleen; Li, liver; Na, nasal epithelium; Th, thymus; Br, brain; Lu, lung; Ki, kidney). The blot was then reprobed with the rat cannabinoid receptor (rCB-R) and then with actin. *c*, PCR analysis of rCX5 expression in sorted rat spleenocytes. cDNA from rat spleenocytes sorted using antibodies against CD11b for macrophages/monocytes, or CD5 for T-cells, was amplified with primers specific for rCX5, with products being removed after the indicated number of cycles (32,36,40). To demonstrate that the rCX5 signal derives from mRNA, cDNA reactions without added reverse transcriptase were amplified for 40 cycles (−) and, as a positive control, cDNAs were amplified with primers specific for elongation factor 1α (E).
METHODS. Total RNA was isolated from HL60 cells by lysis in guanidinium/LiCl, and 7.5 μg per sample was separated on a 1.2% agarose/4% formaldeyhde gel, transferred to nylon (Hybond-N, Amersham) and ultraviolet cross-linked. Parallel blots were probed at 42 °C in 5 × SSPE/50% formamide/100 μg/ml salmon sperm DNA/5x Denhards/0.1% SDS with either CX5 or ICAM-1[29] labelled with ^{32}P by random-priming (Pharmacia). After washing with 1 × SSC at 42 °C, the blot was exposed to Kodak XAR with an intensifying screen, ICAM, 8 days; hCX5, 10 days). The blots were then stripped according to the manufacturer's instructions and reprobed with human γ-actin, exposure 6 h. The fall in expression at the 6 h time point in TPA was reproducible. A rat homologue of CX5 was cloned from genomic DNA by PCR using primers. GGGCTCGAGGTNRAYTTYCAYGTNTT and GAGGGATCCATNSWRCARAANGCRAA that encode sequences in hCX5 which are also found in the cannabinoid receptor but not in other G-proten-coupled receptors (VNFHVF (91–96) and FAFCSM (279–284)). Cloning of PCR products of 600–650 bp produced primarily the rat cannabinoid receptor or a sequence with 88% homology to hCX5, which was termed rCX5 (S.M. unpublished observations). Total RNA extracted by guanidinium lysis from various rat tissues (5–10 μg per lane) was blotted and probed as before with rCX5, rat cannabinoid receptor and γ-actin and then exposed using a phospho-imager (Molecular Dynamics) for rCX5 (9 h), CB-R (4 h) or XAR film (12 h) for actin. For PCR analysis, rat spleenocytes were separated by FACS using MRC OX-42 (CD11b)[31] or MRC OX-19 (CD5)[32] (Serotec). Cytoplasmic RNA prepared from 3×10^5 cells by detergent lysis, (20 μg glycogen added as carrier) was treated with ribonuclease-free DNase (0.5 unit for 10 min; RQ1, Promega), phenol extracted, ethanol precipitated, resuspended in reverse transcriptase buffer with random primers, divided in two and MMLV reverse transcriptase was added to one set (GIBCO). After 60 min at 37 °C, PCR amplification was done with the primers TTTCACGGTGTGGACTCC and TAGGTAGGAGATCAAGCG (rCX5, 214 bp product) or GAAATGCACCATGAAGCT and TTACGATGCATTGTTATC (EF-1α, 645 bp product from spliced transcript[33]) using 94 °C, 1 min 54 °C, 1 min, 72°C 1 min with the manufacturer's buffer (Promega).

and the expression of CX5 examined using PCR. Expression was detected in the macrophage/monocyte population but not in the CD5-positive population used as a control.

The marginal zone is the site through which blood-borne cells and antigens enter the spleen, and the marginal zone macrophages comprise a distinct population of highly phagocytic cells thought to play a role in both digesting and processing bacterial antigens and in directing lymphocyte recirculation[19–21]. We are currently preparing antibodies specific for CX5 to investigate its cellular distribution in more detail, but the *in situ* distribution suggests that its expression is concentrated in these marginal zone macrophages. The *in vivo* function of CX5 is presumably to transduce a signal through a G-protein in response to an endogenous ligand, although we do not yet have direct evidence

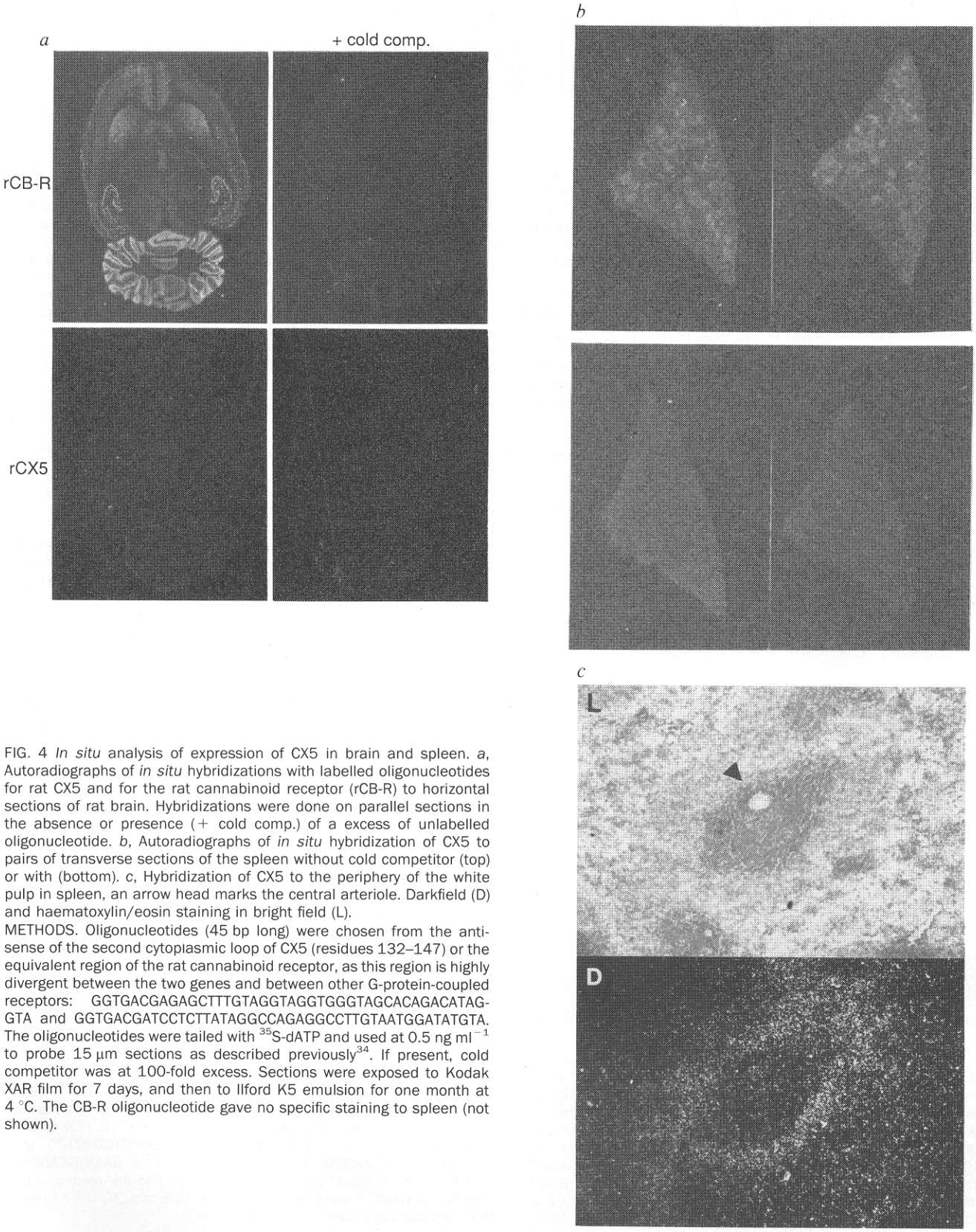


FIG. 4 *In situ* analysis of expression of CX5 in brain and spleen. *a*, Autoradiographs of *in situ* hybridizations with labelled oligonucleotides for rat CX5 and for the rat cannabinoid receptor (rCB-R) to horizontal sections of rat brain. Hybridizations were done on parallel sections in the absence or presence (+ cold comp.) of a excess of unlabelled oligonucleotide. *b*, Autoradiographs of *in situ* hybridization of CX5 to pairs of transverse sections of the spleen without cold competitor (top) or with (bottom). *c*, Hybridization of CX5 to the periphery of the white pulp in spleen, an arrow head marks the central arteriole. Darkfield (D) and haematoxylin/eosin staining in bright field (L).

METHODS. Oligonucleotides (45 bp long) were chosen from the antisense of the second cytoplasmic loop of CX5 (residues 132–147) or the equivalent region of the rat cannabinoid receptor, as this region is highly divergent between the two genes and between other G-protein-coupled receptors: GGTGACGAGAGCTTTGTAGGTAGGTGGGTAGCACAGACATAGGTA and GGTGACGATCCTCTTATAGGCCAGAGGCCTTGTAATGGATATGTA. The oligonucleotides were tailed with ^{35}S-dATP and used at 0.5 ng ml^{-1} to probe 15 μm sections as described previously[34]. If present, cold competitor was at 100-fold excess. Sections were exposed to Kodak XAR film for 7 days, and then to Ilford K5 emulsion for one month at 4 °C. The CB-R oligonucleotide gave no specific staining to spleen (not shown).

for such coupling. Analysis of other seven spanning receptors has suggested that basic, amphipathic helixes at either end of the third cytoplasmic loop are involved in G-protein coupling and appropriate residues are found in these positions both in the CX5 sequence and in the brain receptor, which has been shown to be G-protein coupled[6,22].

There are many reports of cannabinoids exerting suppressive effects on various cells of the immune system, including macrophages[23–25], although the significance of some of these observations has been questioned because of the high doses of drug used[26]. But the location of the CX5 receptor, and its distinct structure from the brain receptor, strongly suggest that the endogenous ligand for these receptors will have an immuno-modulatory role in addition to its neuronal function. Anandamide has been recently identified as a candidate ligand for the cannabinoid receptor[16] and this compound also binds to the CX5 receptor, although with an apparent affinity 30-fold less than that reported for the brain receptor. Anandamide is able to cross the blood brain barrier rapidly[27] but worthwhile speculation as to its function, and possible interactions between the neural and immunological systems, will require the identification of all the sources of this intriguing molecule. Furthermore, the question of further potential ligands from brain remains to the resolved[16]. Even so the existence of the CX5 receptor does have further implications. G-protein-coupled receptors are highly conserved throughout evolution[12], and yet the sequence of CX5 is considerably divergent from that of CB-R. Of the 162 residues in transmembrane sections of the human CB-R, three are different in rat CB-R, but 68 are different in human CX5. This suggests that the two receptors did not diverge recently and furthermore it suggests that it should be possible to identify receptor-specific cannabinoids. The fact that cannabinol appears to have a higher relative affinity for the CX5 receptor than for the brain receptor, may provide the basis for identifying such a ligand for the CX5 receptor. We suggest that in future the two receptors be distinguished by calling the brain receptor CB1 and the CX5 receptor CB2. It has been proposed that the peripheral effects of cannabinoids are either indirect effects of central actions, or reflect interactions with non-receptor proteins such as lipoxygenases[1,8]. It is clearly possible that some of these peripheral effects are in fact mediated through the CB2 receptor and it will be interesting to determine the activities of any cannabinoids specific for this receptor. □

Received 11 June; accepted 14 July 1993.

1. Dewey, W. L. *Pharmac. Rev.* **38,** 151–178 (1986).
2. Howlett, A. C. *et al. Trends Neurosci.* **13,** 420–432 (1990).
3. Hollister, L. E. *Pharmac. Rev.* **38,** 1–20 (1986).
4. Razdan, R. K. *Pharmac. Rev.* **38,** 75–149 (1986).
5. Devane, W. A., Dysarz, F. A., Johnson, M. R., Melvin, L. S. & Howlett, A. C. *Molec. Pharmacol.* **34,** 605–613 (1988).
6. Matsuda, L. A., Lolait, S. J., Brownstein, B. J., Young, A. C. & Bonner, T. I. *Nature* **356,** 561–564 (1990).
7. Martin, B. R. *Pharmac. Rev.* **38,** 45–74 (1986).
8. Reichman, M., Nen, W. & Hokin, L. E. *Molec. Pharmac.* **40,** 547–555 (1991).
9. Collins, S. J. *Blood* **70,** 1233–1244 (1987).
10. Lee, J. *et al. J. biol. Chem.* **267,** 16283–16287 (1992).
11. Zhou, Q. Y. *et al. Proc. natn. Acad. Sci. U.S.A.* **89,** 7432–7436 (1992).
12. Baldwin, J. M. *EMBO J.* **12,** 1693–1703 (1993).
13. Jansen, E. M., Haycock, D. A., Ward, S. J. & Seybold V. S. *Brain Res.* **575,** 93–102 (1992).
14. Devane, W. A. *et al. J. med. Chem.* **35,** 2065–2069 (1992).
15. Herkenham, M. *et al. Proc. natn. Acad. Sci. U.S.A.* **87,** 1932–1936 (1990).
16. Devane, W. A. *et al. Science* **258,** 1946–1949 (1992).
17. Sheets, M. D., Ogg, S. C. & Wickens, M. P. *Nucleic. Acids Res.* **18,** 5799–5805 (1990).
18. Mailleux, P. & Vanderhaeghen, J.-J. *Neuroscience* **48,** 655–668 (1992).
19. Kraal, G. *Int. Rev. Cytol.* **132,** 31–74 (1992).
20. Humphrey, J. H. & Grennan, D. *Eur. J. Immun.* **11,** 221–228 (1981).
21. Keshav, S., Chung, P., Milon, G. & Gordon, S. *J. exp. Med.* **174,** 1049–1058 (1991).
22. Conklin, B. R. & Bourne, H. R. *Cell* **73,** 631–641 (1993).
23. Munson, A. E. & Fehr, K. O. in *Adverse Health and Behavioral Consequences of Cannabis Use* (eds Fehr, K. O. & Kalant, H.) 257–353 (Addiction Research Foundation, Toronto, 1983).
24. Schatz, A. R., Kessler, F. K. & Kaminski, N. E. *Life Sci.* **51,** 25–30 (1992).
25. Lopez-Cepero, M., Friedman, M., Klein, T. & Friedman, H. *J. Leukocyte Biol.* **39,** 679–686 (1986).
26. Hollister, L. E. *J. Psychoactive Drugs* **24,** 159–164 (1992).
27. Fride, E. & Mechoulam, R. *Eur. J. Pharmac.* **231,** 313–314 (1993).
28. Gerard, C. M., Mollereau, C., Vassart, G. & Parmentier, M. *Biochem. J.* **279,** 129–134 (1991).
29. Simmons, D., Makgoba, M. W. & Seed, B. *Nature* **331,** 624–627 (1988).
30. Seed, B. *Nature* **329,** 840–842 (1987).
31. Robinson, A. P., White, T. M. & Mason, D. W. *Immunology* **57,** 239–247 (1986).
32. Dallman, M. J., Thomas, M. L. & Green, J. R. *Eur. J. Immun.* **12,** 511–518 (1982).
33. Shirasawa, T., Sakamoto, K., Akashi, T., Takahashi, H. & Kawashima, A. *Nucleic Acids Res.* **20,** 909 (1992).
34. Wisden, W., Morris, B. J. & Hunt, S. P. in *Molecular Neurobiology: a Practical Approach* (eds Chad, J. & Wheal, H.) 205–225 (IRL at Oxford Univ. Press, Oxford, 1991).

ACKNOWLEDGEMENTS. We thank J. Baldwin and T. Jackson for advice on receptors, D. Simmons for libraries and clones, N. Dear and A. Venkitaraman for RNA samples, R. Mechoulam and R. Pertwee for generously providing a sample of anandamide, D. Gilmore for cell sorting, J. Fogg and T. Smith for oligonucleotides, M. Bretscher, R. Chapman, H. Pelham and D. Zarkower for criticism of the manuscript and D. Cunningham and colleagues at the MRC Laboratory of Molecular Biology for help, guidance and support. K.L.T is funded by the Human Frontiers of Science Program.

Journal of Autism and Developmental Disorders (2020) 50:4258–4266
https://doi.org/10.1007/s10803-020-04494-4

ORIGINAL PAPER

National and State Estimates of Adults with Autism Spectrum Disorder

Patricia M. Dietz[1] · Charles E. Rose[1] · Dedria McArthur[1] · Matthew Maenner[1]

Published online: 10 May 2020

Abstract
U.S. national and state population-based estimates of adults living with autism spectrum disorder (ASD) are nonexistent due to the lack of existing surveillance systems funded to address this need. Therefore, we estimated national and state prevalence of adults 18–84 years living with ASD using simulation in conjunction with Bayesian hierarchal models. In 2017, we estimated that approximately 2.21% (95% simulation interval (SI) 1.95%, 2.45%) or 5,437,988 U.S. adults aged 18 and older have ASD, with state prevalence ranging from 1.97% (95% SI 1.55%, 2.45%) in Louisiana to 2.42% (95% SI 1.93%, 2.99%) in Massachusetts. Prevalence and case estimates of adults living with ASD (diagnosed and undiagnosed) can help states estimate the need for diagnosing and providing services to those unidentified.

Keywords Autism spectrum disorder · Developmental disabilities · Intellectual disability · Prevalence estimates · Modeling

Introduction

In the U.S. approximately 1.5 million children ages 3–17 years have been diagnosed with Autisms Spectrum Disorder (ASD), a developmental disability characterized by deficits in social communication and interaction, as well as restricted, repetitive behaviors (Kogan et al. 2018; American Psychiatric Association 2013). Prevalence estimates for adults are unknown due to a lack of existing surveillance systems to monitor the prevalence. ASD is a life-long disability that can require intensive support throughout life for some but not all with the condition (Roux et al. 2015; Croen et al 2015; Nicolaidis et al. 2014; Murphy et al. 2016). Based on data from 11 surveillance sites, 1.67% of 8-year-old children have ASD (Baio et al. 2018). As children with diagnosed ASD mature into adolescence and early adulthood, parents, service providers, and policy makers can support them by ensuring necessary services for adults with ASD are available to meet the demand.

National and state-based estimates of adults living with ASD could inform planning for programs and services; however, no U.S. estimates currently exist. Without data on ASD in adults, estimates of ASD prevalence among adults can be derived from applying existing data to models. Modeling of estimates has been done for national prevalence of congenital heart disease (Gilboa et al. 2016) and state-based prevalence of hepatitis C virus (Rosenberg et al. 2018). We estimated national and state prevalence of adults living with ASD using existing state-based data for children and adjusting for higher mortality rates among persons with ASD.

Methods

We used unpublished ASD prevalence data from NSCH (2016–2018), published ASD population mortality rates, 1999–2017 U.S. mortality rates by state, age, and sex, and 2017 population to develop an estimator of ASD prevalence and cases by state and sex, and nationally for 2017. For the unpublished ASD prevalence data, we calculated

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10803-020-04494-4) contains supplementary material, which is available to authorized users.

✉ Patricia M. Dietz
pad8@cdc.gov

Charles E. Rose
CVR7@cdc.gov

Dedria McArthur
njk5@cdc.gov

Matthew Maenner
xde8@cdc.gov

[1] National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, 4770 Buford Highway NE, (MS S106-4), Atlanta, GA 30341, USA

ASD prevalence for the age group 3–17 years, consistent with NSCH reports (Kogan et al 2018). In that study the age group 3–5 years had a prevalence of 1.97%, (95% CI 1.41–2.74) compared with 2.61 (95% CI 2.15–3.15) for ages 6–11 years and 2.65 (95% CI 2.27–3.10) for ages 12–17. A sensitivity analysis was run using data for ages 6–17 years to assess the effect of the choice of age group on the estimated number of adults with ASD.

Our estimator of the prevalence and cases of ASD for the *i*th state, *j*th age (year), and *k*th sex used the following equations and begins with the ages 3–17 prevalence estimate (see Supplemental material for derivation).

$$\gamma_{ijk}^{\text{adj}} = N_{ijk}\rho_{i(j-1)k}\frac{S_{ik}^{ASD}}{S_{ijk}^{POP}} \tag{1}$$

$$\rho_{ijk}^{\text{adj}} = \frac{\gamma_{ijk}^{\text{adj}}}{N_{ijk}} = \frac{1}{N_{ijk}}N_{ijk}\rho_{i(j-1)k}\frac{S_{ik}^{ASD}}{S_{ijk}^{POP}} = \rho_{i(j-1)k}\frac{S_{ik}^{ASD}}{S_{ijk}^{POP}} \tag{2}$$

where γ^{adj} is the number of ASD cases adjusted for the survival ratio of the ASD to population, N is the population, ρ is the ASD prevalence, survival rates for the adults with ASD and population are defined by S, and ρ^{adj} is the adjusted state, sex, and age (> 17) ASD prevalence rate. National and state estimates are obtained by summing over all ASD cases for ages 18–84 and then calculating the national and state ASD prevalence estimates. Estimates went up to age 84, reflecting the availability of general population mortality data. Our ASD prevalence estimator assumes that given the age 3–17 prevalence estimate, the prevalence decreases over time as a function of the ASD-to-population survival ratio.

Inputs into the models (Eqs. 1, 2) are presented in Table 1. Inputs assumed to be known are the population and mortality rates whereas the ages 3–17 prevalence and survival ratio are estimated. Using simulation, we incorporate the uncertainty of the ages 3–17 prevalence and survival ratio estimates into the model. Our inputs include 2016–18 state-based ASD numbers by sex for children ages 3–17 years from the National Survey of Children's Health (NSCH). NSCH is an annual, cross-sectional, complex design, address-based survey that collects information on the health and well-being of children ages 0–17 years using both web-based and paper and pencil methodologies. Children whose parents responded "yes" on two ASD questions were included: (1) "Has a doctor or other health care provider ever told you that your child has Autism or Autism Spectrum Disorder? Include diagnoses of Asperger's Disorder or Pervasive Developmental Disorder (PDD)"; (2) "If yes, does this child currently have the condition?" We used a two-step process to estimate the ages 3–17 ASD prevalence by state and sex. First, we used the NSCH ages 3–17 data and study design weights to estimate the logistic regression model regression coefficients and standard errors (SE) by state and sex. Our second stage used the NSCH logistic regression coefficients and SE in a Bayesian hierarchical meta-analysis model to estimate the partially pooled effects for each state and sex. Partial pooling assumes each state and sex has a different prevalence, but the data for all states and sex informs the prevalence estimate of each state and sex. We used partial pooling to reduce the influence of outliers and estimates from states with small numbers of observations, resulting in more statistically robust estimates (Gelman 2013). The 2017 state populations, by sex, were obtained from the National Center for Health Statistics (US DHHS 2018a, b). We estimated ASD prevalence separately for males and females as males are known to have higher rates of ASD diagnoses than females (Kogan et al. 2018; Baio et al. 2018).

Standardized mortality ratio (SMR) is a relative measure of excess mortality for one group compared to the general population. The SMR by sex was estimated using a meta-analysis method based on five studies (Supplement Table 1). We used a Bayesian hierarchical Poisson model with the observed mortality as the outcome and the expected mortality as the offset (Supplemental Methods) to estimate the partially pooled overall SMR by sex. We assumed that the SMR was the same across states because we had no information on state-specific mortality rates. We also assumed the SMR did not change across age groups, although the majority of the mortality studies followed persons with ASD only through middle age.

We used simulation to estimate the 2017 national and state prevalence and 95% simulation interval (SI) of men and women ages 18–84 living with ASD. First, we obtained the mortality rate by state and sex for ages 3–17, and 18–84 by year and strata (state, sex, and age). Second, we obtained the U.S. 2017 population data by state, sex, and age. Next, we estimated ages 3–17 meta-analysis prevalence and associated SE by state and sex and randomly drew 10,000 samples from a normal distribution using our estimated mean and SE meta-analysis estimates by sex for the SMRs (see Supplemental Table 1). Next, we randomly drew 10,000 prevalence samples using our meta-analysis estimates by state and sex. Lastly, we estimate the prevalence and ASD cases by state, sex, and age using Eqs. 1 and 2. Our simulation resulted in 10,000 estimates for the prevalence and ASD cases for age class 18–84 by state and sex, and we summarize these results using the mean and 95% SI.

The male-to-female ASD prevalence ratio (PR) was calculated by state for each simulation and then summarized for all 10,000 simulations with the mean and 95% SI (see Supplement Fig. 1).

Table 1 Data inputs used to estimate ASD prevalence among adults 18–84 years by state and sex

Input	Data source for estimates	Link
2016–2018 estimated state prevalence of male and female children ages 3–17 years with diagnosed ASD reported by a parent	National survey of children's health, 2016–2018	https://mchb.hrsa.gov/data/national-surveys/questionnaires-datasets-supporting-documents
A meta-analysis of mortality studies used to estimate male and female mortality rates among persons with diagnosed ASD	Picket et al. (2006) All ages included among those receiving services in the California Department of Developmental Services 1/83–12/1997, 1/1998–12/2002 with autism diagnoses and died during the study time period. Comparison group adjusted for age	PMID:16565885
	Mouridsen et al. (2008) ASD was a clinical cohort, average age 43 years. Comparison group adjusted for age	PMID:18,579,647 https://doi.org/10.1177/1362361308091653
	Gillberg et al. (2010) Population-based group of persons with ASD followed up to average age of 33 years. Comparison group adjusted for age	PMID:19838782 https://doi.org/10.1007/s10803-009-0883-4
	Hirvikoski et al. (2016) All ages included: median age of death for persons with ASD = 55 years, control population = 70 years	PMID: 26541693 https://doi.org/10.1192/bjp.bp.114.160192
2017 estimate of the state populations by sex	United States Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Health Statistics, Bridged-Race Population Estimates, United States. postcensal population estimates, released by NCHS on 6/27/2018	https://wonder.cdc.gov/bridged-race-v2017.html
1999–2017 state mortality rates	Multiple Cause of Death Files, 1999–2017, as compiled from data provided by the 57 vital statistics jurisdictions through the Vital Statistics Cooperative Program	https://wonder.cdc.gov/ucd-icd10.html

Results

In 2017, we estimated that 2.21% (95% SI 1.95%, 2.45%) or 5,437,988 (95% SI 4,798,561; 6,025,184) U.S. adults aged 18–84 years were living with ASD. State prevalence estimates ranged from 1.97% (95% SI 1.55%, 2.45%) in Louisiana to 2.42% (95% SI 1.93%, 2.99%) in Massachusetts (Table 2). The states with the greatest number of adults estimated to be living with ASD included California (701,669 cases), Texas (449,631), New York (342,280) and Florida (329,131). No obvious geographic pattern for prevalence was found (Fig. 1).

The estimated U.S. ASD prevalence for females was 0.86% (95% SI 0.60, 1.09), and by state ranged from 0.72% (95% SI 0.41, 1.11) in Arkansas to 0.97% (95% SI 0.50, 1.45) in Virginia (Table 3, Fig. 2). The estimated U.S. ASD prevalence among adult males was higher than females, at 3.62%, (95% SI 3.14, 4.04), and state estimates ranged from 3.17% (95% SI 2.33, 4.19) in South Dakota to 4.01% (95% SI 3.07, 5.14) in Massachusetts (Table 4, Fig. 2). State PRs for males versus females estimates ranged from 3.94 (95% SI 2.29, 6.48) in South Dakota to 5.08 (95% SI 2.84, 8.78) in Arkansas (see Supplemental Table 2). The male-to-female prevalence difference ranged from 2.32% points (95% SI 1.40, 3.39) for South Dakota to 3.16% points (95% SI 2.16, 4.35) for Connecticut (see Supplemental Table 2).

We conducted a sensitivity analysis to assess the estimated number of adults with ASD using the ASD estimated prevalence for the age group 6–17 years in the model compared with the age group 3–17 years. The estimated U.S. ASD prevalence was 2.38% (95% SI 2.10, 2.64) using data for the age group 6–17 years in the model compared with 2.21% (95% SI 1.95, 2.45) using data for the age group 3–17 years in the model (see Supplement for state estimates).

Discussion

Using existing data and adjusting for elevated mortality among persons living with ASD, we estimated national and state prevalence of adults 18–84 years of age living with ASD. Our estimate of 2.21% is higher than a study

Table 2 State estimated autism spectrum disorder prevalence among adults ages 18–84 years, cases, and associated 95% simulation interval

State	Cases	95% SI	Prevalence	95% SI
Alabama	78,072	61,527, 96,435	2.12	1.67, 2.61
Alaska	12,000	9559, 14,849	2.19	1.74, 2.71
Arizona	119,924	95,618, 147,485	2.29	1.82, 2.81
Arkansas	45,569	35,644, 56,735	2.03	1.59, 2.53
California	701,669	563,358, 863,471	2.36	1.89, 2.90
Colorado	96,917	78,736, 117,790	2.28	1.85, 2.77
Connecticut	65,337	51,985, 81,354	2.37	1.89, 2.96
Delaware	16,683	13,191, 20,742	2.26	1.79, 2.81
District of Columbia	11,700	9281, 14,425	2.10	1.67, 2.59
Florida	329,131	259,573, 407,473	2.03	1.60, 2.51
Georgia	174,612	139,616, 213,983	2.25	1.80, 2.75
Hawaii	22,797	18,103, 28,324	2.11	1.67, 2.62
Idaho	27,094	21,741, 33,212	2.18	1.75, 2.67
Illinois	223,353	178,832, 274,414	2.32	1.86, 2.85
Indiana	111,067	88,717, 136,349	2.24	1.79, 2.75
Iowa	53,243	43,024, 64,598	2.28	1.84, 2.77
Kansas	46,863	37,387, 57,849	2.19	1.75, 2.71
Kentucky	71,791	56,959, 88,657	2.13	1.69, 2.64
Louisiana	68,819	54,071, 85,662	1.97	1.55, 2.45
Maine	23,910	19,244, 29,167	2.28	1.83, 2.78
Maryland	98,200	78,844, 118,940	2.14	1.72, 2.59
Massachusetts	129,168	103,105, 159,372	2.42	1.93, 2.99
Michigan	164,360	130,831, 201,349	2.17	1.73, 2.66
Minnesota	97,881	80,695, 117,401	2.35	1.94, 2.82
Mississippi	45,911	35,708, 57,883	2.07	1.61, 2.61
Missouri	97,377	77,500, 119,708	2.12	1.68, 2.60
Montana	16,969	13,404, 21,053	2.12	1.68, 2.63
Nebraska	31,417	25,045, 38,775	2.24	1.79, 2.77
Nevada	51,799	41,333, 63,725	2.28	1.82, 2.81
New Hampshire	23,442	19,085, 28,268	2.22	1.81, 2.68
New Jersey	157,245	127,036, 191,192	2.30	1.86, 2.80
New Mexico	31,207	24,166, 39,369	2.00	1.55, 2.52
New York	342,280	276,658, 417,725	2.25	1.82, 2.74
North Carolina	155,953	123,603, 192,285	2.00	1.59, 2.47
North Dakota	11,501	8967, 14,435	2.05	1.60, 2.57
Ohio	185,315	145,971, 228,939	2.11	1.66, 2.60
Oklahoma	61,672	49,304, 75,780	2.13	1.70, 2.61
Oregon	72,727	58,308, 89,294	2.28	1.83, 2.80
Pennsylvania	228,572	180,929, 284,166	2.33	1.85, 2.90
Rhode Island	18,472	15,116, 22,343	2.24	1.83, 2.71
South Carolina	75,985	58,887, 95,248	1.98	1.54, 2.48
South Dakota	12,830	9881, 16,286	2.02	1.56, 2.57
Tennessee	106,083	84,068, 131,132	2.08	1.65, 2.58
Texas	449,631	358,411, 556,627	2.19	1.74, 2.71
Utah	48,818	40,003, 58,452	2.28	1.87, 2.73
Vermont	10,435	8367, 12,764	2.12	1.70, 2.59
Virginia	155,557	125,110, 189,742	2.41	1.94, 2.94
Washington	119,815	95,514, 149,233	2.13	1.70, 2.65
West Virginia	29,083	22,748, 36,322	2.07	1.62, 2.58
Wisconsin	97,977	78,734, 119,841	2.23	1.80, 2.73
Wyoming	9758	7755, 12,036	2.26	1.79, 2.78
Total	5,437,988	4,798,561, 6,025,184	2.21	1.95, 2.45

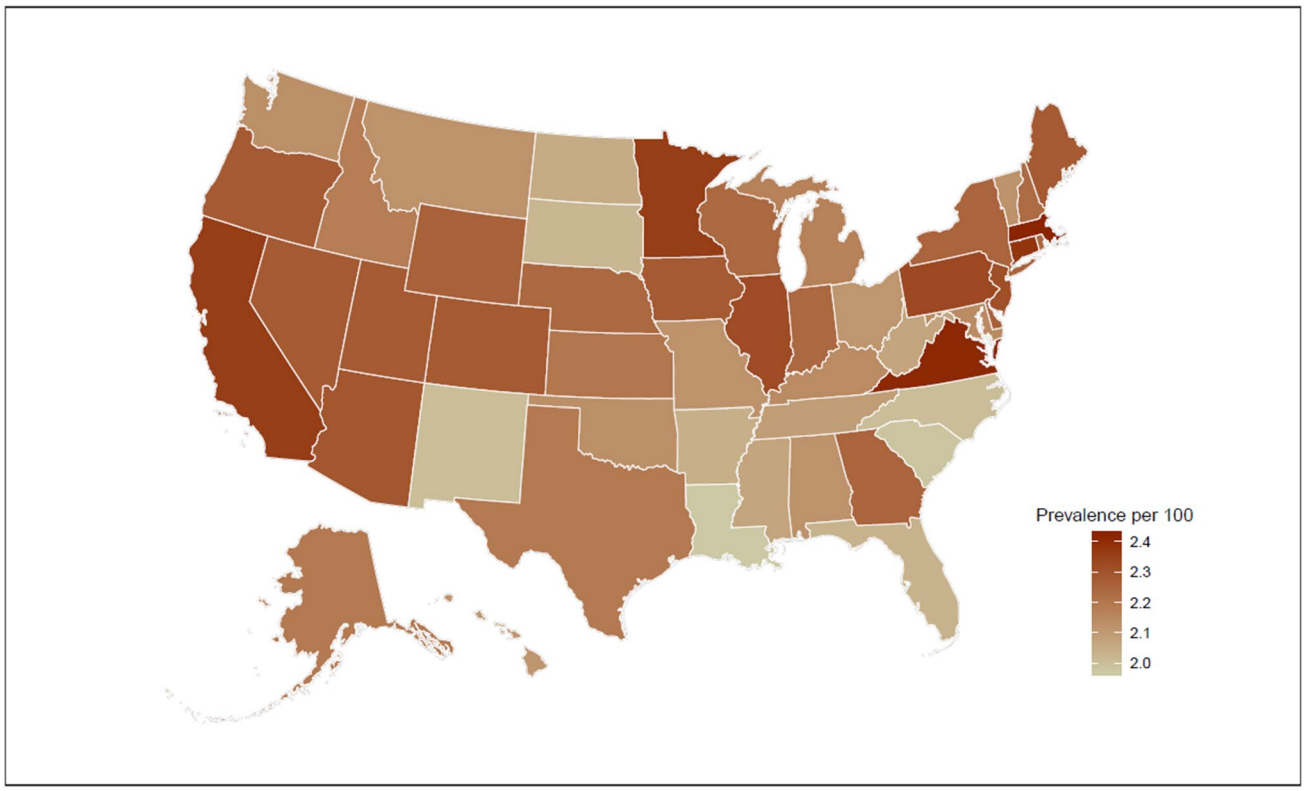


Fig. 1 Estimated autism spectrum disorder prevalence among adults 18–84 years by state, 2017

estimating ASD among adults in a community in England (1.0%) (Brugha et al. 2011). Our estimate may be higher because Brugha et al. was an empirical surveillance study conducted in one community in England among adults whereas the present analysis is a modeling study based on projecting prevalence from parent-report of children diagnosed with ASD in U.S. states to adults.

National and state ASD estimates in this analysis provide a general magnitude of the population of adults living with autism, but they have some important limitations. The prevalence of ASD among children, which was used to estimate prevalence among adults in our analysis, is based on parent report, which may under- or overestimate prevalence. For example, the estimates of ASD prevalence among children ages 3–17 years does not include children with ASD who have not been diagnosed, leading to an underestimate of prevalence. Conversely, it may overestimate prevalence, as parents may falsely report that their child was diagnosed if ASD was suspected or if the child failed a screener but did not receive a diagnosis.

Our assumption that the ASD prevalence among children ages 3–17 years during 2016–2018 (born during 1999–2015) and adults (born before 1999) is similar does not account for the possibility of environmental or gene-environment interactions associated with ASD that may have changed over time. Exposure to some risk factors may have varied among birth cohorts. However, few risk factors have consistently been associated with ASD and those that have been identified have accounted for a very small percent of increases in diagnosed ASD (Schieve et al. 2011; Quinlan et al. 2015). One study found that changes in preterm delivery, small-for-gestational age, multiple births, cesarean delivery, and assisted reproductive technology use contributed to less than 1% of the 57% increase in ASD among 8-year-old children born in 1994 compared to 1998 (Schieve et al. 2011). A study conducted among children in New York City found that changes in maternal and paternal age accounted for only 2.7% of the 143% increase in ASD among children ages 0–3 from 0.03% in 1994 to 0.43% in 2001 (Quinlan et al. 2015). The prevalence of ASD among adults may be equivalent to that among children in that at least one study by Brugha et al. 2011 showed the adult prevalence was comparable to the estimated ASD prevalence among children at the time the study was conducted.

Limited information is available on mortality among adults with ASD. However, studies have shown consistently that adults with ASD have higher mortality rates than those without (Picket et al. 2006; Mouridsen et al. 2008; Gillberg et al. 2010; Hirvikoski et al. 2016). Most of the mortality studies followed persons to an average age of

Table 3 Estimated autism spectrum disorder prevalence among females ages 18–84 years, cases, and associated 95% simulation interval

State	Cases	95% SI	Prevalence	95% SI
Alabama	15,072	8617, 22,982	0.79	0.45, 1.20
Alaska	2275	1399, 3291	0.88	0.54, 1.27
Arizona	22,274	13,559, 32,654	0.84	0.51, 1.23
Arkansas	8230	4690, 12,697	0.72	0.41, 1.11
California	137,645	89,272, 195,797	0.92	0.60, 1.31
Colorado	19,454	12,731, 27,303	0.92	0.60, 1.29
Connecticut	11,799	7494, 16,905	0.84	0.53, 1.20
Delaware	3212	1887, 4743	0.84	0.49, 1.24
District of Columbia	2470	1570, 3634	0.84	0.53, 1.23
Florida	69,038	40,683, 101,477	0.83	0.49, 1.22
Georgia	35,043	21,353, 51,568	0.87	0.53, 1.28
Hawaii	4592	2864, 6629	0.86	0.53, 1.24
Idaho	5241	3278, 7601	0.84	0.53, 1.22
Illinois	44,364	28,116, 63,603	0.90	0.57, 1.29
Indiana	22,492	13,811, 32,950	0.89	0.55, 1.30
Iowa	9822	6255, 14,075	0.84	0.53, 1.20
Kansas	8848	5478, 12,832	0.83	0.51, 1.20
Kentucky	13,109	7528, 19,922	0.77	0.44, 1.16
Louisiana	13,979	8248, 21,019	0.78	0.46, 1.17
Maine	4685	2909, 6775	0.87	0.54, 1.26
Maryland	21,097	12,870, 31,034	0.89	0.54, 1.30
Massachusetts	25,678	16,523, 37,094	0.93	0.60, 1.35
Michigan	32,847	19,687, 49,002	0.85	0.51, 1.27
Minnesota	19,328	12,650, 27,220	0.93	0.61, 1.30
Mississippi	8842	4986, 13,560	0.77	0.43, 1.18
Missouri	19,177	11,751, 28,060	0.82	0.50, 1.19
Montana	3430	1984, 5146	0.87	0.50, 1.30
Nebraska	5774	3615, 8476	0.82	0.52, 1.21
Nevada	9863	6058, 14,443	0.87	0.53, 1.27
New Hampshire	4722	2960, 6732	0.89	0.56, 1.27
New Jersey	30,829	19,381, 44,681	0.88	0.55, 1.27
New Mexico	6215	3548, 9505	0.79	0.45, 1.20
New York	72,438	46,913, 103,195	0.92	0.60, 1.31
North Carolina	33,070	20,403, 47,816	0.82	0.51, 1.19
North Dakota	2129	1308, 3177	0.79	0.48, 1.18
Ohio	37,205	22,486, 54,560	0.83	0.50, 1.21
Oklahoma	11,752	7023, 17,555	0.80	0.48, 1.20
Oregon	13,170	8255, 19,271	0.82	0.51, 1.20
Pennsylvania	43,191	26,449, 62,380	0.86	0.53, 1.25
Rhode Island	3628	2333, 5220	0.85	0.55, 1.23
South Carolina	16,609	9750, 24,644	0.84	0.49, 1.24
South Dakota	2645	1620, 3885	0.85	0.52, 1.24
Tennessee	20,306	12,014, 30,315	0.77	0.46, 1.16
Texas	90,422	57,760, 129,614	0.87	0.56, 1.25
Utah	9968	6557, 13,971	0.93	0.61, 1.31
Vermont	2087	1293, 3040	0.84	0.52, 1.22
Virginia	32,011	19,580, 47,823	0.97	0.59, 1.45
Washington	22,146	13,537, 33,310	0.79	0.48, 1.18
West Virginia	5910	3328, 9096	0.83	0.47, 1.28
Wisconsin	18,244	11,342, 26,895	0.83	0.52, 1.22
Wyoming	1943	1157, 2893	0.92	0.55, 1.37
Total	1,080,322	752,142, 1,359,152	0.86	0.60, 1.09

Table 4 Estimated autism spectrum disorder prevalence among males ages 18–84 years, cases, and associated 95% simulation interval

State	Cases	95% SI	Prevalence	95% SI
Alabama	63,000	48,122, 79,991	3.55	2.71, 4.51
Alaska	9725	7481, 12,391	3.36	2.59, 4.28
Arizona	97,650	75,258, 123,694	3.76	2.89, 4.76
Arkansas	37,339	28,428, 47,714	3.41	2.60, 4.36
California	564,024	436,565, 712,719	3.82	2.96, 4.83
Colorado	77,463	60,754, 96,994	3.61	2.83, 4.52
Connecticut	53,538	41,196, 69,048	3.99	3.07, 5.15
Delaware	13,471	10,262, 17,304	3.80	2.89, 4.88
District of Columbia	9230	7052, 11,767	3.52	2.69, 4.48
Florida	260,093	197,052, 333,489	3.30	2.50, 4.23
Georgia	139,569	107,666, 175,620	3.72	2.87, 4.69
Hawaii	18,205	13,855, 23,302	3.34	2.54, 4.28
Idaho	21,853	16,906, 27,494	3.51	2.71, 4.41
Illinois	178,988	138,499, 226,962	3.79	2.93, 4.80
Indiana	88,575	68,532, 111,810	3.63	2.81, 4.59
Iowa	43,421	33,901, 54,102	3.73	2.91, 4.65
Kansas	38,015	29,328, 48,321	3.57	2.75, 4.54
Kentucky	58,682	44,945, 74,249	3.56	2.72, 4.50
Louisiana	54,840	41,315, 70,670	3.23	2.43, 4.16
Maine	19,225	14,960, 24,183	3.75	2.91, 4.71
Maryland	77,103	60,286, 96,019	3.49	2.73, 4.35
Massachusetts	103,490	79,250, 132,425	4.01	3.07, 5.14
Michigan	131,513	101,753, 166,068	3.54	2.74, 4.47
Minnesota	78,554	62,673, 96,695	3.79	3.03, 4.67
Mississippi	37,069	27,818, 48,132	3.48	2.61, 4.52
Missouri	78,200	60,239, 98,988	3.48	2.68, 4.40
Montana	13,538	10,318, 17,252	3.36	2.56, 4.28
Nebraska	25,642	19,722, 32,701	3.67	2.82, 4.68
Nevada	41,935	32,438, 53,010	3.69	2.86, 4.67
New Hampshire	18,720	14,879, 23,148	3.58	2.85, 4.43
New Jersey	126,416	99,304, 158,245	3.81	2.99, 4.77
New Mexico	24,992	18,581, 32,421	3.25	2.41, 4.21
New York	269,842	210,546, 338,482	3.67	2.86, 4.60
North Carolina	122,883	93,683, 157,051	3.26	2.49, 4.17
North Dakota	9372	7026, 12,121	3.22	2.41, 4.16
Ohio	148,110	111,942, 188,693	3.45	2.61, 4.39
Oklahoma	49,920	38,731, 63,029	3.49	2.71, 4.40
Oregon	59,557	46,065, 75,500	3.78	2.92, 4.79
Pennsylvania	185,382	140,420, 238,656	3.87	2.93, 4.98
Rhode Island	14,844	11,772, 18,395	3.71	2.94, 4.60
South Carolina	59,376	43,786, 77,343	3.22	2.37, 4.19
South Dakota	10,185	7500, 13,455	3.17	2.33, 4.19
Tennessee	85,777	66,069, 108,994	3.48	2.68, 4.42
Texas	359,209	274,707, 459,695	3.53	2.70, 4.52
Utah	38,850	30,928, 47,667	3.63	2.89, 4.46
Vermont	8348	6457, 10,507	3.44	2.66, 4.32
Virginia	123,546	96,564, 154,991	3.91	3.05, 4.90
Washington	97,669	75,180, 125,363	3.47	2.67, 4.45
West Virginia	23,173	17,458, 29,832	3.33	2.51, 4.29
Wisconsin	79,733	62,161, 99,939	3.65	2.85, 4.58
Wyoming	7815	6017, 9936	3.53	2.72, 4.49
Total	4,357,667	3,788,037, 4,867,213	3.62	3.14, 4.04

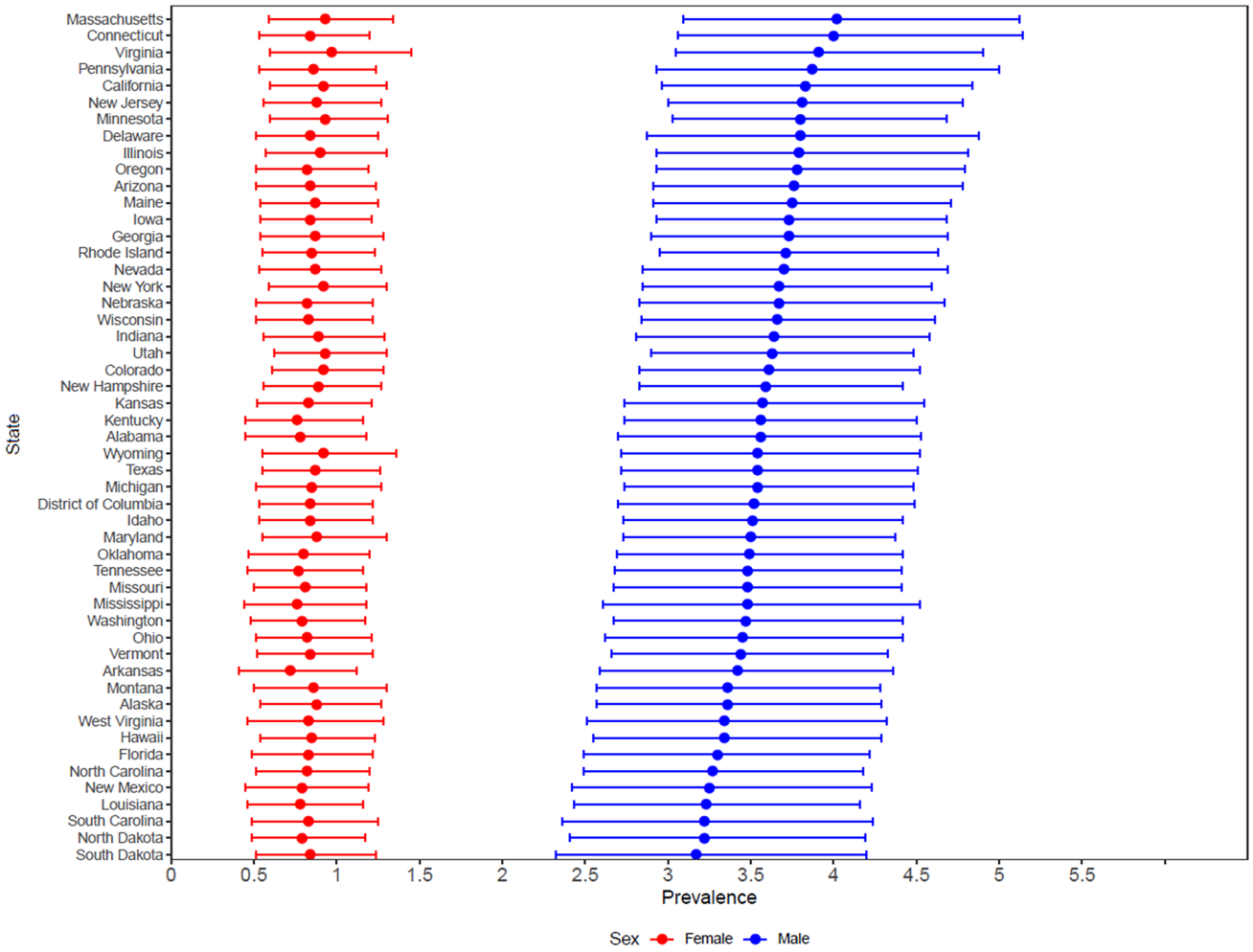


Fig. 2 Estimated state autism spectrum disorder prevalence among adults 18–84 years by sex

30–55 years. We assumed the SMR remained the same for ages above 50 years; however, additional mortality studies that include older persons with ASD are needed to validate this assumption.

There was some variation in the prevalence of ASD by state, with the prevalence ranging from 1.97 to 2.42%. The prevalence estimates were estimated using a partial-pooling hierarchical model that naturally pulls the raw state prevalence estimates towards the mean U.S. estimate and pulls those with less data more towards the mean. Currently, there is no evidence that the prevalence of ASD should vary by geographic location; however, there is evidence that greater availability of screening and diagnostic services will increase the number of persons diagnosed with ASD (Rolthlz et al. 2017; Janvier et al. 2016). Male and female ASD prevalence estimates were substantially different, which is consistent with existing studies (Kogan et al. 2018; Baio et al. 2018). The reason for this difference is unknown but it may reflect, in part, differences in how ASD manifests in boys and girls leading to differential diagnosis by gender.

To date, an empirical study of adult ASD prevalence in the U.S. has not been accomplished, perhaps because any single approach to ascertain adult ASD has challenges. There are no psychometrically validated tests of ASD for adults, which leads to uncertainty for studies using tests designed for children, such as the Autism Diagnostic Observation Schedule. In addition, mixed methods are likely needed in order to reach populations living independently and in group settings. A subset of persons might only be identified through the review of service records of those being served in group settings. Individuals with ASD who live independently may be disinclined to participate in a survey if recruited via phone or in person. Adults with ASD may be more difficult to recruit because they may not be enrolled in services or may not receive services in a wide variety of settings (e.g., schools, health care providers, community-based entities) resulting in challenges to comprehensive

recruitment efforts. Once a validated tool to identify adults with ASD is created, a study could incorporate information from public school classifications or publicly-funded programs that serve individuals with ASD and population-based telephone or community surveys of adults with adjustments to address greater non-response among adults with ASD.

Overall, we estimated that 1 in 45 adults (95% SI, 41, 51), ages 18–84 years, are living with ASD. While these numbers are estimates, they do provide a place for states to think about available services for adults with ASD. We used the most current data available for all states to estimate the ASD prevalence among adults. This analysis may motivate some states to explore state-based data sources that may be more informative than data available for all states, and refine the estimates based on their existing local data.

Disclosure The findings and conclusions in this paper are those of the authors and do not represent the official position of the Centers of Disease Control and Prevention.

Author Contributions PMD conceived of the study, wrote the manuscript except for the methods; CER provided statistical expertise and conducted the analysis, wrote the methods section of the manuscript; DM conducted an independent analysis for quality control, downloaded and prepared data for analysis, and created Figures 1 and 2; MM provided statistical and data expertise. All authors reviewed and approved the final manuscript as submitted and agreed to be accountable for all aspects of the work.

References

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). Washington, D.C: American Psychiatric Association.

Baio, J., Wiggins, L., Christensen, D., et al. (2018). Prevalence of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States, 2014. *MMWR Surveillance Summaries, 67*(6), 1–23.

Brugha, T. S., McManus, S., Bankart, J., et al. (2011). Epidemiology of autism spectrum disorders in adults in the community in England. *Archives of General Psychiatry, 68*(5), 459–465.

Croen, L. A., Zerbo, O., Qian, Y., et al. (2015). The health status of adults on the autism spectrum. *Autism, 19*(7), 814–823.

Gelman, A., Carlin, J. B., Stern, H. S., & Rubin, D. B. (2013). *Bayesian Data Analysis*. New York: CRC Press.

Gilboa, S. M., Devine, O. J., Oster, M. E., et al. (2016). Congenital health defects in the United States. Estimating the magnitude of the affected population in 2010. *Circulation, 134*, 101–109. https://doi.org/10.1161/circulationaha.115.019307.

Gillberg, C., Billstedt, E., Sundh, V., & Gillberg, I. C. (2010). Mortality in autism: A prospective longitudinal community-based study. *Journal of Autism and Developmental Disorders, 40*(3), 352–357. https://doi.org/10.1007/s10803-009-0883-4.

Hirvikoski, T., Mittendorfer-Rutz, E., Boman, M., et al. (2016). Premature mortality in autism spectrum disorder. *British Journal of Psychiatry, 208*(3), 232–238.

Janvier, Y. M., Harris, J. F., Coffield, C. N., et al. (2016). Screening for autism spectrum disorder in underserved communities: Early childcare providers as reporters. *Autism, 20*(3), 364–373. https://doi.org/10.1177/1362361315585055.

Kogan, M. D., Vladutiu, C. J., Schieve, L. A., et al. (2018). The prevalence of parent-reported autism spectrum disorder among US children. *Pediatrics, 142*(6), 1–11.

Mouridsen, S. E., Bronnum-Hansen, H., Rich, B., & Isager, T. (2008). Mortality and causes of death in autism spectrum disorders. *Autism, 12*(4), 403–414.

Murphy, C. M., Wilson, C. E., Roberston, D. M., et al. (2016). Autism spectrum disorder in adults: diagnosis, management, and health services development. *Neuropsychiatr Dis Treat, 2016*(12), 1669–1686.

Nicolaidis, C., Kripke, C. C., & Raymaker, D. (2014). Primary care for adults on the autism spectrum. *Medical Clinics of North America, 98*(5), 1169–1191.

Pickett, J. A., Paculdo, D. R., Shavelle, R. M., et al. (2006). 1998–2002 Update on "Causes of death in autism". *Journal of Autism and Developmental Disorders, 36*(2), 287–288.

Quinlan, C. A., McVeigh, K. H., Driver, C. R., et al. (2015). Prental Age and autism spectrum disorders among New York City children 0–36 months of age. *MCHJ, 19*, 1783–1790.

Rosenberg, E. S., Rosenthal, E. M., Hall, E. W., et al. (2018). Prevalence of hepatitis C virus infection in US states and the District of Columbia, 2013 to 2016. *JAMA Netw Open, 1*(8), e186371.

Rotholz, D. A., Kinsman, A. M., Lacy, K. K., et al. (2017). Improving early identification and intervention for children at risk for autism spectrum disorder. *Pediatrics*. https://doi.org/10.1542/peds.2016-1061.

Roux, A. M., Shattuck, P. T., Rast, J. E., Rava, et al. (2015). *National autism indicators report: Transition into Young Adulthood. Life course Outcomes Research Program*. Philadelphia, PA: A.J. Drexel Autism Institute Drexel University.

Schieve, L. A., Rice, C., Devine, O., et al. (2011). Have secular changes in perinatal risk factors contributed to the recent autism prevalence increase? Development and application of a mathematical assessment model. *Annals of Epidemiology, 21*(12), 930–945.

United States Department of Health and Human Services (US DHHS), Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). (2018a). Bridged-Race Population Estimates, United States July 1st resident population by state, county, age, sex, bridged-race, and Hispanic origin. Post-censal population estimates. Retrieved July 29, 2019, from https://wonder.cdc.gov/bridged-race-v2017.html.

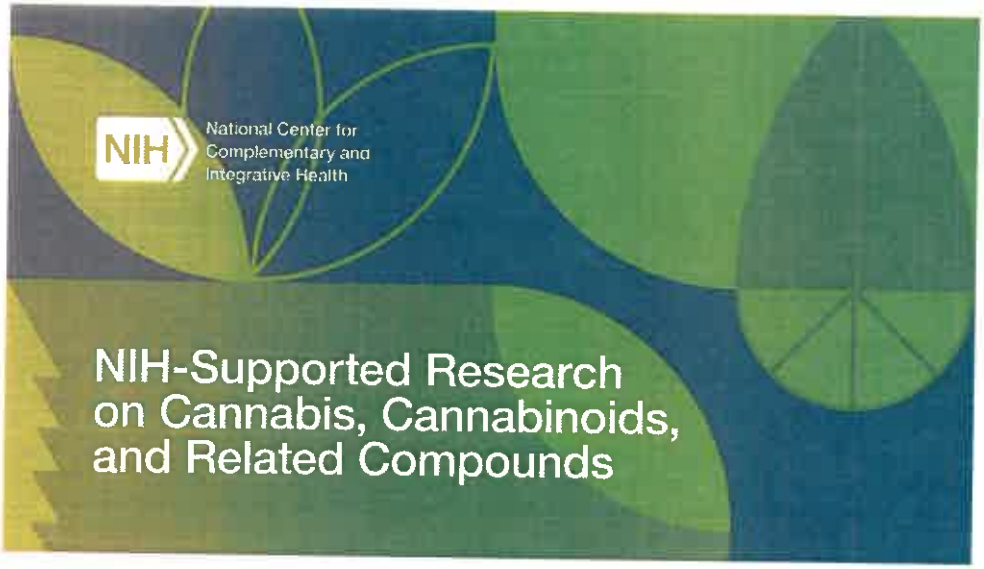
United States Department of Health and Human Services (US DHHS) Centers for Disease Control and Prevention, National Center for Health Statistics. (2018b). Underlying Cause of Death 1999–2017. Retrieved July 29, 2019, from https://wonder.cdc.gov/bridged-race-v2017.html.

U.S. Department of Health and Human Services
National Institutes of Health

National Center for Complementary and Integrative Health

NIH-Supported Research on Cannabis, Cannabinoids, and Related Compounds

NIH-Supported Research on Cannabis, Cannabinoids, and Related Compounds



Background

The National Institutes of Health (NIH) supports a broad portfolio of research on cannabis and cannabis constituents and related compounds, as well as the endocannabinoid system. Specific topics of interest vary among Institutes, Centers, and Offices, but overall the research portfolio includes studies investigating the whole or parts of the *Cannabis sativa* plant, cannabis extracts or enriched extracts, cannabinoid compounds extracted and derived from cannabis extracts, non-cannabinoid constituents of cannabis, synthetic cannabinoids, and the components of the endocannabinoid system (the signaling pathways in the body activated by cannabinoids).

There is considerable interest in the possible therapeutic uses of cannabis and its constituent compounds. In 2015, NIH developed three reporting categories to describe and account for the research efforts underway to examine the chemical, physiological, and therapeutic properties of cannabinoids and the physiological systems they affect.

View examples of NIH research grants in each of the categories below.

- **Cannabinoid Research** – This category reports the total NIH investment in all cannabinoid research including basic research, animal and human preclinical studies, and clinical research. Studies examine cannabis use disorder as well as the societal and/or health impacts of changing cannabis laws and policies; all classes of cannabinoids (purified, synthetic, endocannabinoids, or phytocannabinoids); compounds that modify the activity of consumed cannabis (e.g., cannabinoid receptor allosteric modulators); as well as the physiological systems affected by cannabis (e.g., endocannabinoid system) and modulators thereof (e.g., fatty acid amide hydrolase [FAAH] inhibitors).
- **Cannabidiol Research** – This subset of the Cannabinoid Research category (above) reports all NIH projects examining basic, preclinical, and therapeutic properties of cannabidiol (CBD).
- **Therapeutic Cannabinoid Research** – This subset of the Cannabinoid Research category (above) reports all NIH projects examining the therapeutic properties of all classes of cannabinoids (endocannabinoids, phytocannabinoids, and synthetic).

These categories are publicly accessible on the NIH categorical spending website (NIH RePORTER) and will be updated annually.

RePORTER

Search Results

Export Share

cannabis

Search

Projects Publications Patents Clinical Studies News & More

List Charts Map

Chart Type

Summary By (Chart X Axis)

Administering Institute/Center

Plot By (Chart Y Axis)

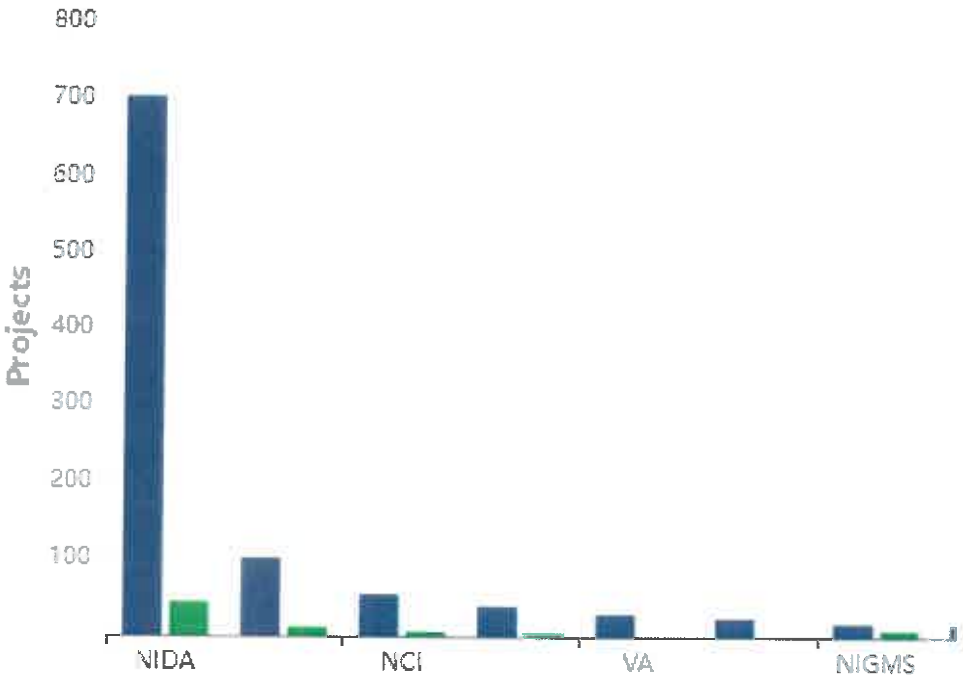
Projects Funding

Filters

Active Projects

Fiscal Years CLEAR

- 2025 (15)
- 2024 (590)
- 2023 (562)
- 2022 (505)
- 2021 (479)
- 2020 (480)
- 2019 (438)
- 2018 (374)
- 2017 (308)
- 2016 (274)
- 2015 (152)
- 2014 (143)
- 2013 (144)
- 2012 (143)
- 2011 (136)
- 2010 (141)
- 2009 (142)
- 2008 (135)
- 2007 (123)
- 2006 (84)



Note: Please note that if the hit list contains both a subproject and its parent grant, the subproject funding is already included in the parent project funding amount.

* Funding data available only for NIH, CDC, FDA, AHRQ, and ACF.

2005 (81)
2004 (81)
2003 (44)
2002 (60)
2001 (46)
2000 (59)
1999 (56)
1998 (47)
1997 (37)
1996 (17)
1995 (27)
1994 (26)

Administering Institute/Center				
NIDA	705	$423,499,851	44	$16,466,903
NIAAA	102	$48,222,878	12	$2,810,244
NCI	55	$33,256,818	6	$2,329,615
NCCIH	39	$17,478,108	4	$2,161,703
VA	29			
NIMH	24	$15,412,973		
NIGMS	17	$12,166,145	8	$3,173,000
NIMHD	15	$5,122,482		
NICHD	14	$11,590,785		
NHLBI	14	$5,760,298		
NIA	11	$5,457,325	1	$655,214
NINDS	11	$3,748,352		
OD	9	$16,098,529		
NIDCR	6	$2,758,621		
NIDDK	4	$1,648,473		
NIEHS	4	$4,593,487		
NCATS	4	$2,757,335		
NIAID	2	$518,196	2	$83,325
FDA	2	$126,000		
NCCDPHP	2	$350,000		
NIBIB	1	$144,998		
AHRQ	1	$398,972		
NINR	1	$778,210		
NCIPC	1	$348,719		
NEI	1	$445,262		
NHGRI	1	$384,910		
Total	**1,075**	**$613,067,727**	**77**	**$27,680,004**

Possible Endocannabinoid Control of Colorectal Cancer Growth

ALESSIA LIGRESTI,* TIZIANA BISOGNO,* ISABEL MATIAS,* LUCIANO DE PETROCELLIS,[†] MARIA GRAZIA CASCIO,* VITTORIO COSENZA,[§] GIUSEPPE D'ARGENIO,[§] GIUSEPPE SCAGLIONE,[‖] MAURIZIO BIFULCO,[¶] ITALO SORRENTINI,[‖] and VINCENZO DI MARZO*

*Endocannabinoid Research Group, Institute of Biomolecular Chemistry, and [†]Institute of Cybernetics, C.N.R., Pozzuoli; [§]Gastroenterologia, Università di Napoli "Federico II," Napoli; [‖]Gastroenterologia, A.O. "Rummo," Benevento; and [¶]Dipartimento di Scienze Farmaceutiche, Università degli Studi di Salerno, Fisciano, Italy

See editorial on page 973.

Background & Aims: **The endocannabinoids anandamide and 2-arachidonoylglycerol (2-AG) inhibit cancer cell proliferation by acting at cannabinoid receptors (CBRs). We studied (1) the levels of endocannabinoids, cannabinoid CB_1 and CB_2 receptors, and fatty acid amide hydrolase (FAAH, which catalyzes endocannabinoid hydrolysis) in colorectal carcinomas (CRC), adenomatous polyps, and neighboring healthy mucosa; and (2) the effects of endocannabinoids, and of inhibitors of their inactivation, on human CRC cell proliferation.** ***Methods:*** **Tissues were obtained from 21 patients by biopsy during colonoscopy. Endocannabinoids were measured by liquid chromatography-mass spectrometry (LC-MS). CB_1, CB_2, and FAAH expression were analyzed by RT-PCR and Western immunoblotting. CRC cell lines (CaCo-2 and DLD-1) were used to test antiproliferative effects.** ***Results:*** **All tissues and cells analyzed contain anandamide, 2-AG, CBRs, and FAAH. The levels of the endocannabinoids are 3- and 2-fold higher in adenomas and CRCs than normal mucosa. Anandamide, 2-AG, and the CBR agonist HU-210 potently inhibit CaCo-2 cell proliferation. This effect is blocked by the CB_1 antagonist SR141716A, but not by the CB_2 antagonist SR144528, and is mimicked by CB_1-selective, but not CB_2-selective, agonists. In DLD-1 cells, both CB_1 and CB_2 receptors mediate inhibition of proliferation. Inhibitors of endocannabinoid inactivation enhance CaCo-2 cell endocannabinoid levels and block cell proliferation, this effect being antagonized by SR141716A. CaCo-2 cell differentiation into noninvasive cells results in increased FAAH expression, lower endocannabinoid levels, and no responsiveness to cannabinoids.** ***Conclusions:*** **Endocannabinoid levels are enhanced in transformed colon mucosa cells possibly to counteract proliferation via CBRs. Inhibitors of endocannabinoid inactivation may prove useful anticancer agents.**

Numerous experimental data indicate that the activation of the endogenous cannabinoid system might represent a potential strategy for the development of new anticancer drugs.[1,2] First, the psychoactive principle of *Cannabis sativa* and marijuana, Δ^9-tetrahydrocannabinol,[3] is known to act mostly by stimulating 2 specific receptors subtypes, the cannabinoid CB_1 and CB_2 receptors[4], and was reported in the past[5] and more recently[6] to have antineoplastic activity in vivo and in vitro.[1,2] Second, endogenous agonists of the cannabinoid receptors (CBRs), i.e., *N*-arachidonoyl-ethanolamine (AEA; anandamide),[7] 2-arachidonoyl-glycerol (2-AG),[8,9] and noladin ether,[10] or their metabolically stable synthetic analogs, were found to inhibit, mostly via CB_1 receptors, the proliferation of breast and prostate cancer cells in vitro[11,12] and of rat thyroid cells transformed by the product of the K-*ras* oncogene in vivo.[13] Finally, stimulation of the 2 CBR subtypes has been found to influence the expression of various genes involved in cell survival, proliferation, and apoptosis via interference with cAMP- and ceramide-mediated signalling, mitogen-activated protein kinases and phosphatidyl-inositol-3-kinase (see Guzman et al.[14] for review).

Apart from the proposed role of CBRs in the control of cancer cell growth, transformation, and death,[2,14] there are at least 3 reasons why the endocannabinoids might be involved in the control of colorectal cancer cell proliferation. First, both AEA and 2-AG are good substrates for cyclooxygenase 2 (COX-2), which seems to play a major role in the development of colorectal carcinoma (CRC).[15,16] Because no molecular target has been reported to date for endocannabinoid COX-2 metabolites, it is possible that AEA, and particularly the more abundant 2-AG, might exert some of their biologic effects, including inhibition of CRC growth, via inhibition, by

Abbreviations used in this paper: AEA, arachidonoylethanolamide (anandamide); 2-AG, 2-arachidonoylglycerol; CBR, cannabinoid receptors; CRC, colorectal carcinoma; COX-2, cyclooxygenase 2; FAAH, fatty acid amide hydrolase; LC-MS, liquid chromatography-mass spectrometry.

0016-5085/03/$30.00
doi:10.1016/S0016-5085(03)00881-3

substrate competition, of COX-2–mediated formation of prostaglandins.[16] Second, it has been reported recently[17] that a particular class of lysophosphatidic acids, molecules known to play a major role in the development of cancer and to be overproduced in several types of tumors, including CRC,[18,19] might serve as biosynthetic precursors for 2-AG. Therefore, this possibly antiproliferative compound[11,12] is likely to be overproduced in CRC as compared with normal colon tissue. Finally, the *ras* family of oncogenes plays a crucial role in the onset and growth of CRC,[20] and we have recently reported that an endocannabinoid analogue inhibits the proliferation of v-K-*ras*–transformed thyroid cells by blocking the activity of p21*ras*, the protein encoded by *ras*.[13]

Based on this background, we have decided to investigate whether endocannabinoids, their receptors, and one of the enzymes deputed to their inactivation, the fatty acid amide hydrolase (FAAH), are present, and with what possible biologic function, in CRCs as well as in colorectal adenomatous polyps that are known to progress into CRC. We compared the levels of AEA, 2-AG, CB_1, CB_2, and FAAH in normal colon mucosa to those in transformed mucosa (adenomas and carcinomas). Furthermore, we studied the effect of the endocannabinoids, of selective CB_1 and CB_2 receptor stimulation, and of selective inhibitors of endocannabinoid inactivation (to augment AEA and 2-AG endogenous levels) on the proliferation of two human CRC cell lines: (1) the CaCo-2 cells, which are widely used for studies on this type of cancer and undergo differentiation when in culture; and (2) DLD-1 cells, which, unlike CaCo-2 cells, do not differentiate in culture. We report data pointing to a tonic limiting action by endocannabinoids and CBRs on the growth of CRC.

Materials and Methods

Drugs

AEA and 2-AG were purchased from Cayman Chemicals, and ACEA, Met-Fluoro-anandamide, and BML-190 from Tocris. HU-210 was a kind gift from Prof. R. Mechoulam, Hebrew University of Jerusalem, and SR14176A and SR144528 were donated by Sanofi Recherche. Indomethacin *N*-methyl-ester was obtained from Sigma. VDM-11, VDM-13, and arachidonoyl-serotonin were synthesized from the corresponding amines and arachidonoyl-chloride, as described previously.[21]

Biopsy

Biopsy specimens were obtained in agreement with current Italian health care rules, by means of biopsy forceps during colonoscopy on both healthy and cancer tissue in 9 patients affected with left-sided colon carcinoma (average age 64.5 ± 10 years, 3 males, 6 females; mitosis = 1.35 ± 0.33; grading = 2.44 ± 0.52; nuclear pleiomorphism 2.55 ± 0.52; means ± SD) and on healthy tissue and adenomatous polyps in 12 patients affected with colonic adenomas (average age 59.9 ± 14 years, 10 males, 2 females). A small piece (15–20 mg wet weight) from the head of each polyp removed by snare polipectomy and aliquots of each biopsy sample were kept at −80°C until processing. Adenomatous polyps and aliquots of all samples were stored in formalin for histology to evaluate tumor grading, mitoses for high-power field, and nuclear pleiomorphism.

Cell Culture and Proliferation and Differentiation Assays

Caco-2 cells were grown in Dulbecco's modified Eagle medium supplemented with 2 mmol/L L-glutamine, 1% nonessential amino acids, and 10% fetal calf serum (FCS). DLD-1 cells were grown in RPMI-1640 medium supplemented with 2 mmol/L L-glutamine and 10% FCS. Sucrase activity was assessed by the method of Dahlquist[22] by measuring the glucose released from saccharose by the enzyme under standardized condition. The sucrase activity was expressed as 1 unit = 1 μmol of glucose released/min at 37°C at pH 7. Cell proliferation assays were carried out in 6-well dishes containing subconfluent cells (5×10^4 cells). Three hours after cell seeding, test substances were added in medium and then daily at each change of medium. After 4 days, cells were treated with trypsin and counted by a hemocytometer. Cell viability was assessed by trypan blue, and no significant decrease was observed with up to 10 μmol/L anandamide. DNA fragmentation of CaCo-2 cells treated for 72 hours with met-fluoro-anandamide (Tocris) was analyzed by flow cytometry using FACScan (Becton Dickinson).[11]

Endocannabinoid Measurement

Tissues or cells were dounce-homogenized with chloroform/methanol/Tris-HCl 50 mmol/L, pH 7.4 (1/1/1 by volume), containing 5 pmol of d_8-anandamide and 50 pmol of d_8-2-AG (Cayman Chemicals) as internal standards. Lipid-containing organic phase was dried down, weighed, and prepurified by open-bed chromatography on silica gel and analyzed by liquid chromatography (LC)-atmospheric pressure chemical ionization (APCI)-mass spectrometry (MS) (LC-APCI-MS) using a Shimadzu HPLC apparatus (LC-10ADVP) coupled to a Shimadzu (LCMS-2010) quadrupole MS via a Shimadzu APCI interface. MS analyses were carried out in the selected ion-monitoring (SIM) mode as described previously.[23] Anandamide and 2-AG quasimolecular ions were quantified by isotope dilution with the above-mentioned deuterated standards and their amounts in pmols normalized per milligram of lipid extract.[23] Data were statistically evaluated by ANOVA followed by the Bonferroni's test (as per StatMost).

Reverse-Transcriptase Polymerase Chain Reaction

The expression of messenger RNA (mRNA) for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), COX-2, FAAH, CB_1, and CB_2 receptors was examined by reverse transcription coupled to the polymerase chain reaction (RT-PCR). The procedures have been widely described in our previous studies.[11–13] The PCR cycles were 35 for CB_1, CB_2, FAAH, and COX-2 and 28 for GAPDH, which were observed to be optimal and in the linear portion of the amplification curve. The specific human oligonucleotides were synthesized on the basis of cloned human cDNA sequences of GAPDH, COX-2, FAAH, CB_1, and CB_2. For GAPDH, the primer sequences were 5′-CCCTTCATTGACCTCAACTACATGGT-3′ (nt 208-233; sense) and 5′-GAGGGCCATCCACAGTCTTCTG-3′ (nt 655-677; antisense). The COX-2 sense and antisense primers were 5′-TGGGAAGCCTTCTCTAACCTCTCCT-3′ (nt 125-132) and 5′-CTTTGACTGTGGGAGGATACATCTC-3′ (nt 246-254), respectively. The FAAH sense and antisense primers were 5′-GTGGTGCT(G/A)ACCCCCATGCTGG-3′ (nt 469-475) and 5′-TCCACCTCCCGCATGAACCGCAGACA-3′ (nt 561-569), respectively. The CB_1 sense and antisense primers were 5′-GATGTCTTTGGGAAGATGAACAAGC-3′ (nt 365-373) and 5′-AGACGTGTCTGTGGACACAGACATGG-3′ (nt 460-468), respectively. For CB_2, the primer sequences were 5′-TTTCCCACTGATCCCCAATG-3′ (nt 182-188; sense) and 5′-AGTTGATGAGGCACAGCATG-3′ (nt 285-291; antisense). The expected sizes of the amplicons were 470 bp for GAPDH, 388 bp for COX-2, 300 bp for FAAH, 309 bp for CB_1, and 329 bp for CB_2. In the presence of contaminant genomic DNA, the expected size of the amplicons would be 1062 bp for GAPDH, 1668 bp for COX-2, and 1335 bp for FAAH, respectively. The GAPDH housekeeping gene expression was used to evaluate any variation in the RNA content and cDNA synthesis in the different preparations. No PCR products were detected when the reverse transcriptase step was omitted (data not shown).

Western Immunoblotting

Western immunoblotting analysis was used to determine the presence of the CB_1 and CB_2 proteins and carried out as described in detail previously.[11–13] Antibodies (both from Cayman Chemicals, Ann Arbor, MI) were used at a dilution of 1:333 for CB_1 and 1:250 for CB_2. Control of specificity was performed by preadsorbing the antibody with the homologous antigen at a concentration of 8 μg/mL of antibody solution.

Anandamide Uptake Assays

Confluent Caco-2 cells (plated in 6-well dishes, 150,000 cells per dish) take up [^{14}C]AEA (5.0 μmol/L, 20,000 cpm) from serum-free medium in a time- and temperature-dependent manner ($t_{1/2}$ = 3.5 minutes, uptake at 37°C − uptake at 4°C = 48.3 ± 4.1% of total uptake). The effect of compounds on [^{14}C]AEA uptake was studied as described previously.[21] Cells were incubated with [^{14}C]AEA for 5 minutes at 37°C, in the presence or absence of varying concentrations of the inhibitors. Residual [^{14}C]AEA in the incubation media after extraction with $CHCl_3$/CH_3OH 2:1 (by volume), determined by scintillation counting of the lyophilized organic phase, was used as a measure of the AEA that was taken up by cells.

Anandamide Hydrolysis Assays

The effect of compounds on the enzymatic hydrolysis of AEA was studied as described previously,[21] using membranes prepared from cells incubated with the test compounds and [^{14}C]AEA (10 μmol/L, 40,000 cpm) in 50 mmol/L Tris-HCl, pH 9, for 30 minutes at 37°C. [^{14}C]Ethanolamine produced from [^{14}C]AEA hydrolysis was measured by scintillation counting of the aqueous phase after extraction of the incubation mixture with 2 volumes of $CHCl_3$/CH_3OH 2:1 (by volume).

Binding Assays

Displacement assays for CB_1 receptors were carried out by using [^{3}H]SR141716A (0.4 nmol/L, 55 Ci/mmol; Amersham) as the high-affinity ligand on membrane preparations (0.4 mg/tube) from frozen male CD rat brains (Charles River, Wilmington, MA) and in the presence of 100 μmol/L PMSF.[21] Specific binding was calculated with 1 μmol/L SR141716A and was 84%. Data are expressed as the K_i, calculated using the Cheng-Prusoff equation from the concentration exerting 50% inhibition of AEA uptake (IC_{50}).

Results

Endocannabinoid Levels and CB_1, CB_2, and FAAH Expression in Human Colorectal Tissues

We found that human colon mucosa tissues contain both AEA and 2-AG (Figure 1*A*), as determined by using an ultrasensitive LC-MS technique, and express mRNA transcripts of the size expected for CB_1 and CB_2 receptors as well as FAAH (Figure 1*B*), as determined by RT-PCR. The finding of CB_1 receptors was also confirmed by Western immunoblot of proteins from biopsy specimens of normal colon mucosa (not shown). The levels of both AEA and 2-AG increased when passing from normal mucosa to transformed mucosa (Figure 1*A*). Although the levels of endocannabinoids in colorectal adenomas and carcinomas could not be determined in the same set of patients, it was possible to observe a stronger increase of the amounts of 2-AG and, particularly, AEA (3-fold vs. 2-fold, respectively) in adenomatous polyps than in CRC tissue as compared with healthy mucosa. Tissues from both normal and transformed mucosa yielded similar amounts of extracted lipids per gram of wet weight, with normal mucosa, adenomas, and CRC

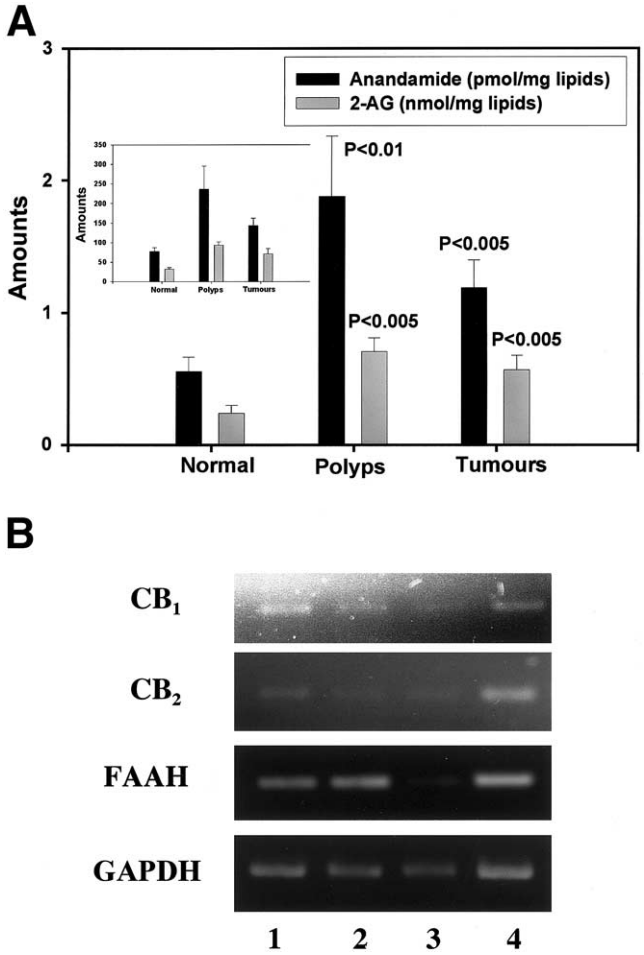


Figure 1. Endocannabinoid levels (*A*) and CB_1, CB_2, and FAAH mRNA expression (*B*) in human colorectal normal mucosa, adenomatous polyps, and carcinomas. (*A*) Anandamide and 2-arachidonoylglycerol (2-AG) levels are expressed as pmol or nmol per mg extracted lipids, respectively, and are means ± SEM of n = 12 samples for adenomas and n = 9 samples for tumors. Data for control mucosa were pooled from the 21 samples. Means were compared by 1-way ANOVA followed by the Bonferroni's test, and the level of significant difference from the respective controls is shown for each histogram. *Inset* shows the same data expressed as pmol or nmol/g wet tissue weight. (*B*) Expression of mRNA transcripts for CB_1 (*upper panel*), CB_2 (*second panel*), FAAH (*third panel*), and the housekeeping gene (GAPDH; *lower panel*) in samples from colorectal carcinomas (*lanes 1* and *2*) and healthy mucosa (*lanes 3* and *4*) from representative patients A (*lanes 1* and *4*) and B (*lanes 2* and *3*). RT-PCR was performed on tissues from 4 more patients with similar results. Amplicons were of the size expected from the type of oligoprimers used (see Materials and Methods section).

tissue containing 130 ± 7, 124 ± 5, and 119 ± 4 mg lipids/g wet tissue weight (means ± SEM, n = 21, 12, and 9, respectively), respectively. Therefore, the ranking of endocannabinoid content in the 3 types of tissues (adenomas>CRC>normal mucosa) did not change when the amounts were expressed as pmol/g wet tissue weight (Figure 1*A*, inset). No consistent differences between the levels of the expression of CB_1, CB_2, and FAAH (the former as assessed by both RT-PCR and immunoblot) were found between normal and CRC tissue (Figure 1*B* and data not shown).

CRC Cell Differentiation and/or Proliferation In Vitro

CaCo-2 cells reached confluence after about 7 days from cell seeding and, starting from the 12th day in culture, started differentiating as assessed by the progressive synthesis of sucrase (Figure 2*A*). Both AEA and 2-AG significantly inhibited the growth of undifferentiated CaCo-2 cells with IC_{50} in the submicromolar range (Figure 2*B*). No toxicity to cells, as assessed by the trypan blue method, and no effect on apoptosis, as assessed by FACScan, was observed up to 5 μmol/L AEA (not shown). The CBR agonist HU-210 inhibited CaCo-2 cell proliferation more potently than the 2 endocannabinoids. Two agonists selective for CB_1 vs. CB_2 receptors, i.e., arachidonoyl-chloro-anandamide (ACEA) and *N*-arachidonoyl-dopamine (NADA), also inhibited CaCo-2 cell proliferation, whereas the CB_2-selective agonist BML-190 was inactive (Figure 2*C*). Importantly, the effect of AEA, HU-210, and NADA was antagonized by the selective CB_1 receptor antagonist SR141716A (0.2 μmol/L) but not by the selective CB_2 receptor antagonist SR144528 (0.2 μmol/L, Figure 2*D* and data not shown). During the course of differentiation, the responsiveness of CaCo-2 cells to AEA and HU-210 changed dramatically, the proliferation of differentiated cells being almost insensitive to treatment with these 2 compounds (Table 1).

We also assessed the effect of AEA, 2-AG, HU-210, and BML-190 on the nondifferentiating DLD-1 cells. In this case, stimulation of both CB_1 and CB_2 receptors led to inhibition of cell growth, although with lower efficacy as compared with CaCo-2 cells (Table 1). The effect of HU-210 was counteracted by both SR141716A and SR144528 (0.2 μmol/L, Table 1).

The selective COX-2 inhibitor indomethacin *N*-methyl ester (0.1-25 μmol/L) inhibited undifferentiated CaCo-2 cell proliferation (Table 1). This effect of was not additive with that exerted by 2-AG, which instead occluded the antiproliferative effect indomethacin *N*-methyl ester (Table 1). The COX-2 inhibitor also blocked the proliferation of DLD-1 cells, and this effect was antagonized by SR141716A (Table 1).

Presence of CBRs in CRC Cells in Culture

By using RT-PCR, we found that CaCo-2 cells express the CB_1 receptor, whereas no evidence for the presence of CB_2 mRNA transcripts was found (Figure 3*A* and data not shown). Western immunoblotting con-

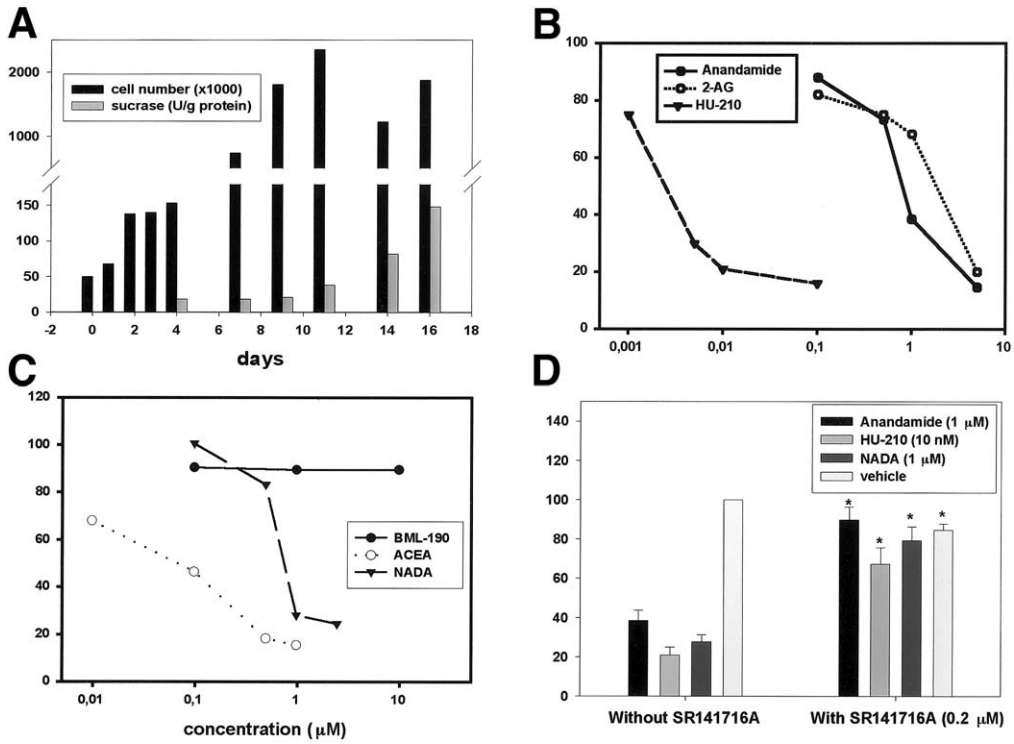


Figure 2. CaCo-2 cell proliferation and differentiation and the effect of cannabimimetics. (*A*) Growth of CaCo-2 cells in culture and sucrase production as an index of differentiation. (*B*) Effect of anandamide, 2-arachidonoyl-glycerol (2-AG), and the potent CB_1/CB_2 agonist HU-210 on the proliferation of undifferentiated (days 0–5) CaCo-2 cells. (*C*) Effect of the selective CB_1 agonists arachidonoylchloroethanolamide (ACEA) and *N*-arachidonoyl-dopamine (NADA) and of the selective CB_2 agonist BML-190 on the proliferation of undifferentiated (days 0–5) CaCo-2 cells. (*D*) Effect of the selective CB_1 antagonist SR141716A on the antiproliferative action of HU-210, anandamide, and NADA. $*P < 0.01$ vs. control, by ANOVA. In *B–D*, data are expressed as percentage of control cell proliferation (100× final treated cell number − initial cell number/final control cell number) and are means of n = 3 experiments carried out in duplicate. In *B* and *C*, SEM bars are not shown for the sake of clarity and were never higher than 5% of the means.

firmed that CB_1 is expressed in these cells, in that 3 immunoreactive bands, sensitive to saturation with the blocking peptide, were found with molecular weight very similar to those found in rat brain homogenates (Figure 3*C*) and corresponding to the truncated, native, and glycosylated forms of the CB_1 receptor. Overall, the levels of CB_1 receptors in CaCo-2 cells appeared to remain constant upon differentiation of the cells, although the amounts of the native form (~53 kilodaltons) of the receptor were significantly lower than in undifferentiated cells (Figure 3*C*). DLD-1 cells were found to express both CB_1 and CB_2 mRNA and protein, although CB_1 receptors appeared to be less abundant in these cells than in CaCo-2 cells (Figure 3*A* and *C*).

Endocannabinoids and Their Inactivation in CRC Cells

By using again our sensitive LC-MS analytical method, we found that undifferentiated CaCo-2 cells contain measurable levels of both AEA (Table 2). We also found that these cells exhibit FAAH activity (93.6 ± 11.4 pmol mg protein^{-1} minute^{-1}, mean ± SD, n = 3) and, like DLD-1 cells, express FAAH mRNA (Figure 3*B*). Intact, undifferentiated CaCo-2 cells also clear [^{14}C]AEA from the incubation medium in a temperature-dependent manner (67.0 ± 3.2 pmol minutes^{-1} per 10^6 cells, corresponding to 111.7 ± 5.3 pmol minutes^{-1} mg protein^{-1}, means ± SD, n = 3). The level of expression of FAAH mRNA in CaCo-2 cells appeared to increase upon differentiation of the cells (Figure 3*B*), thus explaining why the amounts of endocannabinoids in differentiated cells were significantly lower than in undifferentiated cells (Table 2).

The effect of the 2 selective AEA uptake inhibitors VDM11 and VDM13 and of the selective FAAH inhibitor arachidonoyl-serotonin were examined on the uptake and hydrolysis, respectively, of [^{14}C]AEA by CaCo-2 cells. VDM11 and VDM13 efficiently inhibited [^{14}C]AEA uptake with similar IC_{50} values around 3 μmol/L (Figure 4*A* and data not shown). Arachidonoyl-serotonin instead inhibited [^{14}C]AEA hydrolysis with a IC_{50} ~9 μmol/L (Figure 4*B*).

Table 1. Effect of Various Cannabimimetic Agents and of Indomethacin *N*-Methyl Ester on the Proliferation of CRC Cells in Culture

	DLD-1	CaCo-2 (days 1–5)	CaCo-2 (days 14–16)
AEA (2.5 μmol/L)	59.8 ± 5.4	27.0 ± 3.1	96.0 ± 2.2
2-AG (1 μmol/L)	75.0 ± 3.1	41.6 ± 1.5	NT
2-AG (2.5 μmol/L)	59.3 ± 3.8	23.8 ± 0.1	NT
HU-210 (0.1 μmol/L)	100.2 ± 1.5	18.3 ± 1.9	90.6 ± 2.8
HU-210 (1 μmol/L)	57.1 ± 3.5	NT	NT
HU-210 (1 μmol/L) + SR141716A (0.2 μmol/L)	79.8 ± 2.2[a]	NT	NT
HU-210 (1 μmol/L) + SR144528 (0.2 μmol/L)	88.7 ± 4.2[a]	NT	NT
BML-190 (1 μmol/L)	66.5 ± 3.1	90.7 ± 4.5	NT
BML-190 (1 μmol/L) + SR144528 (0.2 μmol/L)	90.5 ± 4.1[b]	NT	NT
INDO (0.1 μmol/L)	100.0 ± 5.2	74.0 ± 0.1	NT
INDO (1 μmol/L)	99.0 ± 3.5	74.2 ± 1.5	NT
INDO (10 μmol/L)	63.0 ± 4.2	62.6 ± 3.3	NT
INDO (25 μmol/L)	22.0 ± 0.9	10.3 ± 2.8	NT
INDO (25 μmol/L) + 2-AG (1 μmol/L)	NT	29.6 ± 3.8[c]	NT
INDO (25 μmol/L) + 2-AG (2.5 μmol/L)	NT	17.7 ± 2.1[c]	NT
INDO (25 μmol/L) + SR141716A (0.2 μmol/L)	61.5 ± 3.1[c]	NT	NT

NOTE. Data are expressed as percentage of control cell proliferation (100×; final treated cell number − initial cell number/final control cell number) and are means of n = 3 experiments carried out in duplicates. Both undifferentiated (days 1–5) and differentiated (days 14–16) CaCo-2 cells were used.

[a,b,c]$P < 0.05$ vs. agonist only (i.e., HU-210, BML-190, or INDO), by ANOVA followed by the Bonferroni test.

AEA, anandamide; 2-AG, 2-aracidonoylglycerol; NT, not tested; INDO, indomethacin *N*-methyl ester.

Effect of Selective Inhibitors of Endocannabinoid Inactivation on CaCo-2 Cell Proliferation

VDM11, VDM13, and arachidonoyl-serotonin were also evaluated for their effect on undifferentiated CaCo-2 cell proliferation. We found that the 3 compounds inhibited proliferation with IC_{50} values almost identical to those observed for the inhibition of [^{14}C]AEA uptake and hydrolysis, respectively (Figure 4*A* and *B* and data not shown). Importantly, the effect on cell proliferation of a 10 μmol/L concentration of the 3 inhibitors was antagonized by SR141716A (0.2 μmol/L; Figure 4*A* and *B*). Accordingly, we found that 24-hour incubation of undifferentiated CaCo-2 cells with either VDM-11 (10 μmol/L) or arachidonoyl-serotonin (10 μmol/L) led to a significant increase of endocannabinoid levels (Table 2).

COX-2 Expression in CRC Cell Lines

A messenger RNA transcript for COX-2 was detected both in undifferentiated CaCo-2 cells and, to a much smaller extent, DLD-1 cells (Figure 5). The level of expression of COX-2 decreased significantly when CaCo-2 cells underwent differentiation (Figure 5).

Discussion

We found that human colon mucosa tissues contain both AEA and 2-AG and express CB_1 and CB_2 receptors as well as FAAH. The endocannabinoids and FAAH previously have been described to occur in mouse and rat whole colon,[24,25] but we found here that the levels of both AEA and 2-AG increase dramatically when passing from normal mucosa to adenomatous polyps and then slightly decrease in CRC tissue. These changes are likely to result in corresponding changes in endocannabinoid tissue concentrations. In fact, considering that, on average, 1 g (and, hence, ~1 mL) of wet tissue weight yields ~125 mg of extracted lipids, it can be calculated that AEA concentrations augment from around 75 pmol/g (i.e., ~75 nmol/L) to 143 and 236 pmol/g (i.e., ~143 nmol/L and ~236 nmol/L) when passing from healthy mucosa to CRC and adenomatous polyps, respectively. Therefore, AEA concentrations are increased to concentrations well above the threshold of CB_1 activation by this lipid (the reported K_i values of AEA for CB_1 receptors are in the 40–200 nmol/L range[4]). Regarding 2-AG, the concentration of this compound augments from about 32 μmol/L, in normal tissue, to 71 and 93 μmol/L, respectively, in CRC and adenomas. Although the levels of 2-AG in healthy mucosa appear to be already sufficient to tonically activate CB_1 receptors (the reported K_i values of 2-AG for CB_1 receptors are in the 250–1200 nmol/L range[4]), it must be kept in account that only a part of 2-AG found in tissues might be used as an endocannabinoid. In fact, this compound, unlike AEA, is also an intracellular intermediate of (phospho)glyceride metabolism and is released outside cells only in part to activate CBRs.[29]

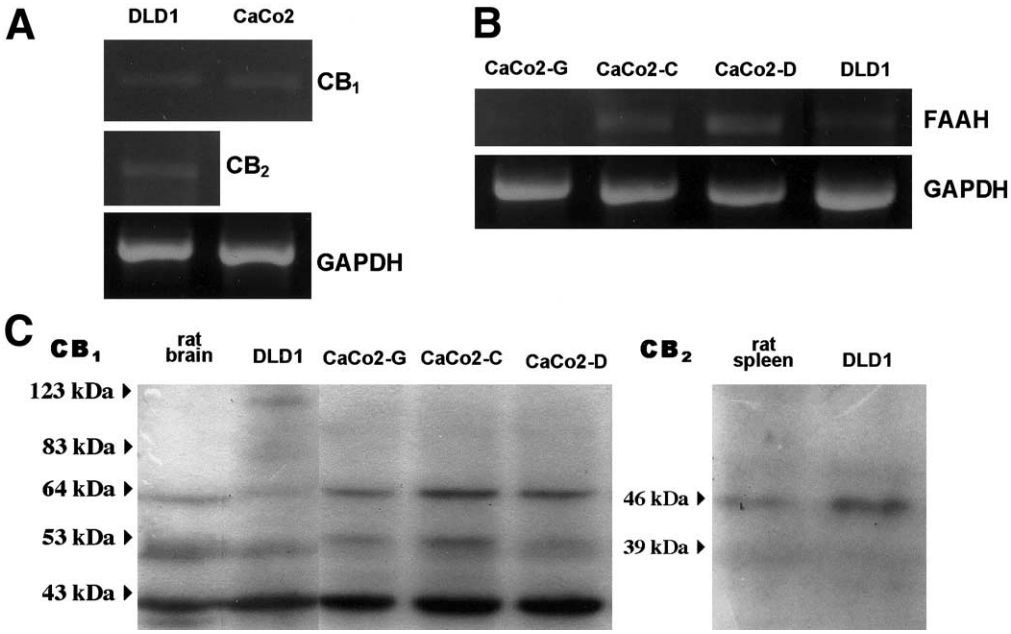


Figure 3. CB_1, CB_2, and FAAH expression in DLD-1 and CRC cells. (*A*) Expression in DLD-1 and CaCo-2 (growing phase, days 0–7) cells of mRNA transcripts with the expected sizes for CB_1 (309 bp) and CB_2 (329 bp). (*B*) Fatty acid amide hydrolase (FAAH) mRNA expression in CaCo-2 (growing phase, *G*, days 0–7; confluent phase, *C*, days 7–12; differentiated phase, *D*, days 14–16) and DLD-1 cells. The expected size of the amplicon was 300 bp. In both *A* and *B*, GAPDH (amplicon size 470 bp) was used as the housekeeping gene. (*C*) Western immunoblotting of protein homogenates from DLD-1 and CaCo-2 cells. Proteins (50 μg/lane) from lysates of rat brain (used as positive control), DLD-1 cells, and CaCo-2 cells (growing phase, *G*, days 0–7; confluent phase, *C*, days 7–12; differentiated phase, *D*, days 14–16), reacted with anti-CB_1 antibody, exhibited 3 immunoreactive bands at ~42 kilodaltons, ~53 kilodaltons, and ~63 kilodaltons, corresponding to the truncated, the native, and the glycosylated forms of the CB_1 receptor. In DLD-1 cells, 2 faint bands at ~83 kilodaltons and ~123 kilodaltons, corresponding to a further glycosylated and dimeric CB_1 receptor protein, respectively, were observed. Proteins (50 μg/lane) from lysates of rat spleen (used as positive control) and DLD-1 cells, reacted with the anti-CB_2 antibody, show 2 immunoreactive bands at ~46 kilodaltons and ~39 kilodaltons, corresponding to the glycosylated and the native forms of the CB_2 receptor, respectively. None of the immunoreactive bands was observed when the antibodies were preadsorbed with the immunizing peptide.

Several biochemical mechanisms might explain the enhancement of endocannabinoid levels in transformed human colon mucosa. First, an up-regulation of 1 of the enzymes responsible for arachidonate mobilization and phospholipid remodelling, the secretory phospholipase A_2, has been described to occur in colorectal adenomas from familial adenomatous polyposis patients.[26] Second, elevated levels of lysophosphatidic acids, possibly also including the *sn*-2-arachidonate-containing species that serve as biosynthetic precursors for 2-AG,[17] have been reported in patients with CRC.[19] These 2 events, albeit suggested to play a causative role in the onset and growth of tumors, via enhanced formation of the precursor for COX-2-catalyzed production of prostaglandins[15] or stimulation of LPA receptors,[18] respectively, might lead at the same time to elevated levels of endocannabinoids with tumor-inhibitory activity.

Table 2. Amounts of Endocannabinoids in Undifferentiated and Differentiated CaCo-2 Cells in Culture

	Anandamide	2-arachidonoyl-glycerol
Differentiated cells + vehicle	10.8 ± 3.0[a]	107.1 ± 3.5[a]
Undifferentiated cells + vehicle	41.0 ± 7.6	208.0 ± 45.1
+VDM11	70.0 ± 2.6[a]	400.0 ± 121.0[a]
+arachidonoyl-serotonin	107.6 ± 36.9[a]	405.0 ± 130.5[a]

NOTE. Table shows the effect of 24-hour cell treatment with vehicle (methanol, 0.1%) and VDM11 (10 μmol/L), and arachidonoyl-serotonin (10 μmol/L) of undifferentiated cells. Data are expressed as pmol/g wet cell weight and are means ± SD of n = 3 determinations.
[a]$P < 0.05$ vs. undifferentiated cells + vehicle, as assessed by the Student *t* test.

To test the above hypothesis, we undertook a series of experiments aimed at investigating whether (1) endocannabinoids do inhibit colon cancer cell growth in vitro; (2) substances that inhibit endocannabinoid inactivation, and hence enhance the amounts of endocannabinoids produced by CRC cells in culture, also inhibit the proliferation of these cells; and (3) changes in endocannabinoid signaling occur in CRC cells also during their differentiation in culture. We used a widely employed cell line for the study of CRC, the CaCo-2 cells, which have the special feature of being capable of differentiating in culture after having reached confluence, thus becoming more similar to enterocytes.[27] We found that

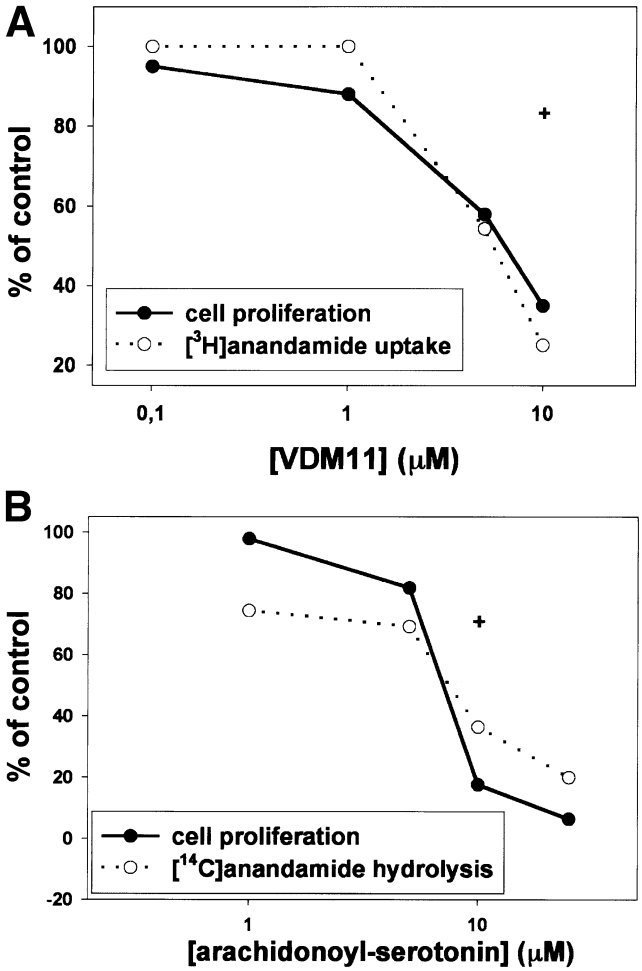


Figure 4. Effect of inhibitors of endocannabinoid metabolism on anandamide uptake and hydrolysis, and on proliferation, in undifferentiated (days 0–4) CaCo-2 cells. (*A*) Effect of the anandamide cellular uptake inhibitor VDM11 on the uptake of [^{14}C]anandamide by intact cells (○) and on cell proliferation (●). Similar data were obtained with VDM13, another anandamide cellular uptake inhibitor. (*B*) Effect of the anandamide enzymatic hydrolysis inhibitor arachidonoyl-serotonin on the hydrolysis of [^{14}C]anandamide by cell membranes (○), and on cell proliferation (●). Data for the effect on cell proliferation are expressed as percentage of control cell proliferation (see Figure 2 legend) and are means of n = 3 experiments carried out in duplicate. The effect of SR141716A (0.2 μmol/L) on the antiproliferative effect of VDM-11 (10 μmol/L) and arachidonoyl-serotonin (10 μmol/L) is shown with a *cross* and was statistically significant ($P < 0.01$ by ANOVA). Data for the inhibition of [^{14}C]anandamide uptake and hydrolysis are means of n = 3 experiments carried out in duplicate. SEM bars are not shown for the sake of clarity and were never higher than 5% of the means.

AEA, 2-AG, and the ultra-potent CBR agonist HU-210 inhibited CaCo-2 cell proliferation with IC_{50} values in the submicromolar range and with relative potencies (HU-210>>AEA≥2-AG) that reflect their relative potencies at cannabinoid CB_1 receptors.[4] Three further observations strongly supported the involvement of this receptor subtype in endocannabinoid antiproliferative effects. First, 2 agonists selective for CB_1 vs. CB_2 receptors, i.e., arachidonoyl-chloro-anandamide (ACEA) and *N*-arachidonoyl-dopamine (NADA), the latter of which is much more stable to enzymatic hydrolysis than AEA, but less potent on CB_1 receptors than AEA,[28] inhibited CaCo-2 cell proliferation with the rank of potency expected from their relative affinity for CB_1 receptors (ACEA>NADA), whereas the CB_2-selective agonist BML-190 was inactive. Second, the effect of AEA, HU-210, and NADA (which is also an agonist for the VR1 receptor for capsaicin[28]) was entirely antagonized by the selective CB_1 receptor antagonist SR141716A but not by the selective CB_2 receptor antagonist SR144528. Finally, we found that CaCo-2 cells express the CB_1 receptor, whereas no evidence for the presence of CB_2 was found. We also assessed the effect of the endocannabinoids and of HU-210 and BML-190 in another CRC cell line, the nondifferentiating DLD-1 cells. In this case, stimulation of both CB_1 and CB_2 receptors led to inhibition of cell growth, in agreement with the presence of both receptor subtypes in these cells. However, CB_1 receptors appeared to be less expressed in DLD-1 cells than in CaCo-2 cells, and this finding, together with the observation that DLD-1 cells were clearly less responsive to cannabimimetics than CaCo-2 cells, might suggest that CB_1 receptors are more important than CB_2 receptors in causing blockade of CRC cell proliferation. In summary, we found that, depending on the CRC cell line, either selective CB_1 receptor stimulation (as previously found for breast and prostate cancer cells[11,12]) or activation of both CB_1 and CB_2 receptors causes inhibition of proliferation. These findings are in agreement with the presence of both CBR subtypes in colon normal mucosa and CRC (Figure 1*B*) and suggest that endocannabinoids, present in high amounts in CRCs and, particularly, colorectal adenomas, might function as endogenous inhibitors of cancer growth.

To further challenge this hypothesis, we started a series of experiments aimed at manipulating pharmacologically the endogenous levels of endocannabinoids in CRC cells without directly activating the CBRs and at evaluating whether these treatments lead to inhibition of

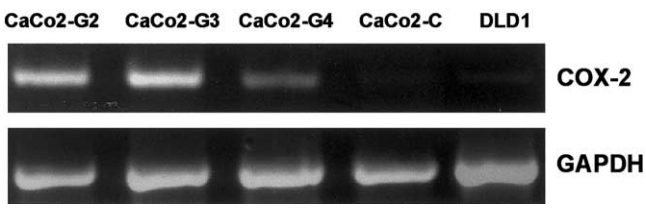


Figure 5. Cyclooxygenase 2 (COX-2) mRNA expression in CaCo-2 cells (growing phase, days 2, 3, and 4; confluent phase, days 7–12) and in DLD-1 cells. The expected sizes of the amplicons were 388 bp for COX-2 and 470 bp for GAPDH, used as the housekeeping gene.

CRC cell proliferation. We found that undifferentiated CaCo-2 cells contain AEA and 2-AG in concentrations (~40 and ~200 nmol/L, respectively) that, although smaller than those found in CRC tissue, are still compatible with a possible tonic submaximal activation of CBRs. Unlike CRC tissue, the amounts of 2-AG in these cells were only about 5-fold higher than those of AEA, in agreement with the hypothesis (see above) that only a part of the 2-AG detected in CRC tissue might derive from cancer cells and be used to activate their CBRs and to inhibit their proliferation. However, if the endocannabinoids do exert a tonic submaximal inhibition of cancer cell growth, agents that inhibit their inactivation should inhibit cancer cell proliferation. Endocannabinoids are inactivated through a 2-step process including cellular reuptake, which is facilitated by the AEA membrane transporter (AMT, a yet-to-be characterized protein facilitating the cellular reuptake not only of AEA but also of 2-AG and other endocannabinoids[28,29]), and intracellular hydrolysis by FAAH, an amidase that, in cell cultures, can also catalyze the hydrolysis of fatty acid esters such as 2-AG (see Di Marzo et al.[29] for a review). We found that undifferentiated CaCo-2 cells express FAAH mRNA and exhibit FAAH activity and that they take up anandamide in a temperature-dependent manner. We, therefore, could examine the effect of 2 selective AMT inhibitors, VDM11 and VDM13,[21] and of a selective FAAH inhibitor, arachidonoyl-serotonin,[30] on the uptake and hydrolysis, respectively, of [^{14}C]AEA by CaCo-2 cells and on CaCo-2 cell proliferation. We found that the 3 compounds inhibited with the same potency the 2 inactivation processes and CaCo-2 cell proliferation and that their antiproliferative effect was antagonized by SR141716A. Because none of these compounds is capable of directly stimulating CB_1 receptors at concentrations lower than 10 μmol/L,[21,30] this finding suggests that VDM-11, VDM-13, and arachidonoyl-serotonin inhibit CaCo-2 cell proliferation by enhancing the endogenous amounts of AEA and/or 2-AG available for CB_1 stimulation. This suggestion was strongly supported by our finding that 24-hour incubation of undifferentiated CaCo-2 cells with either VDM-11 or arachidonoyl-serotonin leads to a significant increase of endocannabinoid levels up to concentrations likely to cause a full activation of CB_1 receptors. These findings strengthen our hypothesis that endocannabinoids tonically inhibit CRC cell proliferation and suggest that inhibitors of endocannabinoid inactivation might represent useful anticancer drugs, although FAAH inhibitors might be less efficacious in vivo than was shown here in vitro, because of the presence of more than 1 hydrolyzing enzyme for 2-AG.[29]

Another approach that we used to gain further support to this hypothesis was to see whether, in CRC cells, responsiveness to (endo)cannabinoids and the extent of endocannabinoid signalling change during cell differentiation. To this purpose, we exploited the capability of CaCo-2 cells to differentiate in culture into enterocytes with a much lower degree of malignancy and invasiveness.[27,31,32] First, we observed that AEA was only efficacious against undifferentiated cells. This finding might have been caused by the fact that cell differentiation was accompanied by an increase of FAAH expression, which is likely to lead to an enhanced AEA degradation in differentiated cells. However, because HU-210, which is not a substrate for FAAH, was inactive on differentiated cells, the increase of FAAH expression might not be the sole cause of the lack of activity of AEA on differentiated cells. We also analyzed the expression of CB_1 receptors in differentiated CaCo-2 cells and found overall CB_1 levels similar to those observed in undifferentiated cells. However, the amounts of the native form (~53 kilodaltons) of the receptor appeared to be significantly lower than in undifferentiated cells. It is, therefore, possible that the smaller antiproliferative effect of HU-210 in differentiated cells is due to a decrease of the levels of functionally active CB_1 or, alternatively, to changes in CB_1-coupled intracellular signalling events during CaCo-2 cell differentiation. We also measured the endocannabinoids in differentiated cells and found significantly lower amounts of both AEA and 2-AG, in agreement with the higher FAAH expression in differentiated cells and with our data obtained with arachidonoyl-serotonin (Table 2). These findings, as well as preliminary data obtained in our laboratory, indicating no significant change in the expression of monoacylglycerol lipase (another 2-AG metobolizing enzyme[29]) during CaCo-2 cell proliferation, suggest that FAAH plays an important role in limiting the levels of both AEA and 2-AG in CaCo-2 cells. It is worthwhile noting that, in agreement with our findings, in human breast cancer cells, whose proliferation is blocked by endocannabinoids via CB_1 receptors,[11] FAAH has been shown very recently to be expressed in up to 30-fold higher levels in those cell lines with a lower degree of invasiveness and malignancy.[33] At any rate, the overall reduction of both the levels and the antiproliferative effects of endocannabinoids in differentiated (and less malignant) vs. undifferentiated (and more malignant) CaCo-2 cells suggests that, also in vitro, endocannabinoid signalling is regulated depending on colorectal cell differentiation and supports our hypothesis that AEA and 2-AG might act as endogenous CRC growth inhibitors.

The higher sensitivity to AEA and 2-AG of undifferentiated CaCo-2 cells as compared with DLD-1 and differentiated CaCo-2 cells might be also explained by the additional action of the 2 endocannabinoids, only in the former cells, on a target different from CB_1. This target might be COX-2 because (1) AEA and 2-AG compete efficaciously with arachidonic acid as COX-2 substrates[16] and might occlude the formation of protumoral prostaglandins, and (2) COX-2 is virtually absent in differentiated CaCo-2 and DLD-1 cells (Figure 5).[27,34] We found that the selective COX-2 inhibitor indomethacin *N*-methyl ester[35] inhibited undifferentiated CaCo-2 cell proliferation at concentrations ($IC_{50} \sim 14$ μmol/L) much higher than those required to inhibit the enzyme in vitro ($IC_{50} = 0.04$ μmol/L[35]). This antiproliferative effect was occluded by 2-AG, thus suggesting that the 2 substances share, at least in part, a similar mechanism of action. However, the COX-2 inhibitor (1) was also active on DLD-1 cells (which express little COX-2) where its effect was antagonized by SR141716A and (2) acted as a CB_1 receptor ligand in binding assays carried out with rat brain membranes ($K_i = 2.8 \pm 0.3$ μmol/L, mean ± SD, n = 3). This indicates that, like 2-AG, indomethacin *N*-methyl ester inhibits CRC cell proliferation by stimulating CBRs. Hence, the reason why (endo)cannabinoids are less potent in DLD-1 cells is not because they cannot act by inhibiting COX-2 expression, as suggested by the observation that HU-210, which does not inhibit COX-2, is also much less efficacious in the same cells (Table 1).

In conclusion, we have shown that endocannabinoids (1) are overproduced in cancerous and, particularly, precancerous (i.e., adenomas) colon tissue and (2) exert a growth-inhibitory effect on CRC cells in culture, in which the extent of their action and levels seems to depend on the degree of differentiation (and malignancy/invasiveness) of these cells. The antiproliferative effects of the endocannabinoids are exerted in a large part by stimulation of CBRs, which are expressed in both colorectal mucosa and CRC cells. However, these compounds might act also by inhibiting COX-2, a possibility that, although not supported by our present data, deserves further investigation. Whatever their mechanism of action, endocannabinoids can be regarded as potential endogenous tumor growth inhibitors as well as possible markers for cancer cells. This hypothesis is strengthened by a recent preliminary study[36] showing that AEA levels are increased in other tumors, including those whose growth was previously shown to be inhibited by endocannabinoids.[11,12] Metabolically stable substances that act by stimulating CBRs directly might exert anticancer actions in this as well as other type of tumors.[1,2] However, in view of (1) the possible multiple mechanisms of action of endocannabinoids, (2) the potential undesired psychotropic effect of CB_1 receptor agonists, and (3) the tonic inhibition on cancer growth suggested here for endocannabinoids, substances that act selectively by enhancing further the tumor levels of AEA and 2-AG, such as inhibitors of their cellular uptake and enzymatic hydrolysis, might provide for a more efficacious and tolerable therapeutic strategy against not only CRC but also other types of cancer.[2,36] In support of this possibility, we have found in a separate study (V. Di Marzo, G. Portella and M. Bifulco, manuscript in preparation) that the growth of transformed thyroid cells in athymic mice[13] is inhibited efficaciously by the AMT inhibitor VDM11 and by the FAAH inhibitor arachidonyl-serotonin via enhancement of tumoral endocannabinoid levels.

References

1. De Petrocellis L, Melck D, Bisogno T, Di Marzo V. Endocannabinoids and fatty acid amides in cancer, inflammation and related disorders. Chem Phys Lipids 2000;108:191–209.
2. Bifulco M, Di Marzo V. Targeting the endocannabinoid system in cancer therapy: a call for further research. Nat Med 2002;8:547–550.
3. Gaoni Y, Mechoulam R. Isolation, structure and partial synthesis of an active constituent of hashish. J Am Chem Soc 1964;86:1646–1656.
4. Pertwee RG. Pharmacology of cannabinoid CB1 and CB2 receptors. Pharmacol Ther 1997;74:129–180.
5. Munson AE, Harris LS, Friedman MA, Dewey WL, Carchman RA. Antineoplastic activity of cannabinoids. J Natl Cancer Inst 1975;55:597–602.
6. Galve-Roperh I, Sanchez C, Cortes ML, del Pulgar TG, Izquierdo M, Guzman M. Anti-tumoral action of cannabinoids: involvement of sustained ceramide accumulation and extracellular signal-regulated kinase activation. Nat Med 2000;6:313–319.
7. Devane WA, Hanus L, Breuer A, Pertwee RG, Sevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 1992;258:1946–1949.
8. Mechoulam R, Ben-Shabat S, Hanus L, Ligunsky M, Kaninski NE, Schatz AR, Gopher A, Almog S, Martin BR, Compton DR, Pertwee RG, Griffin G, Bayewitch M, Barg J, Vogel Z. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinnoid receptors. Biochem Pharmacol 1995;50:83–90.
9. Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K, Yamashita A, Waku K. 2-arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochem Biophys Res Commun 1995;215:89–97.
10. Hanus L, Abu-Lafi S, Fride E, Breuer A, Vogel Z, Shalev DE, Kustanovich I, Mechoulam R. 2-Arachidonyl glyceryl ether, an endogenous agonist of the cannabinoid CB1 receptor. Proc Natl Acad Sci U S A 2001;98:3662–3665.
11. De Petrocellis L, Melck D, Palmisano A, Bisogno T, Laezza C, Bifulco M, Di Marzo V. The endogenous cannabinoid anandamide inhibits human breast cancer cell proliferation. Proc Natl Acad Sci U S A 1998;95:8375–8380.
12. Melck D, De Petrocellis L, Orlando P, Bisogno T, Laezza C, Bifulco M, Di Marzo V. Suppression of nerve growth factor Trk receptors and prolactin receptors by endocannabinoids leads to inhibition

of human breast and prostate cancer cell proliferation. Endocrinology 2000;141:118–126.
13. Bifulco M, Laezza C, Portella G, Vitale M, Orlando P, De Petrocellis L, Di Marzo V. Control by the endogenous cannabinoid system of ras oncogene-dependent tumor growth. FASEB J 2001; 15:2745–2747.
14. Guzman M, Sanchez C, Galve-Roperh I. Control of the cell survival/death decision by cannabinoids. J Mol Med 2001;78:613–625.
15. Dempke W, Rie C, Grothey A, Schmoll HJ. Cyclooxygenase-2: a novel target for cancer chemotherapy? J Cancer Res Clin Oncol 2001;127:411–417.
16. Marnett LJ. Recent developments in cyclooxygenase inhibition. Prostaglandins Other Lipid Mediat 2002;68-69:153–164.
17. Nakane S, Oka S, Arai S, Waku K, Ishima Y, Tokumura A, Sugiura T. Arachidonoyl-sn-glycero-3-phosphate, an arachidonic acid-containing lysophosphatidic acid: occurrence and rapid enzymatic conversion to 2-arachidonoyl-sn-glycerol, a cannabinoid receptor ligand, in rat brain. Arch Biochem Biophys 2002;402:51–58.
18. Huang MC, Graeler M, Shankar G, Spencer J, Goetzl EJ. Lysophospholipid mediators of immunity and neoplasia. Biochim Biophys Acta 2002;1582:161–167.
19. Merchant TE, Kasimos JN, de Graaf PW, Minsky BD, Guerke LW, Glonek T. Phospholipid profiles of human colon cancer using 31P magnetic resonance spectroscopy. Int J Colorectal Dis 1991;6: 121–126.
20. Smith G, Carey FA, Beattie J, Wilkie MJ, Lightfoot TJ, Coxhead J, Garner RC, Steele RJ, Wolf CR. Mutations in APC, Kirsten-ras, and p53—alternative genetic pathways to colorectal cancer. Proc Natl Acad Sci U S A 2002;99:9433–9438.
21. De Petrocellis L, Bisogno T, Davis JB, Pertwee RG, Di Marzo V. Overlap between the ligand recognition properties of the anandamide transporter and the VR1 vanilloid receptor: inhibitors of anandamide uptake with negligible capsaicin-like activity. FEBS Lett 2000;483:52–56.
22. Dahlquist A. Method of assay of intestinal disaccharidases. Anal Biochem 1964;7:18–25.
23. Marsicano G, Wotjak CT, Azad SC, Bisogno T, Rammes G, Cascio MG, Hermann H, Tang J, Hofmann C, Zieglgansberger W, Di Marzo V, Lutz B. The endogenous cannabinoid system controls extinction of aversive memories. Nature 2002;418:530–534.
24. Izzo AA, Fezza F, Capasso R, Bisogno T, Pinto L, Iuvone T, Esposito G, Mascolo N, Di Marzo V, Capasso F. Cannabinoid CB1-receptor mediated regulation of gastrointestinal motility in mice in a model of intestinal inflammation. Br J Pharmacol 2001; 134:563–570.
25. Katayama K, Ueda N, Kurahashi Y, Suzuki H, Yamamoto S, Kato I. Distribution of anandamide amidohydrolase in rat tissues with special reference to small intestine. Biochim Biophys Acta 1997; 1347:212–218.
26. Kennedy BP, Soravia C, Moffat J, Xia L, Hiruki T, Collins S, Gallinger S, Bapat B. Overexpression of the nonpancreatic secretory group II PLA2 messenger RNA and protein in colorectal adenomas from familial adenomatous polyposis patients. Cancer Res 1998;58:500–503.
27. Di Popolo A, Memoli A, Apicella A, Tuccillo C, di Palma A, Ricchi P, Acquaviva AM, Zarrilli R. IGF-II/IGF-I receptor pathway up-regulates COX-2 mRNA expression and PGE2 synthesis in Caco-2 human colon carcinoma cells. Oncogene 2000;19:5517–5524.
28. Huang SM, Bisogno T, Trevisani M, Al-Hayani A, De Petrocellis L, Fezza F, Tognetto M, Petros TJ, Krey JF, Chu CJ, Miller JD, Davies SN, Geppetti P, Walker JM, Di Marzo V. An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. Proc Natl Acad Sci U S A 2002;99: 8400–8405.
29. Di Marzo V, De Petrocellis L, Bisogno T. Endocannabinoids 1. Molecular basis of endocannabinoid formation, action and inactivation, and development of selective inhibitors. Emerg Ther Targets 2001;5:241–266.
30. Bisogno T, Melck D, De Petrocellis L, Bobrov MY, Gretskaya NM, Bezuglov VV, Sitachitta N, Gerwick WH, Di Marzo V. Arachidonoylserotonin and other novel inhibitors of fatty acid amide hydrolase. Biochem Biophys Res Commun 1998;248:515–522.
31. Tsujii M, Kawano S, DuBois RN. Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. Proc Natl Acad Sci U S A 1997;94:3336–3340.
32. Steinert M, Wobus M, Boltze C, Schutz A, Wahlbuhl M, Hamann J, Aust G. Expression and regulation of CD97 in colorectal carcinoma cell lines and tumor tissues. Am J Pathol 2002;161:1657–1667.
33. Jessani N, Liu Y, Humphrey M, Cravatt BF. Enzyme activity profiles of the secreted and membrane proteome that depict cancer cell invasiveness. Proc Natl Acad Sci U S A 2002;99:10335–10340.
34. Tsuji S, Kawano S, Sawaoka H, Takei Y, Kobayashi I, Nagano K, Fusamoto H, Kamada T. Evidences for involvement of cyclooxygenase-2 in proliferation of two gastrointestinal cancer cell lines. Prostaglandins Leukot Essent Fatty Acids 1996;55:179–183.
35. Kalgutkar AS, Crews BC, Rowlinson SW, Marnett AB, Kozak KR, Remmel RP, Marnett LJ. Biochemically based design of cyclooxygenase-2 (COX-2) inhibitors: facile conversion of nonsteroidal antiinflammatory drugs to potent and highly selective COX-2 inhibitors. Proc Natl Acad Sci U S A 2000;97:925–930.
36. Schmid PC, Wold LE, Krebsbach RJ, Berdyshev EV, Schmid HH. Anandamide and other N-acylethanolamines in human tumors. Lipids 2002;37:907–912.

Received January 8, 2003. Accepted April 24, 2003.

Address requests for reprints to: V. Di Marzo, Endocannabinoid Research Group, Institute of Biomolecular Chemistry, C.N.R., Via Campi Flegrei 34, Comprensorio Olivetti, Bldg. 70, Pozzuoli, (NA), Italy. e-mail: vdimarzo@icmib.na.cnr.it; fax: (39) 081-804-1770.

Supported by funds from the Italian National Research Council, MURST (3933 to V.D.M.) and the Associazione Italiana per la Ricerca sul Cancro (to M.B.).

The authors thank Drs. Filomena Fezza and Pierangelo Orlando for valuable assistance and Dr. Gabriella Caruso (I.R.C.C.S. "S. de Bellis," Bari, Italy) for the kind gift of DLD-1 cells.

A.L. and T.B. contributed equally to this work.

Postsecondary Employment Experiences Among Young Adults With an Autism Spectrum Disorder

Anne M. Roux, M.P.H., Paul T. Shattuck, M.S.S.W., Ph.D., Benjamin P. Cooper, M.P.H., Kristy A. Anderson, M.S.W., Mary Wagner, Ph.D., Sarah C. Narendorf, Ph.D.

Objective: We examined postsecondary employment experiences of young adults with an autism spectrum disorder (ASD) and compared these outcomes with those of young adults with different disabilities. **Method:** Data were from Wave 5 of the National Longitudinal Transition Study–2 (NLTS2), a nationally representative survey of young adults who had received special education services during high school. We examined the prevalence of ever having had, and currently having, a paid job at 21 to 25 years of age. We analyzed rates of full-time employment, wages earned, number of jobs held since high school, and job types. **Results:** Approximately one-half (53.4%) of young adults with an ASD had ever worked for pay outside the home since leaving high school, the lowest rate among disability groups. Young adults with an ASD earned an average of $8.10 per hour, significantly lower than average wages for young adults in the comparison groups, and held jobs that clustered within fewer occupational types. Odds of ever having had a paid job were higher for those who were older, from higher-income households, and with better conversational abilities or functional skills. **Conclusions:** Findings of worse employment outcomes for young adults with an ASD suggest that this population is experiencing particular difficulty in successfully transitioning into employment. Research is needed to determine strategies for improving outcomes as these young adults transition into adulthood. J. Am. Acad. Child Adolesc. Psychiatry, 2013;52(9):931–939. **Key Words:** adolescent, autism, employment, outcomes, young adult

A growing population of adolescents diagnosed with an autism spectrum disorder (ASD) is aging toward adulthood. An estimated 50,000 youth with an ASD turn 18 years of age each year in the United States.[1] However, research about the adult stage of life for those with an ASD remains extremely underdeveloped relative to research on young children with an ASD.[2,3] The 2011 Strategic Plan for Autism Spectrum Disorder Research of the Interagency Autism Coordinating Committee emphasized the critical need for longitudinal studies of adult outcomes, such as employment, as a basis for policy recommendations.[4]

This article is discussed in an editorial by Dr. Patricia Howlin on page 897.

Clinical guidance is available at the end of this article.

Parents of youth with an ASD need information about employment prospects and often turn to medical providers for anticipatory guidance regarding the transition to adulthood.[5] The Society for Adolescent Medicine affirms the importance of family–professional partnerships in planning for the transitional care needs of youth, and specifies "vocational progress" as a developmental area that must be addressed.[6] For psychiatrists and mental health practitioners, participation in transition planning is especially important, given that the onset of many commonly co-occurring psychiatric disorders occurs during adolescence and persists into early adulthood.[7,8]

Employment is a socially normative activity that often occupies the majority of adult lives and is a key component of passage into adulthood.[9] Employment for wages contributes to individual economic and social well-being, is linked to positive health outcomes, is a gateway to health insurance, and is a factor in quality of life. For

youth with disabilities, achieving employment through an effective transition process is a primary intended outcome of special education per federal law.[10] Yet, historically, employment has not been easily achieved or maintained for young adults with developmental disabilities.[11] There are few nationally representative findings characterizing the employment experiences of young adults with an ASD.

A cross-sectional study of young adults 18 to 24 years of age conducted in the United States found that 54% were employed in 2011.[12] A separate national survey of adults 26 to 31 years of age asked participants if they ever had at least 1 job between age 18 and 25 years; of the participants, 98.6% replied that they had.[13] Similarly, the vast majority (91%) of young adults with disabilities find some form of paid employment outside the home within the first 8 years after high school.[14]

Young adults with an ASD appear to fare worse in employment outcomes when compared with young adults with other types of disabilities. Shattuck *et al.* (2012) found that 55% of young adults with an ASD had ever worked outside the home for pay at least once in the first 6 years after high school—the lowest rate across comparison disability groups.[1]

Very few details about the characteristics of employment experiences of young adults with an ASD have been published.[15,16] Prior research suggests that few who are employed work independently or in competitive employment, and most are employed in sheltered workshop settings.[17] Difficulty securing and maintaining employment also extends to individuals with autism who have average or above-average intellectual ability.[18,19] Factors related to employment and other outcomes in adults with an ASD include intelligence, communication abilities, adaptive functioning level, co-occurring mental health and medical issues, and family socioeconomic status.[2,3] Evidence about employment of young adults with an ASD is largely derived from samples that are small or not population based, and studies of those who are already engaged with the U.S. Vocational Rehabilitation (VR) system, thereby limiting the generalizability of findings.

This study characterizes employment experiences among postsecondary young adults with an ASD using data collected in 2009 as part of a nationally representative, cohort study of young adults with disabilities who had been receiving special education services when in high school at the start of the study in 2000. We describe rates of employment, wages earned, types of jobs held, and covariate-adjusted group comparisons. Our study compares findings for the ASD group with those of young adults who had previously been served in the special education enrollment categories of mental retardation (MR), learning disability (LD), emotional disturbance (ED), and speech/language impairment (SLI).

METHOD

Study Participants

We used secondary data from wave 5 (collected in 2009) of the National Longitudinal Transition Study–2 (NLTS2), a 10-year prospective study of youth who were receiving special education services at the start of the study. NLTS2 has ended, and it is not possible to contact study participants to collect additional data. Use of these data is governed by an agreement with the U.S. Department of Education (USDE) and was deemed exempt by the Washington University Institutional Review Board. Unweighted sample sizes were rounded to the nearest 10 as required by the USDE.

Sampling and Generalizability

This study used special education classifications for determining groups. Determination of special education eligibility and specific classification within the autism category was made by each student's school district as per federal law.[10] Independent, clinical validation of ASD diagnosis by our team was not an option. The USDE does not require use of diagnostic criteria from the *DSM-IV-TR* in special education eligibility determination. Each state is allowed to operationalize its own eligibility criteria for the special educational category of autism within broad boundaries established in federal law.[20] The federal definition of autism in special education law is consistent with *DSM-IV-TR* criteria, but is much less detailed. Prior research has indicated that 99% of students eligible for special education under the category of autism also meet *DSM-IV*–derived case criteria for an ASD.[21,22] The autism special education category tends to be highly specific and moderately sensitive. Some youth with an ASD might receive special education services under a disability category other than autism, and some might not receive special education. We excluded youth from comparison groups if parents reported that their children had ever received an autism spectrum diagnosis. Youth with an ASD who are not receiving special education services would also not be included in this study's estimates.

NLTS2 findings generalize to the population of youth who were ages 13 through 16 years as of December 1, 2000, and receiving special education services. A multistage process was used to sample by

school district and then by students within the districts, stratified by disability category. A total of 500 districts provided student rosters from which the student sample was selected; approximately 11,280 students were sampled, and 81.9% participated in the first wave of data collection. Large national surveys often use a weighting strategy to ensure that point estimates generalize to the population from which the sample was drawn. NLTS2 survey weights were rigorously calculated at each wave of data collection to ensure that point estimates were representative of the target population. Furthermore, each of the 4 follow-up waves of NLTS2 data was subject to bias analysis to ascertain the extent of difference between the weighted respondent sample for a given wave and both the wave 1 weighted sample and extant data from other sources on the characteristics of the population that NLTS2 was designed to represent.[23,24]

The U.S. Office of Management and Budget approved release of the data after rigorous review of the sampling and adjustment procedures. Detailed information on the NLTS2 sampling strategy, survey weighting, and questionnaire design has been previously published.[25]

Data Collection

The NLTS2 survey instruments incorporated items from several other national, long-standing longitudinal surveys of youth (e.g., The National Longitudinal Study of Adolescent Health, the National Longitudinal Survey of Youth) and involved field testing with parents of youth in several disability categories and with youth themselves.[26] NLTS2 telephone interviews were conducted by a professional contract research firm whose telephone interview staff were rigorously trained and continually monitored. If the parent or guardian had earlier reported that the young adult was capable of answering questions independently, repeated attempts were made to interview both the young adult and the parent or guardian who was most knowledgeable about the young adult.

The focus of this study was 620 young adults who had previously received special education services in secondary school under the autism eligibility category. The retention rate at wave 5 was 67% of the 920 who had participated in wave 1. This response rate exceeded the estimated sample size (n = 452) required to provide data that would achieve an acceptable level of statistical precision.[27]

Young adults previously served in the special education enrollment categories of mental retardation (MR), learning disability (LD), emotional disturbance (ED), or speech/language impairment (SLI) were chosen for comparison because they often share some, but not all, disability features with those who have an ASD and might reasonably be expected to encounter similar challenges with employment. Group labels reported in this study, such as "mental retardation," reflect the NLTS2 survey terminology and federally designated category labels in use at the time of the study. We wish to emphasize that the term "intellectual disability" is currently the preferred term recommended for practice.[28]

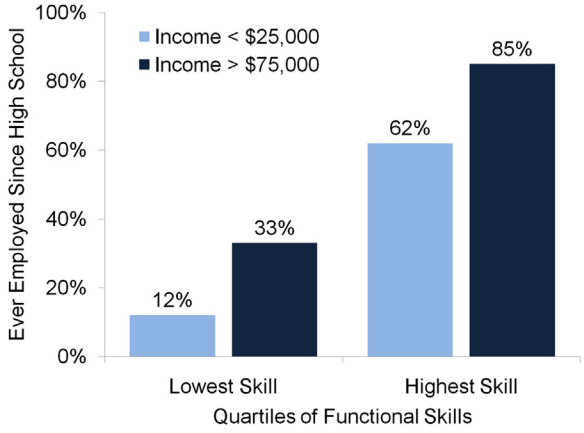
Measures

Employment Outcomes. Data on employment were gathered through a sequence of survey questions that first asked, "At any time since high school did [YOUTH] work for pay other than work around the house?" Participants were also asked "Does [YOUTH] have a paid job now, other than work around the house?" Job type was coded using the U.S. Bureau of Labor's Standardized Occupational Classification (SOC) system for classifying work for pay or profit, excluding volunteer work. The SOC codes use 23 major occupational categories further classified by specific (minor) job types.[29]

Covariates. Demographic variables included age, sex, race, ethnicity, and household income. Overall health status was reported using a 4-point scale ranging from fair/poor to excellent. Conversational impairment was assessed with a question asked of parents: "How well does [YOUTH] carry on a conversation?" Ordinal responses included "Has no trouble," "Has a little trouble," "Has a lot of trouble," or "Doesn't carry on a conversation at all," in which a higher score indicated greater conversational impairment.

A functional skills scale was constructed by summing responses to 8 questions (each with 4-point responses ranging from "not at all well" to "very well"), measured at wave 4 in 2006 to 2007, which assessed the ability to tell time, read and understand common signs, count change, find telephone numbers and use a telephone, navigate to places outside home, use public transportation, purchase clothing at a store, and

FIGURE 1 Marginal estimates of the rate of ever having employment since high school among youth with an autism spectrum disorder, stratified by high and low levels of income and functional skills.



arrange out-of-town travel. The range of possible scores was 8 to 32, with higher scores indicating greater functional skills. This scale was used as a continuous variable in regressions. Categories representing the upper and lower quartiles of the scale scores were used to create Figure 1. Of note, this functional skills scale was not intended to be a proxy for IQ. There was no assessment of IQ in the NLTS2. Functional skills and IQ are distinct constructs.

The rate of missing data for covariates ranged from 0% to 21%. Two variables had more than 10% missing: household income (21%), and the functional skills scale (20%). We used IVEware (version 0.1) to impute 50 implicates through sequential regression for all covariates to prevent bias associated with listwise deletion.[30] We did not impute the employment-dependent variables.

Data Analyses

All analyses were conducted with Stata 12, using survey weighting and appropriate variance adjustment for the complex survey design.[31] Descriptive statistics were estimated for covariates and select employment outcomes for the ASD group (Table 1). We then tested for significant differences in the odds of employment between each group and the ASD group using logistic regression and adjusting for covariates (Table 2). The highest-frequency job types for each disability group were tabulated and rank ordered (Table 3). We used logistic regression to examine the odds of ever having paid employment for the ASD group only (Table 4).

To visually depict the impact of both socioeconomic status and functional abilities, we calculated the estimated percentage of ever having a job for 4 subgroups of youth with an ASD defined by parental household income of less than $25,000 or more than $75,000 and the highest or lowest quartiles of functional skills (Figure 1). This was done using the margins postestimation procedure in Stata after fitting a logistic regression model.[32]

RESULTS

Youth with an ASD were primarily male (85.0%) and white (70.0%), with a mean age of 23.2 years (range = 21–25 years) (Table 1). The majority (87.1%) had been out of high school for more than 2 years. According to parent report, 16.9% could not converse at all.

The probability of employment was independently associated with both higher household income and better functional skills (Figure 1). The majority of young adults (85%) in the highest income/highest skill group had worked for pay since leaving high school, compared with 12% of those in the lowest income/lowest skill group.

TABLE 1 Percentages and 95% CI for Covariates and Employment Outcomes Among Young Adults With an Autism Spectrum Disorder

Variable	Percentage (95% CI)
Male sex	85.0 (79.6–89.2)
Hispanic ethnicity	10.0 (5.9–16.3)
Race	
White	70.0 (62.9–76.2)
African American	18.7 (13.5–25.3)
Mixed/other	11.3 (7.9–16.0)
Household income, $	
≤25,000	16.5 (11.3–23.3)
25,001–50,000	31.5 (25.0–38.8)
50,001–75,000	34.1 (27.3–41.5)
>75,000	17.9 (12.9–24.3)
Conversational ability	
No trouble	12.0 (8.5–16.6)
Little trouble	41.3 (33.7–49.4)
Lots of trouble	29.9 (24.3–36.1)
Not able to converse at all	16.9 (11.7–23.6)
Ever worked for pay since high school	53.4 (44.2–62.5)
Currently employed	33.6 (25.4–41.9)
Employed full-time at current or most recent job	20.9 (13.3–28.6)

Note: National Longitudinal Transition Study–2 (NLTS2), wave 5, 2009. Covariate data (sex, race, ethnicity, income, and conversational ability) are imputed. Number of multiply imputed data sets = 50. Weighted to population levels. Variances adjusted for sampling method.

Of the young adults with an ASD, approximately one-half (53.4%, 95% CI = 44.2–62.5) had ever worked for pay outside the home since leaving high school. A significantly higher ($p < .001$) percentage of young adults in the ED (88.2%, 95% CI = 83.7–92.7), LD (89.8%, 95% CI = 84.8–94.7), and SLI (88.2%, 95% CI = 80.8–95.6) disability categories had ever been employed over the same time span (data not shown). There was no significant difference in ever having worked between the ASD and the MR (62.8%, 95% CI = 56.7–69.0) groups. The stratified rate of employment among those with no trouble conversing was 81.9% (95% CI = 68.2–90.6) and was 18.8% (94% CI = 8.6–36.2) for those with no conversation ability.

Approximately one-third (33.6%, 95% CI = 25.4–41.9) of young adults with an ASD were currently employed at the time of the interview. Among young adults with an ASD who had ever worked for pay since high school, the average number of jobs held was 2.5 (95% CI = 2.1–3.0), significantly fewer ($p < .001$) than for young adults with ED (mean = 4.1, 95% CI = 3.7–4.6),

TABLE 2 Adjusted Odds Ratios (95% CI) of Employment Experiences Among Young Adults With Other Disabilities Compared to Those With Autism, Controlling for Covariates

	Special Education Classification During High School			
	MR	ED	LD	SLI
Employment outcomes				
Ever worked for pay since high school	2.0* (1.1–3.6)	5.2*** (2.7–10.2)	5.4*** (3.0–9.7)	4.8*** (2.6–8.9)
Currently employed	1.5 (0.8–2.6)	1 (0.5–1.7)	2.4** (1.3–4.3)	1.8* (1.1–3.2)
Full-time employee status at current or most recent job	3.8*** (2.0–7.2)	6.9*** (3.5–13.4)	12.0*** (6.1–23.4)	6.7*** (3.5–12.8)

Note: Covariates: sex, age, race, ethnicity, income, overall health, how well youth converses, functional skills. National Longitudinal Transition Study–2 (NLTS2), wave 5, 2009. Weighted to population levels. Variances adjusted for sampling method. ASD = autism spectrum disorder; ED = emotional disturbance; LD = learning disability; MR = mental retardation; SLI = speech/language impairment.

*p < .05, **p < .01, ***p < .001.

LD (mean = 4.0, 95% CI = 3.6–4.4), or SLI (mean = 3.6, 95% CI = 3.2–4.0) ($p < .001$). There was no significant difference in the average number of jobs between the ASD and the MR (mean = 2.8, 95% CI = 2.4–3.1) groups.

Approximately one-fifth of youth with an ASD (20.9%, 95% CI = 13.3–28.6) worked full-time (>35 hours per week) at their current or most recent job, which was about one-half the rate of the MR group (39.7%, 95% CI = 32.0–47.4, $p < .01$) and about one-third of the rate of the ED (61.3%, 95% CI = 54.3–68.2, $p < .001$), LD (73.3%, 95% CI = 67.5–79.0, $p < .001$), and SLI (62.8%, 95% CI = 56.4–69.2, $p < .001$) disability groups. Young adults with an ASD earned a mean wage of $8.10 U.S. dollars per hour (95% CI = 6.20–9.90) at their full-time jobs, which was significantly lower ($p < .01$) than members of the ED ($11.90, 95% CI = 10.60–13.10), LD ($11.20, 95% CI = 10.40–12.00), and SLI ($12.00, 95% CI = 10.70–13.20) groups. There was no significant difference in mean wages between the ASD and the MR ($9.20, 95% CI = 8.10–10.30) groups. Additional analysis revealed no significant differences across disability groups in receipt of job-related health insurance benefits.

After adjusting for covariates (including functional skills and conversational ability) the odds of ever having worked for pay since high school were significantly higher for each group compared with the ASD group (Table 2). The adjusted odds of having full-time employment at a current or most recent job were nearly 4 times higher for young adults with MR ($p < .001$), and 6 to 12 times higher for young adults with ED, LD, and SLI ($p < .001$).

The top 5 occupational classifications for young adults with an ASD or MR accounted for approximately 70% to 80% of all employment at the current or most recent job (Table 3). Young adults with an ASD were most frequently employed in office and administrative support occupations, which most often involved material recording, scheduling, dispatching, and distributing. Transportation and materials moving jobs also were common, as was production work involving assembly, food processing, or work in factories. Other frequent job types included food preparation and serving, and building or grounds cleaning and maintenance. Job types within the ED, LD, and SLI groups were more variable and were less clustered within each group's top 5 occupational classifications.

Young adults with an ASD had higher odds of ever having had a paid job if they were older (odds ratio [OR] = 1.3, 95% CI = 1.0–1.6; $p < .05$), were from higher-income households (OR = 1.1, 95% CI = 1.0–1.3; $p < .05$), or had higher functional skills (OR = 1.1, 95% CI = 1.0–1.1; $p < .01$) (Table 4). Greater conversational impairment was associated with lower odds of ever having had a paid job (OR = 0.5, 95% CI = 0.3–0.7; $p < .001$).

DISCUSSION

We examined key characteristics of postsecondary employment for young adults with an ASD during the first decade after high school, and compared these outcomes with those of young adults with different disabilities. A little more than one-half of young adults with an ASD had ever had a paid job outside the home since high

TABLE 3 Rank-Ordered Major Standard Occupational Classifications Among Postsecondary Young Adults at Their Current or Most Recent Job by Disability Group, Percentage (95% CI)

Rank	ASD	MR	ED	LD	SLI
1	Office, 18.5 (11.3–25.8)	Food prep, 25.3 (17.3–33.3)	Food prep, 19.9 (13.0–26.8)	Sales, 13.1 (8.0–18.2)	Sales, 12.1 (7.6–16.7)
2	Transport, 13.8 (3.6–24.0)	Production, 19.1 (10.9–27.2)	Construction, 11.5 (5.1–17.8)	Food prep, 10.8 (6.2–15.4)	Food prep, 10.5 (6.2–14.9)
3	Production, 13.4 (7.3–19.4)	Cleaning, 14.3 (8.2–20.5)	Cleaning, 9.8 (4.5–15.1)	Service, 9.4 (4.4–14.3)	Office, 10.3 (6.0–14.6)
4	Food prep, 12.1 (6.4–17.7)	Office, 10.0 (3.7–16.3)	Sales, 9.0 (4.2–13.9)	Construction, 9.4 (4.9–13.8)	Transport, 8.5 (4.4–12.5)
5	Cleaning, 11.6 (5.2–18.1)	Transport, 9.4 (3.3–15.4)	Installation, 7.7 (2.4–12.9)	Transport, 8.1 (4.5–11.7)	Service, 8.2 (4.7–11.7)
6	Other 30.6 (20.6–40.6)	Other 22.0 (15.0–29.0)	Other 42.1 (33.4–50.8)	Other 49.3 (41.0–57.6)	Other 50.4 (44.3–56.4)

Note: National Longitudinal Transition Study–2 (NLTS2), wave 5, 2009. Unimputed data. ASD = autism spectrum disorder; Cleaning = building and grounds maintenance; ED = emotional disturbance; Food prep = food preparation and serving; Installation = installation and maintenance; LD = learning disability; MR = mental retardation; Office = office and administrative; Production = production and factory; Service = personal care and service; SLI = speech/language impairment; Transport = transportation and material moving.

school, roughly equivalent to recently published rates.[1,3] However, the covariate-adjusted odds of ever having worked for pay since high school were significantly higher for young adults in the MR, ED, LD, and SLI comparison groups. Better outcomes for young adults from comparison disability types with similar demographic and disability characteristics highlight the achievement gap facing youth with ASDs.

In their current or most recent jobs, the vast majority of young adults with an ASD were working less than full-time, with average earnings of about $8.10 per hour, significantly lower than average wages for young adults in the comparison groups. Our findings also suggested that young adults with an ASD had fewer jobs and less variation in types of jobs than young adults in comparison groups.

Young adults with an ASD who had higher functional skills, better conversational abilities, and higher household incomes were more likely to achieve employment. Higher household income in this population also corresponds to higher rates of service use,[33] and is a marker for social capital—a construct that entails resources to which an individual has access through a network of social relations.[34,35] Social capital is associated with higher rates of employment in the general population.[36]

Even among individuals with an ASD who had the most impaired conversational abilities, approximately one-fifth did become employed. This highlights that employment is potentially feasible even among those with high levels of impairment.

This study had some notable limitations. The age range of participants creates variation in the length of time that young adults had been out of school. Thus, data on variables such as "number of jobs held," which is partly a function of time since high school, need to be interpreted with caution. An additional limitation was the absence of measures of IQ or psychiatric symptoms in the NLTS2. This made it impossible to identify comorbid conditions or to establish their influence on outcomes. The functional skills and conversation impairment survey questions provided some measure of disability severity with high face validity, but have not been validated with the rigor of normed clinical measures.

The results of this study generalize to young adults who were 13 to 16 years of age and were receiving special education under the autism

TABLE 4 Logistic Regression Model of Having Ever Worked for Pay Since High School Among Young Adults With an Autism Spectrum Disorder

Covariate	Odds Ratio (95% CI)
Sex (female)	1.6 (0.8–3.1)
Age	1.3* (1.0–1.6)
Hispanic ethnicity	0.5 (0.2–1.1)
Race (vs. white)	
African American	0.6 (0.3–1.4)
Mixed/other	1 (0.4–2.6)
Parent or guardian household income, $10,000 increments	1.1* (1.0–1.3)
Overall health	1 (0.7–1.4)
Conversational impairment	0.5*** (0.3–0.7)
Functional skills	1.1** (1.0–1.1)

Note: National Longitudinal Transition Study–2 (NLTS2), Wave 5, 2009. Variances adjusted for sampling method. Number of multiply imputed data sets = 50.
*p < .05, **p < .01, ***p < .001.

category in 2000. It is impossible to estimate precisely the degree to which results generalize to the entire population of young adults with an ASD. To the best of our knowledge, there have been no U.S. studies of young adults with an ASD that are perfectly representative of the entire ASD population. Indeed, devising a sampling procedure that would result in a perfectly representative sample of the entire autism spectrum in young adulthood is difficult to conceive. However, the characteristics of the population represented by our sample approximated the male:female ratio, race, and ethnicity distributions of other recent population-based research on autism.[37,38] The wide range of conversational and functional skills impairments in the NLTS2 ASD population is consistent with the spectrum of diagnostic features that characterize ASD diagnosis.[39]

We excluded cases from comparison groups if a parent reported that the youth had ever received an ASD diagnosis. However, it is still possible that some youth in comparison groups would also meet diagnostic criteria for an ASD if clinically assessed. If this were the case, then it is possible that observed group differences might actually underestimate the true magnitude of group differences. As this study was based on secondary survey data, we had no way to go back and contact participants for additional data collection using more rigorous clinical measures.

This study also has several strengths. First, it used nationally representative estimates from a large and recent sample of young adults with an ASD at 21 to 25 years of age. Second, comparison with young adults with other types of disabilities helps to contextualize the findings for ASDs. Third, this study provided results stratified by income and functional skills, providing baseline data on employment in vulnerable subpopulations of young adults with an ASD. Awareness and monitoring of these vulnerable subgroups is critical, as they may be in need of additional services and support to successfully transition into postsecondary employment. Finally, NLTS2 incorporated rigorous methods for developing, field testing, and administering survey protocols, and extensively analyzed and adjusted for nonresponse bias in the sample at each wave of data collection.

Our findings of consistently poorer outcomes for young adults with an ASD relative to other disability groups suggest that this population is experiencing particular difficulty in successfully transitioning to employment in the first years after high school. Overall, patterns of low employment rates, hours worked, and wages, in conjunction with limited job types, appear to be insufficient for supporting independent living for young adults with an ASD. Thus, families likely are bearing increased financial burden for continued dependent support.

This study confirms that employment can occur for many young adults with an ASD over the initial years after school, even for those with severe levels of impairment. Strategies for improving employment outcomes might include providing work experiences during high school,[40] deliberate matching of capabilities to job types during transition planning,[41] attention to the vulnerabilities of disadvantaged youth with ASD during and beyond transition,[2] and increased flexibility of institutional supports provided over the occupational life course.[19] Given expectations for continued growth in the number of young adults with an ASD entering adulthood, urgent consideration should be given to employment preparation that is consistent with the current labor market, to facilitate employment and to minimize costs to society. Future research is needed to determine how training

provided during secondary school, in addition to postsecondary vocational education and rehabilitation services, may contribute to future employment. &

CG Clinical Guidance

- About one-half of young adults with an autism spectrum disorder (ASD) work for pay within the first 8 years after high school.
- The postsecondary employment rate for young adults with an ASD is lower than the rate seen in other disability groups, even after controlling for household income, severity, and other factors.
- The odds of ever having worked for pay were higher for young adults with an ASD who were from higher-income households, and who had better conversational abilities or functional skills.

Accepted June 7, 2013.

Ms. Roux, Dr. Shattuck, and Mr. Cooper are with the George Warren Brown School of Social Work at Washington University in St. Louis. Ms. Anderson is with the Waisman Center at the University of Wisconsin—Madison. Dr. Wagner is with SRI International. Dr. Narendorf is with the Graduate College of Social Work at the University of Houston.

This work was supported by funding to Dr. Shattuck from the Emch Foundation, Autism Speaks, and the National Institute of Mental Health (NIMH; R01 MH086489). Its contents are solely the responsibility of the authors and do not represent the views of NIMH or other funders.

Mr. Cooper and Drs. Shattuck and Wagner served as the statistical experts for this research.

Disclosure: Drs. Shattuck, Wagner, and Narendorf, Ms. Roux, Mr. Cooper, and Ms. Anderson report no biomedical financial interests or potential conflicts of interest.

Correspondence to Anne M. Roux, M.P.H., Washington University in St. Louis, One Brookings Drive, Campus Box 1196, St. Louis, MO 63130; e-mail: aroux@wustl.edu

0890-8567/$36.00/©2013 American Academy of Child and Adolescent Psychiatry

http://dx.doi.org/10.1016/j.jaac.2013.05.019

REFERENCES

1. Shattuck PT, Narendorf SC, Cooper B, Sterzing PR, Wagner M, Taylor JL. Postsecondary education and employment among youth with an autism spectrum disorder. Pediatrics. 2012;129:1042-1049.
2. Shattuck PT, Roux AM, Hudson LE, Lounds Taylor J, Maenner MJ, Trani J-F. Services for adults with an autism spectrum disorder. Can J Psychiatry. 2012;57:284-291.
3. Howlin P, Moss P. Adults with autism spectrum disorders. Can J Psychiatry. 2012;57:275-283.
4. Carbone PS, Behl DD, Azor V, Murphy NA. The medical home for children with autism spectrum disorders: parent and pediatrician perspectives. J Autism Dev Disord. 2010;40:317-324.
5. American Academy of Pediatrics, American Academy of Family Physicians, American College of Physicians-American Society of Internal Medicine. A consensus statement on health care transitions for young adults with special health care needs. Pediatrics (Evanston). 2002;110:1304-1306.
6. Rosen DS, Blum RW, Britto M, Sawyer SM, Siegel DM. Transition to adult health care for adolescents and young adults with chronic conditions: position paper of the Society for Adolescent Medicine. J Adolesc Health. 2003;33:309-311.
7. Kessler RC, Berglund P, Demier O, Jin R, Merikangas KR, Walters EE. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry. 2005;62:593-602.
8. Costello EJ, Mustillo S, Erkanli A, Keeler G, Angold A. Prevalence and development of psychiatric disorders in childhood and adolescence. Arch Gen Psychiatry. 2003;60:837-844.
9. Fussell E, Furstenberg FJ. The transition to adulthood during the twentieth century: race, nativity, and gender. In: Setterson RA. Jr, Furstenberg FF. Jr, Rumbaut RG, eds. On the Frontier to Adulthood: Theory, Research, and Public Policy. Chicago: University of Chicago Press; 2005:29-75.
10. U. S. Department of Education. Individuals with Disabilities Education Act. PL 108-446, Section 1400(c)(14); 2004.
11. Wehman PH. Employment for persons with disabilities: where are we now and where do we need to go? J Vocat Rehabil. 2011;35:145-151.
12. Taylor P, Parker K, Kochhar R, *et al*. Young, underemployed, and optimistic: coming of age, slowly, in a tough economy. Washington, DC: Pew Research Center. Available at: www.pewsocialtrends.org. Accessed May 4, 2013.
13. Bureau of Labor Statistics. America's young adults at 25: school enrollment, number of jobs held and labor market activity: results from a longitudinal survey. Washington DC: U.S. Department of Labor; 2013. Available at: www.bls.gov/nls. Accessed May 4, 2013.
14. Newman L, Wagner M, Knokey A-M, *et al*. The post-high school outcomes of young adults with disabilities up to 8 years after high school. A report from the National Longitudinal Transition Study—2 (NLTS2) (NCSER 2011-3005). Menlo Park, CA: SRI International; 2011.
15. Eaves LC, Ho HH. Young adult outcome of autism spectrum disorders. J Autism Dev Disord. 2008;38:739-747.
16. Taylor JL, Seltzer MM. Employment and post-secondary educational activities for young adults with autism spectrum disorders during the transition to adulthood. J Autism Dev Disord. 2011;41:566-574.
17. Taylor JL, Seltzer MM. A systematic review of vocational interventions for young adults with autism spectrum disorders. Pediatrics (Evanston). 2012;130:531-538.
18. Howlin P, Alcock J, Burkin C. An 8 year follow-up of a specialist supported employment service for high-ability adults with autism or Asperger syndrome. Autism. 2005;9:533-549.
19. Barnhill GP. Outcomes in adults with Asperger syndrome. Focus Autism Other Dev Disabil. 2007;22:116-126.
20. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 4th ed., text rev. Washington, DC: Author; 2000.
21. Yeargin-Allsopp M, Rice C, Karapurkar T, Doernberg N, Boyle C, Murphy C. Prevalence of autism in a US metropolitan area. J Am Med Assoc. 2003;289:49-55.
22. Bertrand J, Mars A, Boyle C, Bove F, Yeargin-Allsopp M, Decoufle P. Prevalence of autism in a United States population: the Brick Township, New Jersey, investigation. Pediatrics (Evanston). 2001;108:1155-1161.
23. Javitz H, Wagner M. Analysis of potential bias in the sample of local education agencies (LEAs) in the National Longitudinal Transition Study—2 (NLTS2) sample. Menlo Park, CA: SRI International, for Office of Special Education Programs, U.S. Department of Education; 2003:54.
24. Javitz H, Wagner M. Analysis of potential bias in the wave 1 and wave 2 respondents to the National Longitudinal Transition Study—2 (NLTS2) sample. Menlo Park, CA: SRI International, for Office of Special Education Programs, U.S. Department of Education; 2005.
25. Wagner M, Kutash K, Duchnowski AJ, Epstein MH. The Special Education Elementary Longitudinal Study (SEELS) and the National Longitudinal Transition Study (NLTS2): Study designs and implications for children and youth with emotional disturbances. J Emot Behav Disord. 2005;13:25-41.
26. Cameto R, Wagner M, Newman L, Blackorby J, Javitz H. National Longitudinal Transition Study II (NLTS2) study design, timeline, and data collection. Menlo Park, CA: SRI International;

2000b:20. Available at: http://nlts2.org/studymeth/nlts2_sampling_plan2.pdf. Accessed May 4, 2013.
27. Cameto R, Wagner M, Newman L, Blackorby J, Javitz H. National Longitudinal Transition Study II (NLTS2) sampling plan. Menlo Park, CA: SRI International; 2000a:20. Available at: http://nlts2.org/studymeth/nlts2_sampling_plan2.pdf. Accessed May 4, 2013.
28. Schalock RL, Luckasson RA, Shogren KA. The renaming of mental retardation: Understanding the change to the term intellectual disability. Intellect Dev Disabil. 2007;45:116-124.
29. Bureau of Labor Statistics. Standard occupational classification (SOC) User Guide. Washington DC: U.S. Department of Labor; 2000. Available at: www.bls.gov. Accessed May 4, 2013.
30. Raghunathan TE, Solenberger PW, Van Hoewyk J. IVEware: imputation and variance estimation software. Ann Arbor, MI: Survey Methodology Program, Survey Research Center, Institute for Social Research, University of Michigan; 2002.
31. StataCorp. Stata: Release 12. Statistical Software. College Station, TX: StataCorp LP; 2011.
32. StataCorp. Stata Base Reference Manual. Volume 2, G-M, Release 12. Stata Press; 2011.
33. Shattuck PT, Wagner M, Narendorf SC, Sterzing PR, Hensley M. Post-high school service use among young adults with an autism spectrum disorder. Arch Pediat Adol Med. 2011;165:141-146.
34. Coleman JS. Social capital in the creation of human capital. Am J Sociol. 1994;94 (Suppl):S95-S120.
35. Adler PS, Kwon S-W. Social capital: prospects for a new concept. Acad Manage Rev. 2002;27:17-40.
36. Granovetter M. Getting a Job: a Study of Contacts and Careers. Chicago: University of Chicago Press; 1995.
37. Levy SE, Giarelli E, Lee L-C, *et al*. Autism spectrum disorder and co-occurring developmental, psychiatric, and medical conditions among children in multiple populations of the United States. J Dev Behav Pediatr. 2010;31:267-275.
38. Schieve LA, Rice C, Yeargin-Allsopp M, *et al*. Parent-reported prevalence of autism spectrum disorders in US-born children: an assessment of changes within birth cohorts from the 2003 to the 2007 National Survey of Children's Health. Matern Child Health J. 2012;16:151-157.
39. Johnson CP, Myers SM. Identification and evaluation of children with autism spectrum disorders. Pediatrics. 2007;120:1183-1215.
40. Hendricks DR, Wehman PH. Transition from school to adulthood for youth with autism spectrum disorders. Focus Autism Other Dev Disabil. 2009;24:77-88.
41. McDonough JT, Revell G. Accessing employment supports in the adult system for transitioning youth with autism spectrum disorders. J Vocat Rehabil. 2010;32:89-100.

Prevalence and Characteristics of Autism Spectrum Disorder Among Children Aged 8 Years — Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2020

Matthew J. Maenner, PhD[1]; Zachary Warren, PhD[2]; Ashley Robinson Williams, PhD[1,3]; Esther Amoakohene, MPH[1]; Amanda V. Bakian, PhD[4]; Deborah A. Bilder, MD[4]; Maureen S. Durkin, DrPH, PhD[5]; Robert T. Fitzgerald, PhD[6]; Sarah M. Furnier, MS[5]; Michelle M. Hughes, PhD[1]; Christine M. Ladd-Acosta, PhD[7]; Dedria McArthur, MPH[1]; Elise T. Pas, PhD[7]; Angelica Salinas, MS[5]; Alison Vehorn, MS[2]; Susan Williams[1]; Amy Esler, PhD[8]; Andrea Grzybowski, MS[9]; Jennifer Hall-Lande, PhD[8]; Ruby H.N. Nguyen, PhD[8]; Karen Pierce, PhD[9]; Walter Zahorodny, PhD[10]; Allison Hudson[11]; Libby Hallas, MS[8]; Kristen Clancy Mancilla[12]; Mary Patrick, MPH[1]; Josephine Shenouda, DrPH[10]; Kate Sidwell[10]; Monica DiRienzo, MA[1]; Johanna Gutierrez[4]; Margaret H. Spivey[7]; Maya Lopez, MD[11]; Sydney Pettygrove, PhD[12]; Yvette D. Schwenk, MS[11]; Anita Washington, MPH[1]; Kelly A. Shaw, PhD[1]

[1]National Center on Birth Defects and Developmental Disabilities, CDC, Atlanta, Georgia; [2]Vanderbilt University Medical Center, Nashville, Tennessee; [3]Oak Ridge Institute for Research and Education, Oak Ridge, Tennessee; [4]University of Utah School of Medicine, Salt Lake City, Utah; [5]University of Wisconsin, Madison, Wisconsin; [6]Washington University, St. Louis, Missouri; [7]Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland; [8]University of Minnesota, Minneapolis, Minnesota; [9]University of California, San Diego, California; [10]Rutgers New Jersey Medical School, Newark, New Jersey; [11]University of Arkansas for Medical Sciences, Little Rock, Arkansas; [12]University of Arizona, Tucson, Arizona

Abstract

Problem/Condition: Autism spectrum disorder (ASD).

Period Covered: 2020.

Description of System: The Autism and Developmental Disabilities Monitoring (ADDM) Network is an active surveillance program that provides estimates of the prevalence of ASD among children aged 8 years. In 2020, there were 11 ADDM Network sites across the United States (Arizona, Arkansas, California, Georgia, Maryland, Minnesota, Missouri, New Jersey, Tennessee, Utah, and Wisconsin). To ascertain ASD among children aged 8 years, ADDM Network staff review and abstract developmental evaluations and records from community medical and educational service providers. A child met the case definition if their record documented 1) an ASD diagnostic statement in an evaluation, 2) a classification of ASD in special education, or 3) an ASD *International Classification of Diseases* (ICD) code.

Results: For 2020, across all 11 ADDM sites, ASD prevalence per 1,000 children aged 8 years ranged from 23.1 in Maryland to 44.9 in California. The overall ASD prevalence was 27.6 per 1,000 (one in 36) children aged 8 years and was 3.8 times as prevalent among boys as among girls (43.0 versus 11.4). Overall, ASD prevalence was lower among non-Hispanic White children (24.3) and children of two or more races (22.9) than among non-Hispanic Black or African American (Black), Hispanic, and non-Hispanic Asian or Pacific Islander (A/PI) children (29.3, 31.6, and 33.4 respectively). ASD prevalence among non-Hispanic American Indian or Alaska Native (AI/AN) children (26.5) was similar to that of other racial and ethnic groups. ASD prevalence was associated with lower household income at three sites, with no association at the other sites.

Across sites, the ASD prevalence per 1,000 children aged 8 years based exclusively on documented ASD diagnostic statements was 20.6 (range = 17.1 in Wisconsin to 35.4 in California). Of the 6,245 children who met the ASD case definition, 74.7% had a documented diagnostic statement of ASD, 65.2% had a documented ASD special education classification, 71.6% had a documented ASD ICD code, and 37.4% had all three types of ASD indicators. The median age of earliest known ASD diagnosis was 49 months and ranged from 36 months in California to 59 months in Minnesota.

Among the 4,165 (66.7%) children with ASD with information on cognitive ability, 37.9% were classified as having an intellectual disability. Intellectual disability was present among 50.8% of Black, 41.5% of A/PI, 37.8% of two or more races, 34.9% of Hispanic, 34.8% of AI/AN, and 31.8% of White children with ASD. Overall, children with intellectual disability had earlier median ages of ASD diagnosis (43 months) than those without intellectual disability (53 months).

Interpretation: For 2020, one in 36 children aged 8 years (approximately 4% of boys and 1% of girls) was estimated to have ASD. These estimates are higher than previous ADDM Network estimates during 2000–2018. For the first time among children aged 8 years, the prevalence of ASD was

Corresponding author: Matthew J. Maenner, National Center on Birth Defects and Developmental Disabilities, CDC. Telephone: 404-498-3072; Email: xde8@cdc.gov.

lower among White children than among other racial and ethnic groups, reversing the direction of racial and ethnic differences in ASD prevalence observed in the past. Black children with ASD were still more likely than White children with ASD to have a co-occurring intellectual disability.

Public Health Action: The continued increase among children identified with ASD, particularly among non-White children and girls, highlights the need for enhanced infrastructure to provide equitable diagnostic, treatment, and support services for all children with ASD. Similar to previous reporting periods, findings varied considerably across network sites, indicating the need for additional research to understand the nature of such differences and potentially apply successful identification strategies across states.

Introduction

Autism spectrum disorder (ASD) is a developmental disability characterized by persistent impairments in social interaction and the presence of restricted, repetitive patterns of behaviors, interests, or activities (*1*) that can cause a wide array of difficulties in social interaction, communication, and participation in daily activities. CDC began monitoring the prevalence of ASD in metropolitan Atlanta, Georgia, in 1996 as part of its Metropolitan Atlanta Developmental Disabilities Surveillance Program (*2*). CDC established the Autism and Developmental Disabilities Monitoring (ADDM) Network in 2000 and used the model developed in metropolitan Atlanta to track ASD prevalence in additional areas of the country. Starting with the 2000 surveillance year, the ADDM Network has reported ASD prevalence for even-numbered years (*3*–*12*). This is the 11th surveillance summary published in *MMWR* and marks a period of 20 years of monitoring ASD in multiple U.S. communities.

During the past two decades, ASD prevalence estimates of children aged 8 years from the ADDM Network have increased markedly, from 6.7 (one in 150) per 1,000 in 2000 to 23.0 (one in 44) in 2018 (*3*,*12*). In addition, overall ASD prevalence among White children was 50% higher than among Black or African American (Black) or Hispanic children in earlier years. (Persons of Hispanic origin might be of any race but are categorized as Hispanic; all racial groups are non-Hispanic). These gaps narrowed over time until ASD prevalence among Black and Hispanic matched prevalence among White children for the first time in 2016 and 2018, respectively (*11*,*12*). Similarly, robust associations between autism prevalence and higher socioeconomic status were observed in ADDM Network sites during 2002–2010 (*13*); however, this association was much more variable in 2018 (*12*). These patterns have largely been interpreted as improvements in more equitable identification of ASD, particularly for children in groups that have less access or face greater barriers in obtaining services (including diagnostic evaluations). However, consistent disparities for co-occurring intellectual disability exist because among all children with ASD, Black children have the largest proportion identified with intellectual disability (*10*–*12*).

This report describes ASD prevalence and characteristics among children aged 8 years from 11 ADDM Network sites in 2020, including prevalence by site and demographic characteristics, median ages when children with ASD were first evaluated or identified, and the co-occurrence of intellectual disability. These data can be used by service providers, educators, communities, researchers, and policymakers to track trends and support efforts to ensure the equitable allocation of needed services and support for all children with ASD.

Methods

Surveillance Sites and Procedures

For 2020, the ADDM Network included 11 sites (Arizona, Arkansas, California, Georgia, Maryland, Minnesota, Missouri, New Jersey, Tennessee, Utah, and Wisconsin) that monitored ASD prevalence. Each site selected a geographic area of its state to monitor ASD among children aged 8 years (Table 1). Children included in this report were born in 2012 and lived in surveillance areas of the 11 sites during 2020. Sites were competitively funded and functioned as public health authorities under the Health Insurance Portability and Accountability Act of 1996 Privacy Rule and met applicable local institutional review board, privacy, and confidentiality requirements under 45 CFR 46 (*14*).

Case Ascertainment and Surveillance Case Definition

The ADDM Network conducts active surveillance of ASD by using multiple sources of information within a community (Table 1). The methods for collecting information and the case definition were unchanged from the 2018 surveillance year (*12*) and were modeled after those developed by CDC's Metropolitan Atlanta Developmental Disabilities Surveillance Program (*3*). Sites request records from community medical, education, and service providers containing specific billing codes from the *International Classification of Diseases, Ninth Revision* (ICD-9) or *International Classification of Diseases, Tenth Revision* (ICD-10) or special education classification. The protocol allowed each site to select the ICD codes that

necessitate record review if those codes closely aligned with program-recommended ICD codes (*11*). All ADDM Network sites used records from medical service providers that evaluated children with developmental disabilities and, for the first time, all sites had at least partial access to public school education records (Table 1). ADDM Network sites received information (including demographic data and ICD codes or special education classifications) for children with one or more of the requested codes or classifications, and ADDM staff manually reviewed the contents of associated (electronic and paper-based) records when available. If any part of the child's record contained information meeting the case definition, ADDM staff abstracted information from the child's developmental evaluations, special education plans, and other documents (e.g., cognitive or IQ tests) from all data sources. At certain sites, full record review could not be completed for all records because of the COVID-19 pandemic or other restrictions on physically accessing the location where records were stored (Table 1).

Children met the ASD case definition if they were aged 8 years in 2020 (born in 2012), lived in the surveillance area for at least 1 day during 2020, and had documentation in their records that they ever received 1) a written ASD diagnostic statement from a qualified professional, 2) a special education classification of autism (either primary exceptionality of ASD or an evaluation reporting criterion for autism eligibility was met) in public school, or 3) an ASD ICD code (ICD-9 codes between 299.00 and 299.99 or ICD-10 codes in the F84 range except for F84.2, Rett syndrome) obtained from administrative or billing information. Five children had an ICD code for Rett syndrome (F84.2) and no other indicators of ASD and did not meet the ASD case definition. ASD-related diagnostic conclusions (including suspected ASD or ruled out ASD) were collected verbatim from evaluations and were reviewed and classified by ADDM Network staff with clinical expertise at each site.

Additional Data Sources and Variable Definitions

Population denominators were obtained from the U.S. Census vintage 2021 county-level single-year-of-age postcensal population estimates for 2020 (*15*). In this report, the Asian and Native Hawaiian or other Pacific Islander categories were combined into Asian or Pacific Islander because current systems often combine these categories or are not explicit whether "Asian" at a given data source includes "Native Hawaiian or other Pacific Islander." Population denominators include categories for American Indian or Alaska Native (AI/AN), Asian or Pacific Islander (A/PI), Black, White, two or more races, and Hispanic ethnicity. In previous ADDM Network reports, the denominator data were based on the National Center for Health Statistics postcensal bridged race estimates (also produced by the Census Bureau) (*16*); the bridged race data set did not include a category for two or more races, which increased counts in the other categories.

Surveillance areas at three sites (Arizona, California, and Minnesota) comprised subcounty school districts. For these sites, county population estimates were adjusted using the National Center for Education Statistics public school enrollment counts and the American Community Survey tract-level ages 5–9 years population estimates described previously (*12*). The primary race and ethnicity and sex information came from medical or education records and, when missing, was augmented by birth certificate linkages (among children born in the state of their residence at age 8 years), administrative, or billing information. Children with missing or unknown race or ethnicity information were excluded from race- and ethnicity-specific prevalence estimates.

Census tract-level median household income (MHI) was measured using the 2020 American Community Survey 5-year estimates (*17*). Population counts of children aged 8 years were estimated by dividing the number of children aged 5–9 years by five for each census tract. The tracts included in the surveillance areas were classified into three approximately equal-sized population groups (i.e., tertiles) of low, medium, and high MHI by using data from all sites. Children meeting the ADDM Network case definition for ASD were geocoded and assigned to a socioeconomic status (SES) group corresponding to their 2020 address. Census tract information was available for 96.0% of children; the remainder could not be linked to a census tract but had service receipt or school attendance that indicated study area residence.

A child was classified as having intellectual disability if they had an IQ score ≤70 on their most recent cognitive test or intellectual disability was indicated in a statement in a developmental evaluation from a qualified professional. Children were classified in the borderline range for IQ if the score on their most recent test was 71–85, and in the average or higher range with most recent IQ score >85 or with a statement their IQ was in the average range without a specific score. Age at first developmental evaluation was limited to children with information on the earliest collected or historically reported evaluation. Age at first ASD diagnosis was based on the earliest documented age when a qualified professional diagnosed ASD.

Analytic Methods

Prevalence was calculated as the number of children with ASD per 1,000 children in the defined population or group. Overall ASD prevalence estimates included all children with

ASD from all 11 sites. Prevalence also was stratified by sex and by race and ethnicity using both the U.S. Census postcensal population estimates as well as the National Center for Health Statistics postcensal bridged race denominators. The Wilson score method was used to calculate 95% CIs. Pearson chi-square tests were used to compare proportions, and the Mantel-Haenszel (Woolf) test of homogeneity compared prevalence ratios across sites. Permutation tests were conducted to test differences in medians. Cochran Armitage tests were used to detect trends in prevalence across SES tertiles. Prevalence estimates with a relative SE >30% (and ratios calculated from those estimates) were considered to have limited statistical precision and were suppressed. Statistical tests with p values <0.05 and prevalence ratio 95% CIs that excluded 1.0 were considered statistically significant. R software (version 4.2; R Foundation) and additional packages were used to conduct analyses (*12*).

Results

ASD Prevalence

The overall ASD prevalence per 1,000 children aged 8 years was 27.6 (one in 36) and ranged from 23.1 in Maryland to 44.9 in California (Table 2). The overall male-to-female prevalence ratio was 3.8, with overall ASD prevalence of 43.0 among boys and 11.4 among girls. The same sites conducted ASD surveillance in 2018 and reported a combined prevalence of 23.0; however, certain sites changed their geographic areas or access to data sources for the current reporting period (Supplementary Table 1, https://stacks.cdc.gov/view/cdc/124397). The two sites with the largest relative changes (Missouri [48.5%] and Wisconsin [49.5%]) from 2018 to 2020 had increased access to education records in 2020 but no change in the geographic areas.

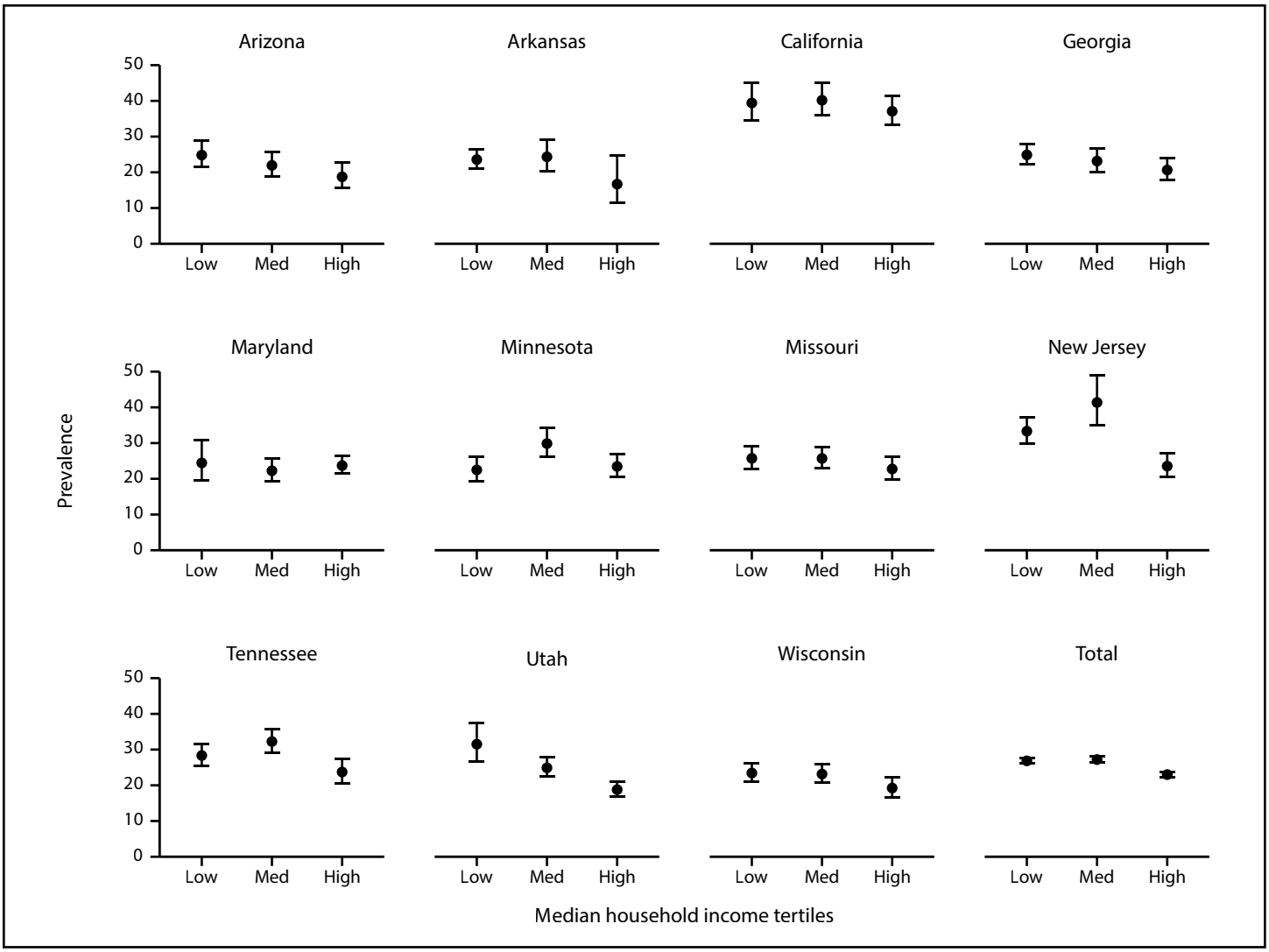
Overall, ASD prevalence per 1,000 children aged 8 years differed by racial and ethnic groups (Table 3); prevalence among White children (24.3) was lower than prevalence among Black, Hispanic, or A/PI children (29.3, 31.6, and 33.4, respectively). Among AI/AN children, ASD prevalence was 26.5 overall and was similar to other groups, but estimates met the 30% relative SE threshold for statistical precision in just one site (Arizona). ASD prevalence among children of two or more races was 22.9, which was not different than among White children but was lower than prevalence among AP/I, Black, and Hispanic children. Missouri was the only site in which White children had higher ASD prevalence than another racial or ethnic group (White compared with two or more races). Additional prevalence ratios comparing racial and ethnic groups are available (Supplementary Table 2, https://stacks.cdc.gov/view/cdc/124397). Prevalence calculations using the bridged-race denominator racial and ethnic categories used in previous reports (Supplementary Table 3, https://stacks.cdc.gov/view/cdc/124397) yielded similar findings of lower ASD prevalence among White children compared with that among Asian, Black, and Hispanic children.

In eight sites, ASD prevalence was not associated with census tract-level MHI, but in three sites (Arizona, New Jersey, and Utah), lower ASD prevalence was observed among children living in census tracts with higher MHI (Figure 1). When all sites were combined, prevalence of ASD was lower among census tracts with higher MHI; however, ASD prevalences for the low, medium, and high SES tertiles were all between 23.0–27.2.

ASD Identification

The percentage of children with diagnostic statements, special education classifications, and ICD codes varied by site (Table 4). Across sites, the percentage of children with ASD who had a documented ASD diagnostic statement was 74.7% overall (range = 60.9% in Wisconsin to 94.7% in New Jersey). ASD prevalence per 1,000 children aged 8 years based exclusively on documented ASD diagnostic statements was 20.6 overall (range = 17.1 in Wisconsin to 35.4 in California) (Figure 2). The overall percentage of children with ASD who had a documented ASD special education classification was 65.2% (range = 44.9% in Utah to 84.9% in Minnesota) (Table 4). The percentage of children with ASD who had a documented ICD code was 71.6% (range = 51.9% in Minnesota to 82.7% in California). A majority of (74.2%) children with ASD had at least two of the three types of ASD identification documented in their records (i.e., ASD diagnostic statement, special education classification, and ASD ICD code) and 37.4% had all three (Figure 3). A majority of children with an ICD code (89.5% of 4,472 children) also had a documented ASD diagnostic statement or ASD special education classification; among all children with ASD, few (7.5% of 6,245 children) met the case definition through having an ICD code only. A majority of children with documents indicating an ASD diagnosis or ASD special education classification had these mentioned multiple times in their records (overall median number of diagnoses documented: two; range: one in Tennessee to six in New Jersey; overall median special education classifications documented: four, site-specific medians ranging from two in Wisconsin and Tennessee to six in California) (Supplementary Table 4, https://stacks.cdc.gov/view/cdc/124397).

FIGURE 1. Prevalence* of autism spectrum disorder among children aged 8 years, by median household income tertile and site — Autism and Developmental Disabilities Monitoring Network, 11 sites, United States, 2020†



* Per 1,000 children aged 8 years. Dots are the point estimates and horizontal lines are the 95% CIs.

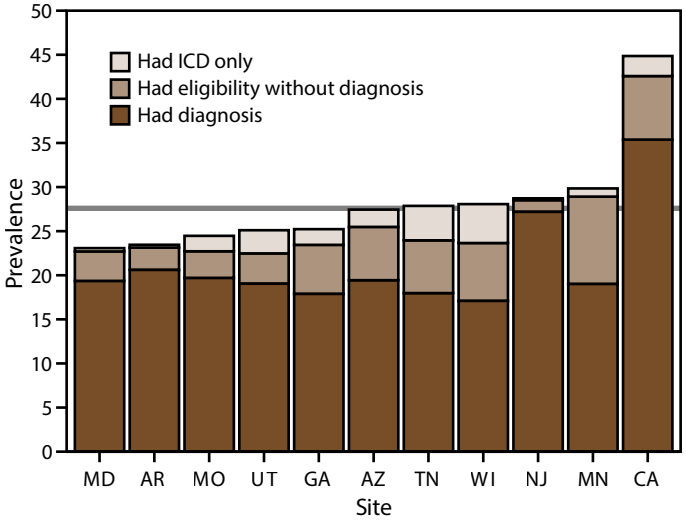
† Cochran Armitage test of trend for association between socioeconomic status tertile and ASD prevalence, by site and overall: Arizona p = 0.03; Arkansas p = 0.3; California p = 0.5; Georgia p = 0.08; Maryland p = 0.9; Minnesota p = 0.8; Missouri p = 0.3; New Jersey p<0.01; Tennessee p = 0.1; Utah p<0.01; Wisconsin p = 0.08; Total p<0.01.

Among children with ASD, 37.4% ever had an evaluation report noting that ASD was suspected but not confirmed (Table 4). Overall, 11.6% of children with ASD had an ASD diagnosis or special education eligibility ruled out (range = 4.3% in Georgia to 29.3% in California). For a majority of children, ASD was confirmed after ASD had previously been ruled out; however, 3.9% (range = 0.2% in New Jersey to 12.8% in California) of all children with ASD had an evaluation ruling out ASD more recently than one confirming ASD.

Cognitive Ability Among Children with ASD

Data on cognitive ability were available for 4,165 (66.7%) children aged 8 years with ASD (range: 39.7% in Wisconsin to 91.2% in Arkansas) (Table 5). Among children with data on cognitive ability, the median age of the most recent cognitive test or examiner impression was 67 months (interquartile range: 51–81 months) (Supplementary Table 5, https://stacks.cdc.gov/view/cdc/124397). Girls with ASD were less likely than boys with ASD to have data on cognitive ability (64.4% versus 67.3%). Similar percentages of Black and White

FIGURE 2. Prevalence* of autism spectrum disorder among children aged 8 years, by identification type and site — Autism and Developmental Disabilities Monitoring Network, 11 sites, United States, 2020†

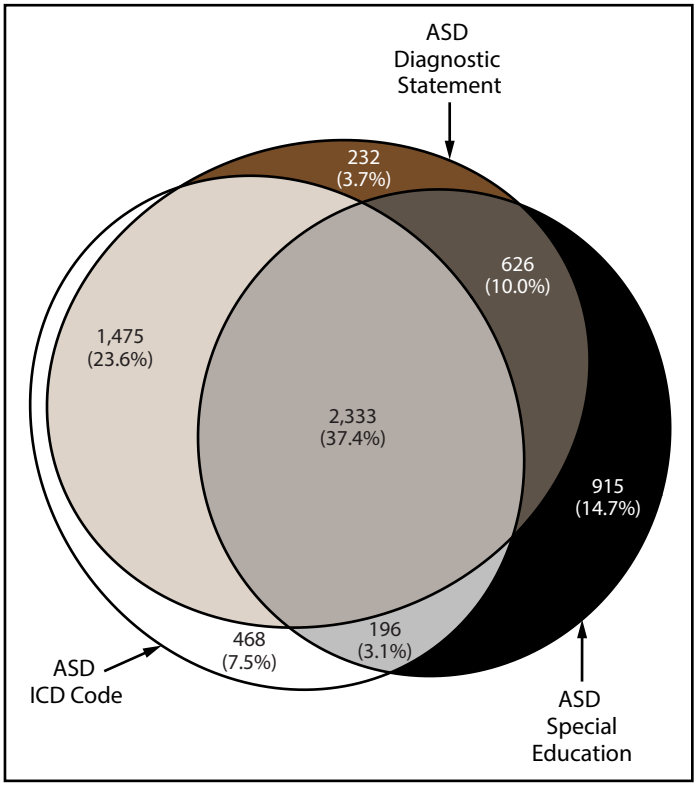


Abbreviation: ICD = International Classification of Diseases.
* Per 1,000 children aged 8 years.
† Horizontal line is the overall Autism and Developmental Disabilities Monitoring Network prevalence of 27.6 per 1,000 children aged 8 years. Children with documented autism spectrum disorder (ASD) statements could also have ASD classifications in special education or ASD ICD codes.

children had data on cognitive ability (66.8% and 65.0%, respectively), but Hispanic children (68.8%) were more likely to have cognitive data than White children. AI/AN (79.3%) and A/PI (71.2%) children and those of two or more races (73.9%) all had cognitive data at least as often as the other groups.

Among children aged 8 years with ASD who had data on cognitive ability, 37.9% were classified as having intellectual disability at their most recent test or examination, 23.5% were classified in the borderline range (IQ 71–85), and 38.6% were classified in the average or higher range (IQ >85) (Table 5). The percentage of children classified as having intellectual disability varied widely among sites (range = 21.7% in California to 51.0% in Tennessee). The median age of most recent test also varied by site (range = 55 months in Wisconsin to 79 months in Arizona) (Supplementary Table 5, https://stacks.cdc.gov/view/cdc/124397). Overall, girls with ASD were more likely to be classified as having an intellectual disability than boys with ASD (42.1% versus 36.9%), and Black children were more likely than Hispanic and White children to be classified as having intellectual disability (50.8%, 34.9%, and 31.8%, respectively). The percentage of children with ASD and intellectual disability among A/PI, two or more races, or AI/AN children was 41.3%, 37.8%, and 34.8%, respectively.

FIGURE 3. Euler diagram of different types of autism spectrum disorder identification among children aged 8 years with autism spectrum disorder* — Autism and Developmental Disabilities Monitoring Network, 11 sites, United States, 2020



Abbreviations: ASD = autism spectrum disorder; ICD = International Classification of Diseases.
* N = 6,245.

Age at First Evaluation and ASD Diagnosis

Among 5,744 children aged 8 years with ASD and recorded evaluations, 49% were evaluated by age 36 months (range = 38.5% in Utah to 59.5% in Maryland) (Table 6). The median age at first recorded evaluation ranged from 32 months in California to 44 months in Utah. Children with ASD with an intellectual disability were more likely to be evaluated by age 36 months compared with children with ASD without an intellectual disability (61.8% versus 46.0%).

Among the 4,663 children aged 8 years with ASD who had an evaluation containing an ASD diagnostic statement, the median age at earliest known diagnosis was 49 months (range = 36 months in California to 59 months in Minnesota) (Table 7). Children with ASD and intellectual disability had a lower median age at diagnosis (43 months) than children without an intellectual disability (53 months). When special education classifications of autism were considered with ASD diagnoses for earliest identification, 5,579 children with ASD were identified with a median age of 52 months (range = 39 months in California and New Jersey to 60 months in Arizona).

Discussion

For 2020, the prevalence estimate of ASD per 1,000 children aged 8 years was 27.6 (range: 23.1 in Maryland to 44.9 in California), which is higher than previous estimates from the ADDM Network. For the first time, the overall ASD prevalence for girls was >1% (11.4); in contrast, the prevalence among boys had already been noted to be higher (11.5) in the first ADDM Network report in 2002 (*4*). The continued variability in prevalence across ADDM sites, as well as the shifting in differences between demographic groups, highlight an ongoing need to better understand the systems and practices that contribute to this variability.

In its earliest years, the ADDM Network consistently reported lower overall ASD prevalence among Black and Hispanic versus White children aged 8 years. The White-Black gap in ASD prevalence narrowed in 2014, and there was no overall difference in ASD prevalence in 2016 or 2018 (Supplementary Figure 1, https://stacks.cdc.gov/view/cdc/124397). ASD prevalence among Asian, Black, and Hispanic children was at least 30% higher in 2020 than 2018, and ASD prevalence among White children was 14.6% higher than in 2018. Although this was the first time the ADDM Network reported lower ASD prevalence among White children than among other groups for children aged 8 years, a similar pattern was observed among children aged 4 years in 2018 (*18*). In addition, similar patterns were reported in analyses of national special education data and of California Developmental Services data, illustrating the prevalence of ASD classifications among Black and Hispanic children catching up and eclipsing that of White children over time (*19*,*20*). These patterns might reflect improved screening, awareness, and access to services among historically underserved groups. ASD prevalence in 2020 also was associated with lower SES, the opposite of what was observed previously (*13*), further supporting progress in identifying children regardless of race and ethnic group. As evidence grows of increased access to identification, attention might shift to what factors, such as social determinants of health, could lead to higher rates of disability among certain populations.

Even with higher ASD prevalence among Black compared with White children, Black children with ASD remained more likely to have co-occurring intellectual disability than White children, a finding that has been observed over multiple ADDM Network surveillance reports and among Black compared with White children without ASD in the United States (*21*). If Black children with ASD have less access to services than White children with ASD, as has been previously reported, the disproportionality in co-occurring intellectual disability might indicate an underascertainment of ASD among Black children without intellectual disability. Continued monitoring of trends is warranted, and it might be appropriate to re-examine potential risk or protective factors that were previously studied when the demographic composition of ASD was different.

During this period of changing demographic differences in ASD prevalence, the ADDM Network implemented two methodological changes. First, a new ASD case definition was adopted for the 2018 surveillance year. The previous case definition relied on reviewing written descriptions of ASD symptoms that were documented in comprehensive developmental evaluations. It could classify children without any formal ASD identification as ASD cases and could exclude children who had ASD diagnosed but lacked sufficient corroborating details in their records. An analysis found that non-White children were more likely to have incomplete records, which could lead to underascertainment of ASD compared with White children (*22*). However, a retroactive application of the current case definition to the 2014 and 2016 surveillance years indicates similar prevalence ratios by race and ethnicity as the previous case definition (*23*). The second change, implemented in 2020, is using population denominators with standardized racial and ethnic categories. The most important difference from the previous (bridged-race) denominators is the inclusion of a category for two or more races, which reduces the size of the denominators among the other racial groups. Nevertheless, prevalence estimates based on the previous bridged-race denominators produced a similar pattern of lower ASD prevalence among White children compared with the other groups (Supplementary Table 3, https://stacks.cdc.gov/view/cdc/124397). Thus, there were qualitatively similar patterns when consistent case definitions and denominator data sets were applied during 2014–2020.

Although ASD can be identified by age 1 year in certain cases (*24*,*25*), as described in this report, a majority of children aged 8 years living in ADDM communities were not identified until they were several years older. The reported median age of identification has not changed much over the years of ADDM Network surveillance, but it does not necessarily indicate a lack of progress in community early identification efforts. In a recent analysis of ADDM Network data during 2002–2016, the median age of diagnosis might mask progress in early detection if more children are identified (i.e., prevalence increases) at all ages and does not include children who might have ASD diagnosed after age 8 years (*26*,*27*). Therefore, the ADDM Network now reports the cumulative incidence of ASD by age 48 months as a measure of early identification and compares children aged 4 years and 8 years living in the same communities as a measure of progress (*28*,*29*). The 2020 report on early identification of ASD found more children were

identified at early ages than in the past, but many are still not identified until they are school-aged (*30*).

CDC maintains a list of peer-reviewed autism prevalence studies with similar metrics to ADDM surveillance reports (https://www.cdc.gov/ncbddd/autism/data/autism-data-table.html). Other federal programs reporting ASD prevalence information in the United States include the National Survey for Children's Health (NSCH) and the National Health Interview Survey. The ASD prevalence estimate based on the 2020 and 2021 NSCH was 2.9% and the 95% CI (2.7%–3.1%) included the 2020 ADDM Network ASD prevalence estimate (2.76%) (*31*). These surveys aim to produce nationally representative estimates among children aged 3–17 years old and ascertain information about ASD through parental report, whereas the ADDM Network estimates are not intended to be nationally representative and are generated from empirical data collected from multiple sources among participating communities. The active surveillance approach used by the ADDM Network allows reporting of when and where children are identified with ASD and affords comparisons between and within diverse U.S. communities and is not dependent on parental survey participation and ASD reporting. To facilitate comparisons between different data sources, CDC maintains an interactive website that presents U.S. state-based ASD prevalence data from four data systems (ADDM Network, NSCH, Medicaid, and special education) (https://www.cdc.gov/ncbddd/autism/data/index.html).

Limitations

The findings in this report are subject to at least seven limitations. First, the methods rely on the availability, quality, and completeness of existing information and records to ascertain ASD cases and other indicators. Although all sites had access to special education classification data, certain sites did not have access to education records for their entire population, limiting the ability to identify children with ASD exclusively identified and served through their schools. Sites requested records from public school special education programs but did not review private school education records. Incomplete information could lead to misclassifying children's cognitive ability, overestimating the age when they were first evaluated or when ASD was diagnosed, or failing to ascertain that the children were identified as having ASD. Sex information reflects what is represented in children's records and might not reflect their gender identity. Second, the case definition for intellectual disability was measured using a child's latest cognitive test or examiner statement of a child's cognitive ability. Diagnostic and special education eligibility criteria for intellectual disability requires concurrent adaptive functioning deficits (*32*). IQ scores are not necessarily stable measures of intellectual ability over time, can increase among children with ASD in response to intensive early therapeutic interventions (*33*), and might be unstable during early childhood (*34*). The age at which children had their most recent test or examiner impression of cognitive ability varied by site. Third, the ADDM Network sites are not intended to be representative of the states in which the sites are located. ADDM Network sites are selected through an objective and competitive process, and findings do not necessarily generalize to all children aged 8 years in the United States. Interpretations of temporal trends can be complicated by changing surveillance areas, case definitions, data source access, and diagnostic practices. Fourth, small numbers result in imprecise estimates for certain sites and subgroups, and estimates falling below the selected threshold for statistical precision were suppressed. Fifth, the surveillance data system does not collect the number of ASD ICD codes a child received at a specific source, limiting comparability to analyses of claims/billing databases that consider number of ICD codes received. Sixth, the COVID–19 pandemic resulted in reduced access to records from some sources at certain sites; it was often possible to electronically obtain some data elements from these sources but not manually review the full contents of records. Disruptions in services and school closures during 2020 might have resulted in less documentation of ASD in records, which could decrease ASD ascertainment by ADDM sites. Finally, the prevalence of undetected ASD in each community as well as false-positive ASD diagnoses and classifications are unknown.

Future Directions

For the 2022 and 2024 surveillance years, the ADDM Network will continue to monitor ASD prevalence among children aged 8 years; progress in early ASD identification among children aged 4 years; and the health status of, needs of, and planning for adolescents with ASD as they prepare to transition to adulthood. The 2020 early identification ADDM Network report documents the impact of the COVID-19 pandemic on early evaluation and detection of ASD; the effects of the pandemic on ASD identification also will be examined among children aged 4 and 8 years in future years of surveillance. Additional analyses are needed to better understand changing patterns in ASD prevalence and differences between groups; for example, changes between 2010 (when higher income was associated with higher ASD prevalence) to the present findings of higher prevalence among lower-SES neighborhoods are comparable to studies from

France and Sweden (*35*,*36*). In the future, it might be possible to link the Social Vulnerability Index to children ascertained through the ADDM Network to better describe disparities within communities.

Conclusion

Findings from the ADDM Network 2020 surveillance year indicate higher ASD prevalence than previous estimates from the ADDM Network and continuing evidence of a marked shift in the demographic composition of children identified with ASD compared with previous years. Although earlier ADDM Network reports have shown higher prevalence among higher-SES White children compared with other groups, the latest data indicate consistently higher prevalence among Black and Hispanic children compared with White children, and no consistent association between ASD and SES. Furthermore, this is the first ADDM Network report in which the prevalence of ASD among girls has exceeded 1%. Since 2000, the prevalence of ASD has increased steadily among all groups, but during 2018–2020, the increases were greater for Black and Hispanic children than for White children. These data indicate that ASD is common across all groups of children and underscore the considerable need for equitable and accessible screening, services, and supports for all children.

Acknowledgments

Community partners at clinical and educational data sources shared data that enabled the generation of these estimates, as well as the following staff from sites:

Arkansas: Julie N. Fulmer, Stefanie L. Jernigan, Sandra K. Walker, J. Michelle Cantrell Kelley; California: Martha "Rebecca" Johnson; Georgia: Patricia Colan, Katrina Langston, Cindy Schley, Angela Wells, Natalyn Jennings; Maryland: Belina Onomake, Jade Lewis, Kelly King, and Thomas (David) Graham; Minnesota: Ryan Ferguson, Courtney Higginbotham, Madilyn Bliss, Shelby Flannagan, and Drake Bauer, John Westerman, Isabel Ricke; Missouri: Carolyn Skowyra, Gregory Tolbert, Christina Mirth; New Jersey: Michael Verile, Cindy Cruz-Alvarez, Isabella Gallo, Christine Shelton, and Paul Zumoff; Tennessee: Ambar Muñoz Lavanderos, Alex Miceli, Jaylaan Parker; Utah: Hunter Pons, Madison Mitamura, Somi Lee, Danli Chen; Wisconsin: Jacqueline Hansen, Erika Hans, Jennifer L.S. Huffman, Ricardo Ortiz, Shannon O'Neill.

Conflicts of Interest

All authors have completed and submitted the International Committee of Medical Journal Editors form for disclosure of potential conflicts of interest. No potential conflicts of interest were disclosed.

References

1. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 5th ed. Arlington, VA: American Psychiatric Association; 2013.
2. Yeargin-Allsopp M, Rice C, Karapurkar T, Doernberg N, Boyle C, Murphy C. Prevalence of autism in a US metropolitan area. JAMA 2003;289:49–55. PMID:12503976 https://doi.org/10.1001/jama.289.1.49
3. Autism and Developmental Disabilities Monitoring Network Surveillance Year 2000 Principal Investigators. Prevalence of autism spectrum disorders—Autism and Developmental Disabilities Monitoring Network, six sites, United States, 2000. MMWR Surveill Summ 2007;56(No. SS–1).
4. Autism and Developmental Disabilities Monitoring Network Surveillance Year 2002 Principal Investigators. Prevalence of autism spectrum disorders—Autism and Developmental Disabilities Monitoring Network, 14 sites, United States, 2002. MMWR Surveill Summ 2007;56(No. SS–1).
5. Autism and Developmental Disabilities Monitoring Network Surveillance Year 2006 Principal Investigators. Prevalence of autism spectrum disorders (ASDs)—Autism and Developmental Disabilities Monitoring (ADDM) Network, United States, 2004. MMWR Surveill Summ 2009;58(No. SS–10).
6. Autism and Developmental Disabilities Monitoring Network Surveillance Year 2006 Principal Investigators. Prevalence of autism spectrum disorders—Autism and Developmental Disabilities Monitoring Network, United States, 2006. MMWR Surveill Summ 2009;58(No. SS–10).
7. Autism and Developmental Disabilities Monitoring Network Surveillance Year 2008 Principal Investigators. Prevalence of autism spectrum disorders—Autism and Developmental Disabilities Monitoring Network, 14 sites, United States, 2008. MMWR Surveill Summ 2012;61(No. SS–3).
8. Autism and Developmental Disabilities Monitoring Network Surveillance Year 2010 Principal Investigators. Prevalence of autism spectrum disorder among children aged 8 years—Autism and Developmental Disabilities Monitoring Network, 11 sites, United States, 2010. MMWR Surveill Summ 2014;63(No. SS–2).
9. Christensen DL, Baio J, Van Naarden Braun K, et al. Prevalence and characteristics of autism spectrum disorder among children aged 8 years—Autism and Developmental Disabilities Monitoring Network, 11 sites, United States, 2012. MMWR Surveill Summ 2016;65(No. SS–3):1–23. PMID:27031587 https://doi.org/10.15585/mmwr.ss6503a1
10. Baio J, Wiggins L, Christensen DL, et al. Prevalence of autism spectrum disorder among children aged 8 years—Autism and Developmental Disabilities Monitoring Network, 11 sites, United States, 2014. MMWR Surveill Summ 2018;67(No. SS–6):1–23. PMID:29701730 https://doi.org/10.15585/mmwr.ss6706a1
11. Maenner MJ, Shaw KA, Baio J, et al. Prevalence of autism spectrum disorder among children aged 8 years—Autism and Developmental Disabilities Monitoring Network, 11 sites, United States, 2016. MMWR Surveill Summ 2020;69(No. SS–4):1–12. PMID:32214087 https://doi.org/10.15585/mmwr.ss6904a1
12. Maenner MJ, Shaw KA, Bakian AV, et al. Prevalence and characteristics of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 sites, United States, 2018. MMWR Surveill Summ 2021;70(No. SS–11):1–16. PMID:34855725 https://doi.org/10.15585/mmwr.ss7011a1
13. Durkin MS, Maenner MJ, Baio J, et al. Autism spectrum disorder among US children (2002–2010): socioeconomic, racial, and ethnic disparities. Am J Public Health 2017;107:1818–26. PMID:28933930 https://doi.org/10.2105/AJPH.2017.304032
14. Public Welfare, Protection of Human Subjects. C.F.R. 45 Part 46 (2010).

15. Census Bureau. Annual county postcensal resident population by single year of age (0–84,85+), sex, race (11 groups), and Hispanic origin for April 1, 2020 (population estimates base) and July 1, 2020 to July 2021 (Vintage 2021).
16. CDC. Vintage 2020 bridged-race postcensal population estimates for April 1, 2010, July 1, 2010–July 1, 2020, by year, county, single-year of age (0 to 85+ years), bridged-race, Hispanic origin, and sex. Atlanta, GA: US Department of Health and Human Services, CDC; 2021. https://www.cdc.gov/nchs/nvss/bridged_race.htm
17. Census Bureau. 2020 American Community Survey 5-year estimates. Washington, DC: US Department of Commerce, Census Bureau; 2022. https://data.census.gov/cedsci
18. Shaw KA, Maenner MJ, Bakian AV, et al. Early identification of autism spectrum disorder among children aged 4 years—Autism and Developmental Disabilities Monitoring Network, 11 sites, United States, 2018. MMWR Surveill Summ 2021;70(No. SS–10):1–14. PMID:34855727 https://doi.org/10.15585/mmwr.ss7010a1
19. Nevison C, Zahorodny W. Race/ethnicity–resolved time trends in United States ASD prevalence estimates from IDEA and ADDM. J Autism Dev Disord 2019;49:4721–30. PMID:31435818 https://doi.org/10.1007/s10803-019-04188-6
20. Winter AS, Fountain C, Cheslack-Postava K, Bearman PS. The social patterning of autism diagnoses reversed in California between 1992 and 2018. Proc Natl Acad Sci U S A 2020;117:30295–302. PMID:33199592 https://doi.org/10.1073/pnas.2015762117
21. Patrick ME, Shaw KA, Dietz PM, et al. Prevalence of intellectual disability among eight-year-old children from selected communities in the United States, 2014. Disabil Health J 2021;14:101023. PMID:33272883 https://doi.org/10.1016/j.dhjo.2020.101023
22. Imm P, White T, Durkin MS. Assessment of racial and ethnic bias in autism spectrum disorder prevalence estimates from a US surveillance system. Autism 2019;23:1927–35. PMID:30892923 https://doi.org/10.1177/1362361319827510
23. Maenner MJ, Graves SJ, Peacock G, Honein MA, Boyle CA, Dietz PM. Comparison of two case definitions for ascertaining prevalence of autism spectrum disorder among 8-year-old children. Am J Epidemiol 2021;190:2198–207. PMID:33847734 https://doi.org/10.1093/aje/kwab106
24. Pierce K, Gazestani V, Bacon E, et al. Get SET early to identify and treatment refer autism spectrum disorder at 1 year and discover factors that influence early diagnosis. J Pediatr 2021;S0022–3476(21)00392–9.
25. Pierce K, Gazestani VH, Bacon E, et al. Evaluation of the diagnostic stability of the early autism spectrum disorder phenotype in the general population starting at 12 months. JAMA Pediatr 2019;173:578–87. PMID:31034004 https://doi.org/10.1001/jamapediatrics.2019.0624
26. Shaw KA, McArthur D, Hughes MM, et al. Progress and disparities in early identification of autism spectrum disorder: autism and developmental disabilities monitoring network, 2002–2016. J Am Acad Child Adolesc Psychiatry 2022;61:905–14. PMID:34838692 https://doi.org/10.1016/j.jaac.2021.11.019
27. Sheldrick RC. Editorial: Evaluating the success of early detection of autism: It's time to move beyond the median. J Am Acad Child Adolesc Psychiatry 2022;61:860–1. PMID:34921909 https://doi.org/10.1016/j.jaac.2021.12.002
28. Shaw KA, Maenner MJ, Baio J, et al. Early identification of autism spectrum disorder among children aged 4 years—Early Autism and Developmental Disabilities Monitoring Network, six sites, United States, 2016. MMWR Surveill Summ 2020;69(No. SS–33):1–11. PMID:32214075 https://doi.org/10.15585/mmwr.ss6903a1
29. Shaw KA, Maenner MJ, Bakian AV, et al. Early identification of autism spectrum disorder among children aged 4 years—Autism and Developmental Disabilities Monitoring Network, 11 sites, United States, 2018. MMWR Surveill Summ 2021;70(No. SS–10):1–14. PMID:34855727 https://doi.org/10.15585/mmwr.ss7010a1
30. Shaw KA, Bilder DA, McArthur D, et al. Early identification of autism spectrum disorder among children aged 4 years—Early Autism and Developmental Disabilities Monitoring Network, 11 sites, United States, 2020. MMWR Surveill Summ 2023;72(No. SS-1).
31. Child and Adolescent Health Measurement Initiative. 2020–2021 National Survey of Children's Health data query. Data Resource Center for Child and Adolescent Health. Washington DC: US Department of Health and Human Services, Health Resources and Services Administration, Maternal and Child Health Bureau; 2021. http://www.childhealthdata.org
32. Schalock RL, Luckasson R, Tassé MJ. Twenty questions and answers regarding the 12th edition of the AAIDD manual: intellectual disability: definition, diagnosis, classification, and systems of supports. Silver Spring, MD: American Association on Intellectual and Developmental Disabilities; 2021.
33. Reichow B, Hume K, Barton EE, Boyd BA. Early intensive behavioral intervention (EIBI) for young children with autism spectrum disorders (ASD). Cochrane Database Syst Rev 2018;5:CD009260. PMID:29742275 https://doi.org/10.1002/14651858.CD009260.pub3
34. Pickles A, McCauley JB, Pepa LA, Huerta M, Lord C. The adult outcome of children referred for autism: typology and prediction from childhood. J Child Psychol Psychiatry 2020;61:760–7. PMID:31957035 https://doi.org/10.1111/jcpp.13180
35. Delobel-Ayoub M, Ehlinger V, Klapouszczak D, et al. Socioeconomic disparities and prevalence of autism spectrum disorders and intellectual disability. PLoS One 2015;10:e0141964. PMID:26540408 https://doi.org/10.1371/journal.pone.0141964
36. Rai D, Lewis G, Lundberg M, et al. Parental socioeconomic status and risk of offspring autism spectrum disorders in a Swedish population-based study. J Am Acad Child Adolesc Psychiatry 2012;51:467–476. PMID:22525953 https://doi.org/10.1016/j.jaac.2012.02.012

TABLE 1. Surveillance sites and data sources used for surveillance in each site — Autism and Developmental Disabilities Monitoring Network, 11 sites, United States, 2020

Site	Surveillance area description	Total population aged 8 yrs	% American Indian or Alaska Native*	% Asian or Pacific Islander	% Black	% Hispanic	% White	% Two or more races	Types of data sources used†	Education data sources (% population coverage)§	% of requested records fully accessible for chart review
Arizona	Part of one county in metropolitan Phoenix	13,118¶	3.1	2.9	6.8	41.8	40.3	5.1	Health, education, Medicaid	100	100
Arkansas	21 counties in central Arkansas	15,432	0.3	1.3	24.2	9.1	60.8	4.2	Health, education	100	100
California	Part of one county in metropolitan San Diego	15,828¶	0.3	11.9	7.1	49.4	23.1	8.3	Health, education, state developmental disability services	100	100
Georgia	Two counties in metropolitan Atlanta	21,921	0.1	7.4	51.1	11.8	25.7	3.9	Health, education	97.6	85.9
Maryland	Five counties in suburban Baltimore	21,278	0.2	9.5	23.9	9.0	51.2	6.1	Health, education, early intervention	100	71.5
Minnesota	Parts of three counties in the Twin Cities metropolitan area	16,150¶	1.1	16.3	23.3	10.9	41.8	6.6	Health, education	100	100
Missouri	Five counties in metropolitan St. Louis	24,561	0.1	3.4	23.8	4.8	63.0	4.8	Health, education	50.3	99.9
New Jersey	Two counties in New York metropolitan area	18,940	0.2	6.3	30.5	33.6	26.6	2.8	Health, education	100	95.8
Tennessee	11 counties in middle Tennessee	25,588	0.2	3.4	17.2	13.5	60.4	5.3	Health, education	100	66.3
Utah	Three counties in northern Utah	24,734	0.6	4.2	1.8	20.7	68.4	4.2	Health, education, early intervention	100	87.6
Wisconsin	Eight counties in southeastern Wisconsin	28,789	0.3	5.5	17.0	17.4	54.8	5.0	Health, education, early intervention, Medicaid claims, state-funded long-term care program	100	100
Total		**226,339**	**0.5**	**6.3**	**20.8**	**18.5**	**48.7**	**5.1**	—	**99.9**	**91.8**

* Persons of Hispanic origin might be of any race but are categorized as Hispanic; all racial groups are non-Hispanic.
† Health sources include records from medical and service providers that evaluate children with developmental disabilities.
§ For public schools in the surveillance area. In the absence of direct access to education sources, education data could be collected if they were included in a child's medical or service records.
¶ Denominator excludes school districts that were not included in the surveillance area, calculated from National Center for Education Statistics enrollment counts of third graders during the 2020–21 school year.

TABLE 2. Prevalence* of autism spectrum disorder among children aged 8 years, overall and by sex — Autism and Developmental Disabilities Monitoring Network, 11 sites, United States, 2020

Site	Overall†			Male prevalence (95% CI)	Female prevalence (95% CI)	Male-to-female prevalence ratio (95% CI)§
	No. with ASD	Total population	Prevalence (95% CI)			
Arizona	360	13,118	27.4 (24.8–30.4)	43.8 (39.2–49.0)	10.3 (8.1–13.1)	4.3 (3.3–5.5)
Arkansas	362	15,432	23.5 (21.2–26.0)	36.3 (32.4–40.6)	9.6 (7.6–12.1)	3.8 (2.9–4.9)
California	710	15,828	44.9 (41.7–48.2)	69.4 (64.1–75.1)	19.1 (16.3–22.4)	3.6 (3.0–4.3)
Georgia	553	21,921	25.2 (23.2–27.4)	40.2 (36.7–44.0)	9.7 (8.0–11.7)	4.2 (3.4–5.1)
Maryland	491	21,278	23.1 (21.1–25.2)	36.9 (33.5–40.6)	8.6 (7.0–10.6)	4.3 (3.4–5.4)
Minnesota	482	16,150	29.8 (27.3–32.6)	47.8 (43.4–52.6)	11.0 (9.0–13.6)	4.3 (3.4–5.4)
Missouri	601	24,561	24.5 (22.6–26.5)	38.7 (35.4–42.2)	9.3 (7.8–11.2)	4.1 (3.4–5.1)
New Jersey	544	18,940	28.7 (26.4–31.2)	44.5 (40.5–48.7)	12.2 (10.2–14.7)	3.6 (3.0–4.5)
Tennessee	713	25,588	27.9 (25.9–30.0)	43.9 (40.5–47.5)	11.1 (9.4–13.1)	4.0 (3.3–4.8)
Utah	621	24,734	25.1 (23.2–27.1)	37.6 (34.4–41.1)	11.8 (10.0–13.9)	3.2 (2.6–3.8)
Wisconsin	808	28,789	28.1 (26.2–30.0)	42.6 (39.4–45.9)	13.0 (11.2–15.0)	3.3 (2.8–3.9)
Total	**6,245**	**226,339**	**27.6 (26.9–28.3)**	**43.0 (41.9–44.2)**	**11.4 (10.7–12.0)**	**3.8 (3.6–4.0)**

Abbreviation: ASD = autism spectrum disorder.
* Per 1,000 children aged 8 years.
† All children are included in the total regardless of sex or race and ethnicity.
§ Wilson score 95% CIs exclude 1.0 in all sites, indicating significantly higher prevalence among males than among females; Mantel Haenszel (Woolf) test of homogeneity of prevalence ratios across sites, p value = 0.15, indicating little heterogeneity in prevalence ratios across sites.

TABLE 3. Prevalence* of autism spectrum disorder among children aged 8 years, by race and ethnicity† — Autism and Developmental Disabilities Monitoring Network, 11 sites, United States, 2020

Site	Prevalence (95% CI)					Prevalence Ratio (95% CI)			
	A/PI	Black	Hispanic	White	Two or more races	Black to White	Hispanic to White	A/PI to White	Two or more races to White
Arizona	—§	25.9 (17.3–38.6)	26.6 (22.7–31.2)	29.7 (25.5–34.7)	20.9 (12.5–34.8)	0.9 (0.6–1.3)	0.9 (0.7–1.1)	—	0.7 (0.4–1.2)
Arkansas	58.8 (34.0–100.0)	23.9 (19.4–29.3)	31.0 (23.2–41.4)	22.5 (19.7–25.7)	—	1.1 (0.8–1.4)	1.4 (1.0–1.9)¶	2.6 (1.5–4.6)¶	—
California	56.5 (46.9–67.9)	44.4 (33.8–58.1)	45.3 (40.9–50.1)	38.3 (32.6–45.1)	39.7 (30.4–51.7)	1.2 (0.8–1.6)	1.2 (1.0–1.4)	1.5 (1.2–1.9)¶	1.0 (0.8–1.4)
Georgia	25.3 (18.7–34.1)	28.6 (25.7–31.8)	25.2 (19.8–32.0)	19.0 (15.7–22.9)	17.6 (10.7–28.9)	1.5 (1.2–1.9)¶	1.3 (1.0–1.8)	1.3 (0.9–1.9)	0.9 (0.5–1.6)
Maryland	36.5 (29.2–45.6)	33.6 (29.0–39.0)	17.2 (12.2–24.0)	16.8 (14.5–19.4)	19.1 (13.0–28.1)	2.0 (1.6–2.5)¶	1.0 (0.7–1.5)	2.2 (1.7–2.8)¶	1.1 (0.8–1.7)
Minnesota	24.3 (19.1–30.9)	27.9 (23.1–33.7)	40.4 (32.2–50.7)	30.0 (26.2–34.4)	31.0 (22.1–43.2)	0.9 (0.7–1.2)	1.3 (1.0–1.8)¶	0.8 (0.6–1.1)	1.0 (0.7–1.5)
Missouri	34.3 (24.0–48.9)	28.1 (24.2–32.7)	16.8 (10.9–25.8)	23.4 (21.1–25.9)	10.2 (5.8–7.7)	1.2 (1.0–1.4)¶	0.7 (0.5–1.1)	1.5 (1.0–2.1)¶	0.4 (0.2–0.8)¶
New Jersey	27.5 (19.6–38.3)	32.9 (28.6–37.8)	32.7 (28.6–37.3)	19.7 (16.2–23.9)	—	1.7 (1.3–2.1)¶	1.7 (1.3–2.1)¶	1.4 (0.9–2.1)	—
Tennessee	38.3 (27.4–53.3)	32.9 (28.0–38.6)	26.3 (21.5–32.2)	25.2 (22.9–27.8)	25.7 (18.5–35.6)	1.3 (1.1–1.6)¶	1.0 (0.8–1.3)	1.5 (1.1–2.2)¶	1.0 (0.7–1.4)
Utah	27.9 (19.5–39.8)	—	23.6 (19.8–28.1)	24.8 (22.5–27.2)	18.2 (11.7–28.3)	—	1.0 (0.8–1.2)	1.1 (0.8–1.6)	0.7 (0.5–1.2)
Wisconsin	29.2 (22.0–38.7)	23.8 (19.9–28.5)	35.6 (30.8–41.1)	25.9 (23.5–28.5)	30.0 (22.4–40.2)	0.9 (0.8–1.1)	1.4 (1.2–1.6)¶	1.1 (0.8–1.5)	1.2 (0.9–1.6)
Total	**33.4 (30.5–36.4)**	**29.3 (27.9–30.9)**	**31.6 (30.0–33.3)**	**24.3 (23.4–25.2)**	**22.9 (20.3–25.8)**	**1.2 (1.1–1.3)¶**	**1.3 (1.2–1.4)¶**	**1.4 (1.2–1.5)¶**	**0.9 (0.8–1.1)**

Abbreviation: A/PI = Asian or Pacific Islander.
* Per 1,000 children aged 8 years. Overall American Indian/Alaska Native autism spectrum disorder prevalence per 1,000 was 26.5 (95% CI = 18.5–37.8). Arizona was the only Autism and Developmental Disabilities Monitoring Network site meeting the threshold for statistical precision for American Indian/Alaska Native autism spectrum disorder prevalence; the site-specific prevalence per 1,000 was 26.8 (95% CI = 15.0–47.3). None of ratios with AI/AN children that met the threshold for suppression were statistically significant.
† Persons of Hispanic origin might be of any race but are categorized as Hispanic; all racial groups are non-Hispanic.
§ Dash indicates estimate was suppressed because SE for prevalence was ≥30% of estimate, or prevalence ratio was based on an estimate that was suppressed.
¶ 95% CI does not include 1.0.

TABLE 4. Autism spectrum disorder identification information among children aged 8 years meeting case definition, by site — Autism and Developmental Disabilities Monitoring Network, 11 sites, United States, 2020

		Part of ASD case definition*			Evaluation in addition to meeting ASD case definition		
Site	No. with ASD	% with ASD ICD code	% with ASD special education classification	% with ASD diagnostic statement	% with ASD with an evaluation summary diagnosis of suspected ASD	% with ASD with an evaluation summary ever ruling out ASD (diagnosis or special education classification)†	% with ASD ruled out (diagnosis or special education) more recently than documented ASD diagnosis or classification†
Arizona	360	60.8	70.8	70.8	62.8	16.4	5.8
Arkansas	362	63.0	75.7	87.8	59.7	13.0	2.5
California	710	82.7	74.5	78.9	24.5	29.3	12.8
Georgia	553	61.1	64.2	70.9	51.4	4.3	1.1
Maryland	491	60.7	74.3	83.9	70.1	13.4	2.2
Minnesota	482	51.9	84.9	63.7	8.5	6.4	1.9
Missouri	601	72.2	54.4	80.5	23.0	11.0	3.0
New Jersey	544	73.2	70.0	94.7	32.9	5.0	0.2
Tennessee	713	79.1	59.5	64.5	37.0	10.4	4.3
Utah	621	80.2	44.9	75.8	39.3	6.0	2.7
Wisconsin	808	81.4	58.4	60.9	28.0	10.5	3.3
Total	**6,245**	**71.6**	**65.2**	**74.7**	**37.4**	**11.6**	**3.9**

Abbreviations: ASD = autism spectrum disorder; ICD = International Classification of Diseases.
* ICD code, special education, and diagnosis can be interpreted as the individual sensitivity of each component related to the entire case definition.
† Includes children who had ASD ruled out and never had either a documented ASD diagnosis or special education classification (i.e., had an ASD ICD code only).

TABLE 5. Availability and distribution of IQ scores among children aged 8 years with autism spectrum disorder, by site, sex, and race and ethnicity — Autism and Developmental Disabilities Monitoring Network, 11 sites, United States, 2020

		With IQ information	Cognitive level		
Site/Characteristic	Total no. with ASD	No. (%)	IQ ≤70 (%)	IQ 71–85 (%)	IQ >85* (%)
Site					
Arizona	**360**	291 (80.8)	30.9	29.2	39.9
Arkansas	**362**	330 (91.2)	48.2	22.4	29.4
California	**710**	617 (86.9)	21.7	26.9	51.4
Georgia	**553**	398 (72.0)	46.2	23.6	30.2
Maryland	**491**	295 (60.1)	46.8	21.0	32.2
Minnesota	**482**	414 (85.9)	31.6	15.0	53.4
Missouri	**601**	364 (60.6)	31.9	23.6	44.5
New Jersey	**544**	342 (62.9)	38.9	30.4	30.7
Tennessee	**713**	478 (67.0)	51.0	22.0	27.0
Utah	**621**	315 (50.7)	29.2	25.4	45.4
Wisconsin	**808**	321 (39.7)	48.9	19.3	31.8
Total	**6,245**	**4,165 (66.7)**	**37.9**	**23.5**	**38.6**
Sex					
Female	**1,255**	808 (64.4)†	42.1§	21.2	36.8
Male	**4,984**	3,357 (67.3)	36.9	24.1	39.0
Race/Ethnicity¶,**					
AI/AN	**29**	23 (79.3)	34.8	39.1	26.1
A/PI	**476**	340 (71.4)	41.5	21.8	36.8
Black	**1,384**	925 (66.8)	50.8	25.1	24.1
Hispanic	**1,331**	916 (68.8)	34.9	27.5	37.6
White	**2,680**	1,743 (65.0)††	31.8	20.7	47.5
Two or more races	**261**	193 (73.9)	37.8	24.9	37.3

Abbreviations: AI/AN = American Indian or Alaska Native; A/PI = Asian or Pacific Islander; ASD = autism spectrum disorder.
* Includes three children stated to have an IQ score in the average range but specific score was not given.
† Pearson chi-square test for proportion of males versus females with ASD and IQ information (p = 0.049).
§ Pearson chi-square test for proportion of males versus females with IQ ≤70 among children with ASD (p = 0.007).
¶ Statistically significant differences for Pearson chi-square tests for proportion of non-Hispanic Black versus non-Hispanic White children with IQ ≤70 among children with ASD (p<0.001); proportion of non-Hispanic Black versus Hispanic children with IQ ≤70 among children with ASD (p<0.001); proportion of non-Hispanic White versus non-Hispanic A/PI children with IQ ≤70 among children with ASD (p = 0.001); proportion of non-Hispanic Black versus non-Hispanic A/PI children with IQ ≤70 among children with ASD (p = 0.007).
** Persons of Hispanic origin might be of any race but are categorized as Hispanic; all racial groups are non-Hispanic.
†† Pearson chi-squared tests for proportion of non-Hispanic Black versus non-Hispanic White children with ASD and IQ information (p = 0.27); proportion of non–Hispanic Black versus Hispanic children with IQ information (p = 0.17); proportion of non-Hispanic White versus Hispanic children with IQ information (p = 0.03).

TABLE 6. Number and percentage of children aged 8 years with autism spectrum disorder who received a developmental evaluation by a qualified professional at age ≤36 months,* by site and intellectual disability status — Autism and Developmental Disabilities Monitoring Network, 11 sites, United States, 2020

Site	Total no. with ASD	Total with recorded evaluation			IQ ≤70			IQ >70			IQ unknown		
		No. with recorded evaluation	% evaluated by age 36 mos	Median age at earliest recorded evaluation (mos)	No. with recorded evaluation	% evaluated by age 36 mos	Median age at earliest recorded evaluation (mos)	No. with recorded evaluation	% evaluated by age 36 mos	Median age at earliest recorded evaluation (mos)	No. with recorded evaluation	% evaluated by age 36 mos	Median age at earliest recorded evaluation (mos)
Arizona	**360**	349	49.0	37	90	64.4	29	201	44.3	38	58	41.4	57
Arkansas	**362**	359	42.6	39	159	56.6	34	171	31.0	45	29	34.5	41
California	**710**	701	58.2	32	134	61.2	30	483	61.1	31	84	36.9	44
Georgia	**553**	488	46.7	39	182	51.1	36	213	46.5	40	93	38.7	42
Maryland	**491**	474	59.5	33	138	74.6	28.5	157	65.0	31	179	43.0	40
Minnesota	**482**	474	42.2	40	131	61.1	34	283	38.5	43	60	18.3	52
Missouri	**601**	591	39.8	43	116	55.2	36	245	27.3	53	230	45.2	39.5
New Jersey	**544**	537	58.3	34	133	60.9	34	208	60.6	34	196	54.1	35
Tennessee	**713**	611	43.9	41	225	61.8	31	202	41.1	43.5	184	25.0	58.5
Utah	**621**	579	38.5	44	87	48.3	39	214	30.8	49	278	41.4	42
Wisconsin	**808**	581	57.5	34	152	82.2	27	158	49.4	37.5	271	48.3	37
Total	**6,245**	**5,744**	**49.0**	**37**	**1,547**	**61.9**	**33.0**	**2,535**	**46.0**	**39**	**1,662**	**41.6**	**41**

Abbreviation: ASD = autism spectrum disorder.
* Permutation test comparing median age of earliest known evaluation for children with known IQ score ≤70 versus known IQ score >70 (p<0.001).

TABLE 7. Median age at earliest known autism spectrum disorder diagnosis among children aged 8 years, by intellectual disability status — Autism and Developmental Disabilities Monitoring Network, 11 sites, United States, 2020

Site	Total no. with ASD	All children with an ASD diagnostic statement			Children with an ASD diagnostic statement and IQ score ≤70		Children with an ASD diagnostic statement and IQ score >70		Children with either an ASD diagnostic statement or ASD special education classification	
		No. with documented ASD diagnosis	Prevalence of ASD with documented diagnosis	Median age at earliest known diagnosis (mos)	No. with documented ASD diagnosis	Median age at earliest known diagnosis (mos)	No. with documented ASD diagnosis	Median age at earliest known diagnosis (mos)	No. with documented ASD diagnosis or ASD special education classification	Median age at earliest known ASD identification (mos)
Arizona	**360**	255	19.4	57	66	50.5	139	58	333	60
Arkansas	**362**	318	20.6	56	144	49.5	147	63	350	58
California	**710**	560	35.4	36	121	39	384	35.5	673	39
Georgia	**553**	392	17.9	50	147	48	166	52.5	476	51
Maryland	**491**	412	19.4	49	127	38.0	138	49	477	53
Minnesota	**482**	307	19.0	59	105	44	171	65	467	56
Missouri	**601**	484	19.7	51.5	99	50	194	65	556	56
New Jersey	**544**	514	27.1	38	131	37	197	39	538	39
Tennessee	**713**	458	17.9	48	192	36.5	161	56	611	58
Utah	**621**	471	19.0	56	73	52	168	65	528	58
Wisconsin	**808**	492	17.1	43	140	35.5	140	50	570	46
Total	**6,245**	**4,663**	**20.6**	**49**	**1,345**	**43***	**2,005**	**53***	**5,579**	**52**

Abbreviation: ASD = autism spectrum disorder.
* Permutation test comparing median age of earliest known diagnosis for children with known IQ score ≤70 versus known IQ score >70 (p<0.001).

South African Journal of Psychiatry
ISSN: (Online) 2078-6786, (Print) 1608-9685

Prevalence of caregiver burden, depressive and anxiety symptoms in caregivers of children with psychiatric disorders in Durban, South Africa

Authors:
Saeeda Paruk[1]
Mayuri Ramdhial[1]

Affiliations:
[1]Department of Psychiatry, University of Kwazulu-Natal, South Africa

Corresponding author:
Saeeda Paruk,
myamod@telkomsa.net

How to cite this article:
Paruk S, Ramdhial M. Prevalence of caregiver burden, depressive and anxiety symptoms in caregivers of children with psychiatric disorders in Durban, South Africa. S Afr J Psychiat. 2018;24(0), a1314. https://doi.org/10.4102/sajpsychiatry.v24i0.1314

Background: There is increased caregiver burden, depressive and anxiety symptoms associated with the care of mentally ill children. This may be influenced by child or caregiver factors such as socio-demographic and clinical factors and has not been explored in the South African context.

Aim: To describe the prevalence of depression, anxiety symptoms and caregiver burden in caregivers of children treated at psychiatric outpatient services at two public sector hospitals.

Methods: A cross-sectional questionnaire study of 121 adult primary caregivers of children aged 1–17 years with mental illness using a socio-demographic questionnaire, Patient Health Questionnaire (PHQ-9), Generalised Anxiety Disorder-7 Questionnaire (GAD-7), and the Child and Adolescent Impact Assessment (CAIA) to assess caregiver burden.

Results: The caregivers were predominantly female (n = 96, 79.5%) and married (n = 72, 59.5%), with a mean age of¬34.99 years (SD 10.38), and 74% were mothers. Among the children, there was a predominance of boys with a 1:4 ratio of girls to boys. The most common diagnoses in the children were attention deficit hyperactivity disorder (ADHD) (n = 56, 59.6%) and autism spectrum disorder (n = 22, 23.4%). Fifty-four (44%) caregivers were depressed with a mean PHQ9 score of 5.75 (SD 5.98), and 65 (54 %) reported anxiety symptoms with a mean GAD7 score of 5.71 (SD 5.03). Mothers reported significantly higher levels of anxiety (p = 0.045) and experienced higher impact on feelings of personal well-being on the CAIA (p = 0.004) in comparison with fathers. Caregiver burden was predominantly reported in the domains of restrictions in activities (n = 40, 32.8%), feelings of personal well-being (n = 37, 30.7%) and economic impact (n = 21, 17.4%).

The caregivers of children with ADHD reported higher anxiety levels (p = 0.023) than for autistic children. A diagnosis of autistic spectrum disorder was associated with higher income impact (p = 0.004) and restrictions impact (p = 0.001) than for children with ADHD diagnosis in terms of caregiver burden.

Conclusion: The high prevalence of depression and anxiety symptoms reported amongst caregivers suggests the need for improved mental health screening and psycho-social support programmes for caregivers, particularly mothers. Programmes should consider the impact of caregiving, particularly on mental health, income and social restrictions of caregivers.

Note: A selected abstract from papers presented at the 19th National Congress of the South African Society of Psychiatrists in 'Professional Psychiatric Practice: Medical, Socio-Economic & Cultural Perspectives', 21–24 September 2018, at the CSIR, Pretoria, South Africa. The congress is hosted by South African Society of Psychiatrists (SASOP).

Current Psychiatry Reports (2021) 23: 79
https://doi.org/10.1007/s11920-021-01292-2

AUTISM SPECTRUM DISORDERS (ES BRODKIN, SECTION EDITOR)

Recent Updates in Psychopharmacology for the Core and Associated Symptoms of Autism Spectrum Disorder

Robyn P. Thom[1,2,3] · Joseph A. Pereira[4] · Danielle Sipsock[5,6] · Christopher J. McDougle[1,2,3]

Accepted: 3 September 2021 / Published online: 13 October 2021

Abstract

Purpose of Review Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by core deficits in social communication and restricted, repetitive patterns of behavior. This article aims to review the recent literature pertaining to psychopharmacology for the core and associated symptoms of ASD including social impairment, repetitive behaviors, irritability, and language impairment.

Recent Findings Recent medication trials targeting social impairment in ASD have focused on neuropeptides (oxytocin and vasopressin) and memantine. None of these three medications has demonstrated consistent benefit for social impairment in ASD; however, additional studies are underway. Two double-blind, placebo-controlled studies on selective serotonin reuptake inhibitors (SSRIs) provide evidence against the use of SSRIs for repetitive behaviors in youth with ASD. Preliminary studies have investigated cannabidiol (CBD) for irritability in ASD but further studies are needed to demonstrate safety and efficacy. Finally, three double-blind, placebo-controlled studies provide preliminary evidence for folinic acid for the treatment of verbal language deficits in children with ASD.

Summary The identification of safe and effective pharmacological treatments to ameliorate the core and associated symptoms of ASD has proven difficult.

Keywords Autism spectrum disorder · Psychopharmacology · Social impairment · Repetitive behaviors · Irritability · Language impairment

Introduction

Autism spectrum disorder (ASD) is a heterogenous neurodevelopmental disorder characterized by core deficits in social communication and restricted, repetitive patterns of behavior [1]. While there are currently no uniformly effective behavioral or psychopharmacological therapies for ASD, management includes early intensive behavioral intervention to improve social functioning and developmental outcomes [2]. Other nonpharmacologic treatments may include intervention for impaired language and communication, social skills training, occupational therapy, and special education. No pharmacologic approaches have been clearly demonstrated to reverse the core symptoms of ASD; however, risperidone and aripiprazole, both second-generation antipsychotic medications, are approved by the United States Food and Drug Administration (FDA) for the treatment of irritability in children and adolescents with ASD. The use of psychopharmacology to treat the associated behavioral symptoms of ASD and co-occurring psychiatric conditions such as

This article is part of the Topical collection on *Autism Spectrum Disorders*

✉ Christopher J. McDougle
cmcdougle@mgh.harvard.edu

1 Massachusetts General Hospital, 55 Fruit St, Boston, MA 02114, USA

2 Lurie Center for Autism, 1 Maguire Road, Lexington, MA 02421, USA

3 Department of Psychiatry, Harvard Medical School, 25 Shattuck St, Boston, MA 02115, USA

4 Department of Psychiatry, Columbia University, 630 W 168th St, New York, NY 10032, USA

5 Center for Autism and Developmental Disorders, Maine Behavioral Healthcare, 78 Atlantic Place, South Portland, ME 04106, USA

6 Maine Medical Center Research Institute, Scarborough, ME, USA

attention-deficit/hyperactivity disorder, anxiety disorders, and mood disorders is common among individuals with ASD. In fact, a recent large population-based, nationwide, managed health plan claims database study of children and adults with ASD in the USA demonstrated that 59.6% of study participants received a prescription for a psychotropic medication during the 6-year study period [3].

There is an ongoing need for the development of safe and effective pharmacologic treatments for ASD. Psychopharmacology research in ASD over the past 3 years has focused on re-evaluating the efficacy of selective serotonin reuptake inhibitors (SSRIs) for the core symptoms of ASD, assessing whether repurposing medications from other fields of medicine may be an effective approach, and beginning to utilize biologically defined subtypes of ASD to predict treatment response. This article aims to review the recent literature pertaining to psychopharmacology for the core and associated symptoms of ASD including social impairment, repetitive behaviors, irritability, and language impairment. The results of recently published randomized, placebo-controlled trials of psychiatric medications for the core and associated symptoms of ASD are summarized in Table 1.

Social Impairment

Persistent social impairment occurring across multiple contexts is a core symptom of ASD. No pharmacologic treatments have been clearly demonstrated to provide benefit for social impairment in ASD. As such, early intensive behavioral intervention, which has been found to improve learning, communication, and social skills in young children with ASD, remains the standard of care. Several medication trials targeting social impairment in ASD have been published recently, most of which have focused on neuropeptides (oxytocin and vasopressin) and memantine.

Oxytocin is a neuropeptide associated with interpersonal bonding, parenting behaviors, and forming social attachments. Animal models relevant to ASD suggest that dysfunction of the oxytocin system may be involved in the social deficits of ASD and have demonstrated that exogenous oxytocin treatment results in increased social behaviors [4]. Three randomized controlled trials (RCTs) of intranasal oxytocin in adults with ASD have been published in the past 3 years. The first trial was a randomized double-blind, placebo-controlled cross-over trial that enrolled 15 males with ASD and 24 healthy controls (18–26 years) [5]. The participants received either one dose of intranasal oxytocin 20 international units (IU) or placebo spray in a random order on two consecutive days. Unlike the control group, individuals with ASD demonstrated enhanced social learning when given oxytocin compared to placebo. The second study was a 4-week randomized double-blind, placebo-controlled trial with parallel design conducted by Bernaerts et al. where 40 adult males with ASD were randomized to either intranasal oxytocin 24 IU per day or placebo [6]. The primary outcome of interest was social impairment, as measured by self- and informant-rated scores on the Social Responsiveness Scale (SRS) total score. Although improvement on the SRS occurred in both the oxytocin and placebo groups, there was no significant between-group differences ($p = 0.37$). There were significant differences between oxytocin and placebo observed in two of the secondary outcomes measured: reduced feelings of avoidance towards others ($p = 0.03$) and reduced repetitive behaviors ($p = 0.04$) which both persisted one year post-treatment. The third and largest study was a 6-week double-blind, placebo-controlled trial, which randomized 106 adult males (18–48 years) with ASD to either intranasal oxytocin 48 IU per day or placebo [7•]. The primary outcome was the Autism Diagnostic Observation Schedule (ADOS) reciprocity subscale. Similar to results from the trial completed by Bernaerts et al., improvement was observed in both the oxytocin and placebo groups, but oxytocin was not found to be superior to placebo ($p = 0.69$). Oxytocin was, however, associated with a statistically significant improvement compared to placebo in two of the secondary outcome measures: the ADOS repetitive behaviors subscale and duration of gaze fixation on socially relevant eye regions as measured by eye tracking software. Finally, a large, 6-month multi-site phase two trial investigating flexibly dosed intranasal oxytocin (maximum dose 80 IU per day) in 290 children and adolescents (3–17 years) with ASD [8] has completed enrollment and publication of the results is pending. Preliminary indications are that there was no significant difference between oxytocin and placebo on the primary outcome measure, the Aberrant Behavior Checklist (ABC) Social Withdrawal subscale. Taken together, the results from these four trials do not provide convincing evidence to support the use of intranasal oxytocin for the treatment of the core social deficits of ASD.

Vasopressin is a neuropeptide which primarily serves to regulate water reabsorption in the kidneys and increase peripheral vascular resistance. Recent research suggests that vasopressin may also act centrally to regulate and promote social behavior. Two trials assessing vasopressin for the treatment of social deficits in ASD have been published in the past 3 years. Bolognani et al. conducted a randomized placebo-controlled trial of balovaptan, a selective vasopressin V1a receptor antagonist, in 223 adult males (15–40 years) with ASD. Participants were randomized to placebo or one of three balovaptan doses (1.5 mg, 4 mg, or 10 mg per day). Balovaptan treatment was not associated with changes in the SRS-2 [9]. Parker et al. conducted a separate pilot 4-week randomized, double-blind, placebo-controlled trial which included 30 children (6–12 years) with ASD. The maximum dosage of intranasal arginine vasopressin (AVP) was 24 IU per day for children ≤ 9.5 years

Table 1 Select recent randomized, placebo-controlled trials of psychiatric medications in ASD with minimum sample size of 30 subjects

Target symptom	Publication	Medication	Population	Study design	Outcomes
Social impairment	Bernaerts et al. [6]	Oxytocin	40 adult males with ASD	4 weeks Parallel groups	No between-group differences on SRS
	Yamasue et al. [7]	Oxytocin	106 adult males with ASD	6 weeks Parallel groups	No between-group differences on ADOS reciprocity subscale
	Bolognani et al. [9]	Balovaptan	223 adult males with ASD	12 weeks Parallel groups	No between-group differences on SRS-2
	Parker et al. [10]	Vasopressin	30 children (6–12 years) with ASD	4 weeks Parallel groups	Vasopressin group had greater improvement in SRS-2 and CGI-I scores
	Hardan et al. [11]	Memantine	379 children (6–12 years) with ASD who responded to open-label memantine	12 weeks Withdrawal trial	No between-group difference in loss of therapeutic response on SRS
	Karahmadi et al. [12]	Memantine as adjunct to ABA therapy	60 children (< 14 years) with ASD	3 months Parallel groups	Memantine group had greater improvement in GARS total score and social interactions subscale, but no between-group differences on the communication subscale
Repetitive behaviors	Reddihough et al. [16]	Fluoxetine	146 children (7–18 years) with ASD	16 weeks Parallel groups	No between-group differences on CY-BOCS-PDD after controlling for covariates
	Herscu et al. [17]	Fluoxetine	158 children (5–17 years) with ASD	14 weeks Parallel groups	No between-group differences on CY-BOCS-PDD
	Politte et al. [22]	Extended-release guanfacine	62 children (5–14 years) with ASD	8 weeks Parallel groups; analysis of repetitive behaviors as secondary outcome measure	Extended-release guanfacine group had decreased repetitive behaviors on CY-BOCS-ASD
	Sprengers et al. [23]	Bumetanide	92 children (7–15 years) with ASD	13 weeks Parallel groups	No between-group differences on SRS-2
Irritability	Aran et al. [32]	CBD and whole-plant cannabis extract	150 individuals (5–21 years) with ASD	12 weeks Crossover design	CBD was not associated with improvement in disruptive behaviors compared to placebo. Whole-plant cannabis extract resulted in improvement in disruptive behaviors compared to placebo

Table 1 (continued)

Target symptom	Publication	Medication	Population	Study design	Outcomes
Language impairment	Frye et al. [38]	Folinic acid	48 children (3–15 years) with ASD	12 weeks Parallel groups	Folinic acid group had greater improvement in verbal language as measured by the CELF
	Batebi et al. [40]	Folinic acid as adjuvant to risperidone	55 children (4–12 years) with ASD	10 weeks Parallel groups	Folinic acid group had greater improvement on Inappropriate Speech subscale of ABC

ASD, autism spectrum disorder; *SRS*, Social Responsiveness Scale; *ADOS*, Autism Diagnostic Observation Schedule; *CGI-I*, Clinical Global Impressions-Improvement; *ABA*, applied behavior analysis; *GARS*, Gillian Autism Rating Scale; *CY-BOCS-PDD*, Children's Yale-Brown Obsessive Compulsive Scale, modified for pervasive developmental disorder; *CY-BOCS-ASD*, Children's Yale-Brown Obsessive Compulsive Scale, modified for autism spectrum disorder; *CBD*, cannabidiol; *CELF*, Clinical Evaluation of Language Fundamentals; *ABC*, Aberrant Behavior Checklist

and 32 IU per day for children 9.6–12.9 years. In this study, AVP was associated with improved caregiver-reported SRS-2 scores and a clinician-rated Clinical Global Impression-Improvement (CGI-I) score anchored to social-communication abilities compared to placebo ($p=0.005$) [10]. Arginine vasopressin was well tolerated and there was no difference in the rate of reported adverse effects between AVP and placebo. The findings of this study are tempered by several important limitations including small sample size and reliance on subjective outcome measures.

Memantine, a *N*-methyl-D-aspartate (NMDA) receptor antagonist with FDA approval for the treatment of Alzheimer disease, has been another medication of interest for the treatment of social impairment in ASD. Results from a series of three well-powered, multi-site phase two trials investigating extended-release memantine in children (6–12 years) with ASD were reported by Hardan et al. [11]. The first was a 50-week open-label trial ($n=906$), the second was a 12-week randomized double-blind placebo-controlled withdrawal trial which enrolled responders from the first trial ($n=479$), and the third was a ≤ 48 week open-label safety and tolerability extension trial ($n=747$). Sixty percent of participants demonstrated improvement in SRS scores after 12 weeks of treatment in the open-label phase. In the withdrawal trial, participants were randomized to weight-based full-dose memantine or a dose reduction by $\geq 50\%$. There was no difference in loss of therapeutic response on the SRS however, between memantine (69%) and placebo (66.7%) in the withdrawal phase. No new safety concerns were observed in the open-label extension trial. In a separate study, Karahmadi et al. investigated memantine as an adjunct to applied behavior analysis (ABA) therapy for children in a small randomized single-blind trial in children (< 14 years) with ASD [12]. Sixty participants were randomized to memantine 2.5 mg twice daily or placebo during a three-month course of ABA therapy. The participants were blinded to treatment assignment. The Gillian Autism Rating Scale (GARS) was completed by the participants' caregiver pre- and post-intervention. The memantine group had a statistically significant improvement compared to placebo in total GARS scores and the GARS social interactions subscale, but there was no between-group difference in the communication subscale. The conclusions of this study are significantly limited by small sample size and the lack of gold standard social and communication measures. Finally, a 12-week RCT of memantine for the treatment of social deficits in youth (8–18 years) with non-verbal learning disorder, high-functioning ASD, and related conditions is currently underway [13]. The results of the double-blind, placebo-controlled withdrawal trial are consistent with the negative results from the original 12-week randomized double-blind, placebo-controlled study of memantine for social impairment in 104 children (6–12 years) with ASD which demonstrated no significant between-group difference on the caregiver-rated SRS [14].

These recently published studies add to our knowledge base of psychopharmacology for social impairment, but do not significantly change current standards of care. The trials assessing oxytocin largely provide evidence against its use in adult males and preliminary indications of the results of a large phase 2 trial in children and adolescents also do not support its efficacy [8]. While recent studies of vasopressin were somewhat promising, there is currently insufficient evidence to support its use clinically. Although the more recent memantine trials demonstrated mixed results [11, 12], it may be worth investigating further and a large RCT of memantine for the treatment of social deficits in youth is currently underway [13].

Repetitive Behaviors

Restricted, repetitive patterns of behavior comprise the second core symptom domain of ASD and include repetitive motor movements, repetitive use of language, circumscribed preoccupations, ritualistic behaviors, difficulty coping with change, and unusual sensory interests. Similar to social impairment, there are no medications currently approved by the FDA for repetitive behavior in ASD. Randomized controlled trials of second-generation antipsychotics, particularly risperidone and aripiprazole, have demonstrated some benefit for repetitive behaviors. A recently published systematic review and meta-analysis including 21 RCTs ($n = 1309$) concluded that antisychotics "probably slightly reduce" repetitive behaviors in children and adolescents with ASD [15]. In light of this weak evidence and the significant side effect burden associated with second-generation antipsychotics including weight gain, metabolic problems, and extrapyramidal symptoms, they are not commonly used for this indication in clinical practice. Alternative safe and effective treatment options remain greatly needed.

The use of SSRIs for the treatment of repetitive behavior in ASD has received significant attention over the years with mixed results. Two recent randomized double-blind, placebo-controlled trials investigating fluoxetine in children and adolescents with ASD add new evidence that SSRIs do not provide benefit for repetitive behaviors compared to placebo as measured by the Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS). Reddihough et al. conducted a 16-week randomized double-blind, placebo-controlled, multi-site trial including 146 children and adolescents (mean age 11.2 years) with ASD [16••]. The primary outcome was the total score on the CY-BOCS, modified for pervasive developmental disorder (CY-BOCS-PDD). Forty-one percent of participants in the fluoxetine group and 36% of participants in the control group discontinued participation. The most common reasons for discontinuation included parent decision to discontinue, adverse events, and clinician decision to discontinue. The most common adverse events were mood disturbance, particularly irritability; gastrointestinal problems; and sleep disorders. Although the between-group mean difference in CY-BOCS-PDD score was statistically significant ($p = 0.03$) at endpoint, fluoxetine was not found to be superior to placebo after controlling for pre-specified covariates including sex, verbal ability, and imbalances in baseline and demographic variables ($p = 0.21$). Similar results were reported by Herscu et al. after completing a 14-week multi-site randomized double-blind, placebo-controlled trial of fluoxetine in 158 children (5–17 years) with ASD [17••]. No significant between-group differences were observed on the CY-BOCS-PDD ($p = 0.06$) and high rates of behavioral activation and adverse events were reported in both groups. Both trials may have encountered limitations inherent to the use of the CY-BOCS-PDD in individuals with ASD [18]. Individuals with ASD experience a range of repetitive behaviors, some of which are pleasurable and others of which are distressing. It is not clear that the CY-BOCS-PDD measures improvement in some of the repetitive behaviors of ASD which may be pleasurable or serve an adaptive function for the individual, yet cause impairment in daily living. Notably, the results from these two trials contrast with results from previous trials which included adults with ASD, suggesting that SSRIs may be more beneficial and better tolerated in post-pubertal individuals [19, 20].

Other pilot studies published within the past 3 years have investigated several other medications including guanfacine, bumetanide, and baclofen for repetitive behaviors in ASD. An analysis of secondary outcome measures of a previously reported 8-week randomized double-blind, placebo-controlled trial of extended-release guanfacine, an alpha-$_2$ agonist, for hyperactivity in 62 youth with ASD (5–14 years) [21], demonstrated significant between-group differences in repetitive behaviors as measured by the CY-BOCS-ASD [22]. Bumetanide, a loop diuretic thought to impact GABA through regulating chloride homeostasis, was assessed in a 91-day randomized double-blind, placebo-controlled trial in 92 youth (7–15 years) with ASD. Bumetanide was not superior to placebo for the primary outcome, the SRS-2, but was superior to placebo for one of the secondary outcomes, the Repetitive Behavior Scale-Revised [23]. Baclofen, a selective $GABA_B$ receptor agonist, was investigated as an adjuvant therapy to risperidone in a 10-week randomized double-blind, placebo-controlled trial including 64 children (3–12 years) with ASD [24]. Improvements in all the ABC subscales were observed in both treatment groups; the adjuvant baclofen group was superior to placebo plus risperidone only on the ABC hyperactivity subscale, indicating that it did not provide additional benefit beyond risperidone for repetitive behaviors. Finally, as discussed in the previous section, two recently published double-blind, placebo-controlled trials of oxytocin for social deficits in ASD noted improvements in repetitive behaviors [6, 7].

In summary, there is no strong evidence for any medication or class of medications for the treatment of repetitive behaviors in ASD. While antipsychotics may be somewhat efficacious, clinical use is limited by their side effect profile. The two recently published double-blind, placebo-controlled studies on SSRIs provide additional evidence against the use of SSRIs for repetitive behaviors in children and adolescents with ASD. There is, however, some new evidence to support the use of extended-release guanfacine for repetitive behaviors.

Irritability

Irritability is common among individuals with ASD and encompasses aggression, self-injurious behavior, property destruction, and severe tantrums. A practice pathway published in *Pediatrics* highlights the need to identify and address the medical, psychiatric, communicative, behavioral, and social factors that may be contributing to disruptive behaviors [25]. If such interventions fail, psychopharmacologic treatments may be necessary. As stated earlier, risperidone and aripiprazole are FDA approved for the treatment of irritability in children and adolescents with ASD. Risperidone's FDA approval was based upon two landmark 8-week randomized double-blind, placebo-controlled trials which each demonstrated significant reductions in irritability in youth with ASD associated with risperidone compared to placebo [26, 27]. Similarly, aripiprazole received its FDA indication for irritability in youth with ASD following the publication of two 8-week randomized double-blind, placebo-controlled trials demonstrating its efficacy [28, 29]. The considerable side effect burden of second-generation antipsychotics and lack of response in some patients have led to an ongoing search for alternative treatments.

Recent investigations have focused on the potential role of cannabidiol (CBD) in the treatment of irritability in ASD. Two prospective, open-label trials and one double-blind, placebo-controlled trial of CBD for irritability in ASD have been published within the past 3 years. The first prospective, open-label trial included 53 individuals (4–22 years) with ASD who received CBD for a median duration of 2 months [30]. Each participant received a preparation of 30% CBD in a ratio of 20:1 cannabidiol:Δ9-tetrahydrocannabinol (THC). Parents reported a change in symptoms of hyperactivity, sleep problems, self-injury, and anxiety on a three-point scale (improvement, no change, or worsening of symptoms). Sixty-eight percent of participants experienced parent-reported improvements in self-injury. The most frequently reported adverse effects included somnolence (22.6%) and appetite suppression (11.3%). The second prospective, open-label study investigated the effectiveness of a medical cannabis product among 188 participants with ASD (mean age 12.9 years) [31]. Most participants used oil with 30% CBD and 1.5% THC sublingually three times daily; however, the route of administration, preparation, dose, and schedule varied among patients. Changes in ASD-associated symptoms including restlessness, rage attacks, agitation, sleep problems, and anxiety were measured at 6 months using a seven-point caregiver scale. Rage attacks and agitation improved among 89% and 84% of participants, respectively. Notably, the dosages of other psychotropic medications were altered in 43% of patients in this study, possibly confounding the effect of the cannabis products. The main limitation of both these studies was the measurement of symptom improvement using unblinded parent reports without the use of a validated scale. The increasingly popular perception that cannabis-derived products have established efficacy for a wide range of conditions suggests that an underlying placebo effect may be partially influencing the results of these two prospective studies. Finally, the variability in the cannabis products, dosage, and route of administration further limits the conclusions that can be drawn from these two prospective, open-label studies.

Aran et al. conducted a 12-week randomized double-blind, placebo-controlled single-site trial studying the efficacy of CBD in 150 individuals (5–21 years) with ASD [32]. Participants were randomized to either whole-plant cannabis extract, pure CBD, or placebo followed by a washout and single crossover period. All participants received a cannabis product in at least one of the two treatment arms. The co-primary outcomes were change in total scores of the Home Situation Questionnaire-ASD (HSQ-ASD) and disruptive behavior measured using the CGI-I. Forty-nine percent of participants who received whole-plant cannabis extract experienced improvement in disruptive behavior (CGI-I = 1 "very much improved" or 2 "much improved"), which was significantly higher than the placebo response rate of 21% ($p = 0.005$). However, the improvement in disruptive behaviors for those treated with pure CBD (38%) was not superior to placebo ($p = 0.08$). Both whole-plant cannabis extract and CBD were generally well tolerated, with somnolence, decreased appetite, tiredness, euphoria, and anxiety being the most commonly reported adverse effects. Since this trial used a particular standardized formulation of CBD, the results may not be generalizable to other CBD oils derived from alternative strains. Limitations of this study include the heterogenous sample, limiting the ability to determine whether a particular subgroup may benefit the most from the use of cannabis-derived products. Additionally, while the crossover design allowed all participants to receive at least one type of cannabis formulation, the researchers were not able to utilize within-participant analyses as they noted a higher change from baseline in the first treatment period compared to the second, suggesting a placebo effect.

At this time, there is insufficient evidence to suggest that CBD be used to target irritability in individuals with ASD. Encouragingly, cannabis-derived products were generally well tolerated, and in fact may be associated with weight loss [32•], a potential advantage over second-generation antipsychotics. However, the relatively short durations of these studies limit the ability to detect potential long-term adverse effects which may be of particular salience in child and adolescent populations. These may include effects on cognition, increased risk of cannabis or other substance use disorders, and other psychiatric disorders (e.g. psychosis, mood disorders, anxiety). There are several challenges in designing high quality clinical trials assessing CBD including the vast

heterogeneity of compounds available and the observation that participants may be able to readily distinguish cannabis products from placebo due to its potentially psychoactive effects. A large, multi-center randomized placebo-controlled trial is warranted to help parcel out potential biases, develop an efficacious standardized formulation, and better assess long-term adverse effects. Until then, the magnitude of potential risks and benefits remain largely unknown. Currently, there are three double-blind randomized controlled trials and one open-label trial underway.

Language Impairment

Current estimates suggest that about a quarter to a third of children with ASD have verbal language deficits [33, 34]. The inability to fully communicate can lead to distress and discomfort that may manifest as agitation and irritability. Currently, the most effective treatment for language impairment associated with ASD includes early intensive behavioral, speech, and educational interventions [35]. At this time, there are no FDA-approved medications for language deficits in ASD. As such, the search for an effective and tolerable pharmacological intervention remains of critical importance. Folinic acid has emerged as a potentially promising candidate [36, 37]. Biochemically, folate metabolism abnormalities have been linked to ASD, including an association with cerebral folate deficiency [37]. Furthermore, the presence of folate receptor-α autoantibodies (FRAAs) may help predict treatment response [36].

Three randomized double-blind, placebo-controlled trials assessing folinic acid for the treatment of language impairment in children with ASD have been published. The first trial included 48 children (3–15 years) with ASD with a wide range of language impairment who were randomized to receive 12 weeks of either high dose folinic acid (two mg/kg per day, maximum 50 mg per day) or placebo [38•]. Baseline and 12-week verbal language skills were assessed using the age-appropriate version of the Clinical Evaluation of Language Fundamentals (CELF). The results of this trial demonstrated a statistically significant improvement in verbal communication in the folinic acid group compared to placebo ($p=0.02$). Folate receptor-α autoantibody status was predictive of response to treatment. Folinic acid was well tolerated and was not associated with any serious adverse effects.

The second placebo-controlled trial assessed commercially available low dose folinic acid (five mg twice daily) for 12 weeks [39]. The study included 19 children (3–10 years) with ASD and documented language impairments. Similar to the Frye et al. trial [38•], individuals were not excluded based on the severity of their language impairment. The primary outcome measure was the change in ADOS score and secondary outcome measures included the ADOS communication and social interaction subscales. A statistically significant difference in change in global ADOS score between groups was not observed; however, statistically significant greater improvements in the ADOS communication ($p=0.02$) and social interaction ($p=0.019$) subscale scores were observed in the folinic acid group compared to placebo.

The third 10-week randomized double-blind, placebo-controlled trial investigated folinic acid as an adjuvant to risperidone for inappropriate speech in 55 children (4–12 years) with ASD [40]. All participants received risperidone plus either high dose folinic acid (2 mg/kg, up to 50 mg per day) or placebo. The primary outcome was change in the Inappropriate Speech subscale of the ABC-Community (ABC-C). Greater improvements in the ABC-C Inappropriate Speech subscale were observed in the folinic acid group compared to the placebo group ($p=0.045$). Unlike the aforementioned studies, Batebi and colleagues reported a higher rate of adverse effects, including increased appetite and diarrhea, although these observations may be confounded by the concurrent use of risperidone.

Overall, these three studies provide promising preliminary evidence for folinic acid for the treatment of verbal language deficits in children with ASD. However, larger studies of longer duration are needed to provide conclusive evidence, optimize dosing, and further characterize the population (age, type of language deficit) that may be most responsive to treatment. Finally, since these three trials were all single-site studies, multi-site studies are needed to increase generalizability. Encouragingly, there are three ongoing multi-center trials currently registered with clinicaltrials.gov that have yet to publish results. Nevertheless, the overall positive outcomes presented in these early studies and the safety profile of folinic acid are encouraging.

Conclusion

The identification of safe and effective pharmacological treatments to ameliorate the core and associated symptoms of ASD has proven difficult. Psychopharmacology research in this area faces several significant challenges including the heterogeneity of ASD without biologically defined endophenotypes and the inherent difficulty of using a psychotropic medication to reverse neurobiological pathophysiology that likely occurs either prenatally or very early in development. The results from recent clinical trials research over the past few years also underscore high rates of placebo response in this population and the need for outcome measures that are both specific to the target symptoms of interest and capable of detecting change as important factors in study design. Despite these challenges, the field continues to make progress. Recent research has provided more conclusive evidence against the use of some medications such as oxytocin

for social impairment in children, adolescents, and adults and SSRIs for repetitive behaviors in children, as well as some new promising avenues for further study including folinic acid for language impairment.

Acknowledgements DS receives research support from the Thrasher Research Fund. This work was supported, in part, by the Nancy Lurie Marks Family Foundation.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
• Of importance
•• Of major importance

1. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 5th ed. Arlington. VA: APA. 2013.
2. Reichow B, Hume K, Barton EE, Boyd BA. Early intensive behavioral intervention (EIBI) for young children with autism spectrum disorders (ASD) [Internet]. Cochrane Database Syst Rev John Wiley and Sons Ltd. 2018 [cited 2021 Jun 13]. Available from: https://pubmed.ncbi.nlm.nih.gov/29742275/.
3. Feroe AG, Uppal N, Gutiérrez-Sacristán A, Mousavi S, Greenspun P, Surati R, et al. Medication use in the management of comorbidities among individuals with autism spectrum disorder from a large nationwide insurance database. JAMA Pediatr [Internet]. 2021 [cited 2021 Jun 13]; Available from: https://jamanetwork.com/journals/jamapediatrics/fullarticle/2780352.
4. Harony-Nicolas H, Kay M, du Hoffmann J, Klein ME, Bozdagi-Gunal O, Riad M, et al. Oxytocin improves behavioral and electrophysiological deficits in a novel Shank3-deficient rat. Elife [Internet]. eLife Sci Public Ltd. 2017 [cited 2021 Jun 11];6. Available from: https://pubmed.ncbi.nlm.nih.gov/28139198/.
5. Kruppa JA, Gossen A, Oberwelland Weib E, Kohls G, Grobheinrich N, Cholemkery H, et al. Neural modulation of social reinforcement learning by intranasal oxytocin in male adults with high-functioning autism spectrum disorder: a randomized trial. NPP [Internet] NPG. 2019 [cited 2021 Jun 10];44:749–56. Available from: https://pubmed.ncbi.nlm.nih.gov/30390065/.
6. Bernaerts S, Boets B, Bosmans G, Steyaert J, Alaerts K. Behavioral effects of multiple-dose oxytocin treatment in autism: a randomized, placebo-controlled trial with long-term follow-up. Mol Autism [Internet] BioMed Central Ltd. 2020 [cited 2021 Jun 10];11. Available from: https://pubmed.ncbi.nlm.nih.gov/31969977/.
7.• Yamasue H, Okada T, Munesue T, Kuroda M, Fujioka T, Uno Y, et al. Effect of intranasal oxytocin on the core social symptoms of autism spectrum disorder: a randomized clinical trial. Mol Psychiatry [Internet] Springer Nature. 2020 [cited 2021 Jun 10];25:1849–58. Available from: https://pubmed.ncbi.nlm.nih.gov/29955161/. **This is the largest study to have examined the effect of oxytocin on social impairment in adults with autism spectrum disorder.**
8. Spanos M, Chandrasekhar T, Kim SJ, Hamer RM, King BH, McDougle CJ, et al. Rationale, design, and methods of the Autism Centers of Excellence (ACE) network Study of Oxytocin in Autism to improve Reciprocal Social Behaviors (SOARS-B). Contemp Clin Trials [Internet]. Elsevier Inc. 2020 [cited 2021 Jun 10];98. Available from: https://pubmed.ncbi.nlm.nih.gov/32777383/.
9. Bolognani F, Del Valle Rubido M, Squassante L, Wandel C, Derks M, Murtagh L, et al. A phase 2 clinical trial of a vasopressin V1a receptor antagonist shows improved adaptive behaviors in men with autism spectrum disorder. Sci Transl Med [Internet] AAAS. 2019 [cited 2021 Jun 10];11. Available from: https://pubmed.ncbi.nlm.nih.gov/31043521/.
10. Parker KJ, Oztan O, Libove RA, Mohsin N, Karhson DS, Sumiyoshi RD, et al. A randomized placebo-controlled pilot trial shows that intranasal vasopressin improves social deficits in children with autism. Sci Transl Med [Internet] AAAS. 2019 [cited 2021 Jun 10];11. Available from: https://pubmed.ncbi.nlm.nih.gov/31043522/.
11. Hardan AY, Hendren RL, Aman MG, Robb A, Melmed RD, Andersen KA, et al. Efficacy and safety of memantine in children with autism spectrum disorder: results from three phase 2 multicenter studies. Autism [Internet] SAGE Publications Ltd. 2019 [cited 2021 Jun 10];23:2096–111. Available from: https://pubmed.ncbi.nlm.nih.gov/31027422/.
12. Karahmadi M, Tarrahi M, Vatankhah Ardestani S, Omranifard V, Farzaneh B. Efficacy of memantine as adjunct therapy for autism spectrum disorder in children aged 14 years. Adv Biomed Res [Internet] Medknow. 2018 [cited 2021 Jun 10];7:131. Available from: https://pubmed.ncbi.nlm.nih.gov/30320040/.
13. Memantine for the treatment of social deficits in youth with disorders of impaired social interactions - full text view - ClinicalTrials.gov [Internet]. [cited 2021 Jun 10]. Available from: https://clinicaltrials.gov/ct2/show/NCT03553875.
14. Aman MG, Findling RL, Hardan AY, Hendren RL, Melmed RD, Kehinde-Nelson O, et al. Safety and efficacy of memantine in children with autism: randomized, placebo-controlled study and open-label extension. J Child Adolesc Psychopharmacol [Internet]. Mary Ann Liebert Inc.; 2017 [cited 2021 Jun 14];27:403–12. Available from: https://pubmed.ncbi.nlm.nih.gov/26978327/.
15. D'Alò GL, De Crescenzo F, Amato L, Cruciani F, Davoli M, Fulceri F, et al. Impact of antipsychotics in children and adolescents with autism spectrum disorder: a systematic review and meta-analysis. Health Qual Life Outcomes [Internet] BioMed Central Ltd. 2021 [cited 2021 Jun 10];19. Available from: https://pubmed.ncbi.nlm.nih.gov/33494757/.
16.•• Reddihough DS, Marraffa C, Mouti A, O'Sullivan M, Lee KJ, Orsini F, et al. Effect of fluoxetine on obsessive-compulsive behaviors in children and adolescents with autism spectrum disorders: a randomized clinical trial. JAMA - J Am Med Assoc [Internet] Am Med Assoc. 2019 [cited 2021 Jun 12];322:1561–9. Available from: https://pubmed.ncbi.nlm.nih.gov/31638682/. **This is a randomized double-blind, placebo-controlled, multisite trial which assessed fluoxetine for repetitive behaviors in children with autism spectrum disorder. Fluoxetine was not found to be superior to placebo after controlling for prespecified covariates.**
17.•• Herscu P, Handen BL, Arnold LE, Snape MF, Bregman JD, Ginsberg L, et al. The SOFIA Study: Negative multi-center study of low dose fluoxetine on repetitive behaviors in children and adolescents with autistic disorder. J Autism Dev Disord

[Internet] Springer. 2020 [cited 2020 Sep 28];50:3233–44. Available from: https://pubmed.ncbi.nlm.nih.gov/31267292/. **This is another randomized double-blind, placebo-controlled, multisite trial which assessed fluoxetine for repetitive behaviors in children with autism spectrum disorder. No significant between-group differences were detected and fluoxetine was poorly tolerated.**

18. King BH. Fluoxetine and repetitive behaviors in children and adolescents with autism spectrum disorder [Internet]. JAMA - J Am Med Assoc Am Med Assoc. 2019 [cited 2021 Jun 12]. p. 1557–8. Available from: https://pubmed.ncbi.nlm.nih.gov/31638657/.
19. Hollander E, Soorya L, Chaplin W, Anagnostou E, Taylor BP, Ferretti CJ, et al. A double-blind placebo-controlled trial of fluoxetine for repetitive behaviors and global severity in adult autism spectrum disorders. Am J Psychiatry [Internet] Am Psych Assoc. 2012 [cited 2021 Jun 10];169:292–9. Available from: https://pubmed.ncbi.nlm.nih.gov/22193531/.
20. McDougle CJ, Naylor ST, Cohen DJ, Volkmar FR, Heninger GR, Price LH. A double-blind, placebo-controlled study of fluvoxamine in adults with autistic disorder. Arch Gen Psychiatry [Internet] Am Med Assoc. 1996 [cited 2021 Jan 17];53:1001–8. Available from: https://pubmed.ncbi.nlm.nih.gov/8911223/.
21. Scahill L, McCracken JT, King BH, Rockhill C, Shah B, Politte L, et al. Extended-release guanfacine for hyperactivity in children with autism spectrum disorder. Am J Psych [Internet] Am Psych Assoc. 2015 [cited 2021 Jun 12];172:1197–206. Available from: https://pubmed.ncbi.nlm.nih.gov/26315981/.
22. Politte LC, Scahill L, Figueroa J, McCracken JT, King B, McDougle CJ. A randomized, placebo-controlled trial of extended-release guanfacine in children with autism spectrum disorder and ADHD symptoms: an analysis of secondary outcome measures. NPP [Internet]. Nature Publish Group. 2018 [cited 2021 Jun 10];43:1772–8. Available from: https://pubmed.ncbi.nlm.nih.gov/29540864/.
23. Sprengers JJ, van Andel DM, Zuithoff NPA, Keijzer-Veen MG, Schulp AJA, Scheepers FE, et al. Bumetanide for core symptoms of autism spectrum disorder (BAMBI): a single center, double-blinded, participant-randomized, placebo-controlled, phase-2 superiority trial. J Am Acad Child Adolesc Psych [Internet] Elsevier Inc. 2020 [cited 2021 Jun 10]; Available from: https://pubmed.ncbi.nlm.nih.gov/32730977/.
24. Mahdavinasab SM, Saghazadeh A, Motamed-Gorji N, Vaseghi S, Mohammadi MR, Alichani R, et al. Baclofen as an adjuvant therapy for autism: a randomized, double-blind, placebo-controlled trial. Eur Child Adolesc Psychiatry [Internet]. Dr. Dietrich Steinkopff Verlag GmbH and Co KG. 2019 [cited 2021 Jun 10];28:1619–28. Available from: https://pubmed.ncbi.nlm.nih.gov/30980177/.
25. McGuire K, Fung LK, Hagopian L, Vasa RA, Mahajan R, Bernal P, et al. Irritability and problem behavior in autism spectrum disorder: a practice pathway for pediatric primary care. Pediatr Am Acad Pediatr. 2016;137:S136–48.
26. McCracken JT, McGough J, Shah B, Cronin P, Hong D, Aman MG, et al. Risperidone in children with autism and serious behavioral problems. N Engl J Med [Internet]. 2002 [cited 2018 Oct 17];347:314–21. Available from: http://www.nejm.org/doi/abs/https://doi.org/10.1056/NEJMoa013171.
27. Shea S, Turgay A, Carroll A, Schulz M, Orlik H, Smith I, et al. Risperidone in the treatment of disruptive behavioral symptoms in children with autistic and other pervasive developmental disorders. Pediatrics [Internet]. 2004 [cited 2018 Nov 6];114:e634–41. Available from: http://pediatrics.aappublications.org/cgi/doi/https://doi.org/10.1542/peds.2003-0264-F.
28. Owen R, Sikich L, Marcus RN, Corey-Lisle P, Manos G, McQuade RD, et al. Aripiprazole in the treatment of irritability in children and adolescents with autistic disorder. Pediatr [Internet]. 2009 [cited 2018 Oct 24];124:1533–40. Available from: http://pediatrics.aappublications.org/cgi/doi/https://doi.org/10.1542/peds.2008-3782.
29. Marcus RN, Owen R, Kamen L, Manos G, McQuade RD, Carson WH, et al. A Placebo-controlled, fixed-dose study of aripiprazole in children and adolescents with irritability associated with autistic disorder. J Am Acad Child Adolesc Psychiatry [Internet]. 2009 [cited 2018 Oct 17];48:1110–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19797985.
30. Barchel D, Stolar O, De-Haan T, Ziv-Baran T, Saban N, Fuchs DO, et al. Oral cannabidiol use in children with autism spectrum disorder to treat related symptoms and co-morbidities. Front Pharmacol [Internet] Frontiers Media S.A. 2019 [cited 2021 Jun 13];9. Available from: https://pubmed.ncbi.nlm.nih.gov/30687090/.
31. Bar-Lev Schleider L, Mechoulam R, Saban N, Meiri G, Novack V. Real life experience of medical cannabis treatment in autism: analysis of safety and efficacy. Sci Rep. 2019;9:200.
32.• Aran A, Harel M, Cassuto H, Polyansky L, Schnapp A, Wattad N, et al. Cannabinoid treatment for autism: a proof-of-concept randomized trial. Mol Autism [Internet] BioMed Central Ltd. 2021 [cited 2021 Jun 10];12. Available from: https://pubmed.ncbi.nlm.nih.gov/33536055/. **This is a randomized double-blind, placebo-controlled trial assessing CBD for irritability in individuals with ASD.**
33. Brignell A, Morgan AT, Woolfenden S, Klopper F, May T, Sarkozy V, et al. A systematic review and meta-analysis of the prognosis of language outcomes for individuals with autism spectrum disorder. Autism Dev Lang Impair. 2018;3.
34. Norrelgen F, Fernell E, Eriksson M, Hedvall Å, Persson C, Sjölin M, et al. Children with autism spectrum disorders who do not develop phrase speech in the preschool years. Autism. 2015;19.
35. Parsons L, Cordier R, Munro N, Joosten A, Speyer R. A systematic review of pragmatic language interventions for children with autism spectrum disorder. PLoS One. 2017;12:e0172242.
36. Frye RE, Sequeira JM, Quadros E V, James SJ, Rossignol DA. Cerebral folate receptor autoantibodies in autism spectrum disorder. Mol Psychiatry [Internet]. 2013 [cited 2018 Sep 13];18:369–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22230883.
37. Ramaekers VT, Rothenberg SP, Sequeira JM, Opladen T, Blau N, Quadros E V., et al. Autoantibodies to folate receptors in the cerebral folate deficiency syndrome. N Engl J Med. 2005;352.
38.• Frye RE, Slattery J, Delhey L, Furgerson B, Strickland T, Tippett M, et al. Folinic acid improves verbal communication in children with autism and language impairment: a randomized double-blind placebo-controlled trial. Mol Psychiatry. 2018;23:247–56. **This is the first double-blind, placebo-controlled trial demonstrating that high dose folinic acid was associated with improvement in verbal communication in children with autism spectrum disorder.**
39. Renard E, Leheup B, Guéant-Rodriguez RM, Oussalah A, Quadros E V., Guéant JL. Folinic acid improves the score of Autism in the EFFET placebo-controlled randomized trial. Biochimie [Internet] Elsevier B.V. 2020 [cited 2021 Jun 10];173:57–61. Available from: https://pubmed.ncbi.nlm.nih.gov/32387472/.
40. Batebi N, Moghaddam HS, Hasanzadeh A, Fakour Y, Mohammadi MR, Akhondzadeh S. Folinic acid as adjunctive therapy in treatment of inappropriate speech in children with autism: a double-blind and placebo-controlled randomized trial. Child Psychiatry Hum Dev [Internet]. Springer; 2020 [cited 2021 Jun 13]; Available from: https://pubmed.ncbi.nlm.nih.gov/33029705/

Psychoneuroendocrinology (2013) 38, 2952—2961

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/psyneuen

Reductions in circulating endocannabinoid levels in individuals with post-traumatic stress disorder following exposure to the world trade center attacks

Matthew N. Hill [a,b,1,*], Linda M. Bierer [c,1], Iouri Makotkine [c], Julia A. Golier [c], Sandro Galea [d], Bruce S. McEwen [b], Cecilia J. Hillard [e], Rachel Yehuda [c]

[a] The Hotchkiss Brain Institute, Department of Cell Biology & Anatomy and Psychiatry, University of Calgary, Calgary, AB, Canada
[b] Laboratory of Neuroendocrinology, The Rockefeller University, New York, NY, USA
[c] Traumatic Stress Studies Division, Mount Sinai School of Medicine and James J. Peters Veterans Affairs Medical Center, Bronx, NY, USA
[d] Department of Epidemiology, Columbia Mailman School of Public Health, New York, NY, USA
[e] Neuroscience Research Center and Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI, USA

Received 16 May 2013; received in revised form 3 August 2013; accepted 18 August 2013

KEYWORDS
Stress;
PTSD;
Trauma;
Endocannabinoid;
HPA axis;
Cortisol;
N-arachidonylethanolamine;
Anandamide;
2-Arachidonoylglycerol;
Anxiety

Summary Endocannabinoid (eCB) signaling has been identified as a modulator of adaptation to stress, and is integral to basal and stress-induced glucocorticoid regulation. Furthermore, interactions between eCBs and glucocorticoids have been shown to be necessary for the regulation of emotional memories, suggesting that eCB function may relate to the development of post-traumatic stress disorder (PTSD). To examine this, plasma eCBs were measured in a sample (n = 46) drawn from a population-based cohort selected for physical proximity to the World Trade Center (WTC) at the time of the 9/11 attacks. Participants received a structured diagnostic interview and were grouped according to whether they met diagnostic criteria for PTSD (no PTSD, n = 22; lifetime diagnosis of PTSD = 24). eCB content (2-arachidonoylglycerol (2-AG) and anandamide (AEA)) and cortisol were measured from 8 a.m. plasma samples. Circulating 2-AG content was significantly reduced among individuals meeting diagnostic criteria for PTSD. The effect of reduced 2-AG content in PTSD remained significant after controlling for the stress of exposure to the WTC collapse, gender, depression and alcohol abuse. There were no significant group differences for AEA or cortisol levels;

* Corresponding author at: Hotchkiss Brain Institute, University of Calgary, 3330 Hospital Drive NW, Calgary, AB, Canada T2N 4N1. Tel.: +1 403 220 8466.
E-mail address: mnhill@ucalgary.ca (M.N. Hill).
[1] These authors contributed equally to the writing of this manuscript.

0306-4530/$ — see front matter
http://dx.doi.org/10.1016/j.psyneuen.2013.08.004

however, across the whole sample AEA levels positively correlated with circulating cortisol, and AEA levels exhibited a negative relationship with the degree of intrusive symptoms within the PTSD sample. This report shows that PTSD is associated with a reduction in circulating levels of the eCB 2-AG. Given the role of 2-AG in the regulation of the stress response, these data support the hypothesis that deficient eCB signaling may be a component of the glucocorticoid dysregulation associated with PTSD. The negative association between AEA levels and intrusive symptoms is consistent with animal data indicating that reductions in AEA promote retention of aversive emotional memories. Future work will aim to replicate these findings and extend their relevance to clinical pathophysiology, as well as to neuroendocrine and molecular markers of PTSD.

1. Introduction

The development of post-traumatic stress disorder (PTSD) is related to abnormalities in the regulation of biological stress response systems, specifically the hypothalamic–pituitary–adrenal (HPA) axis and sympathetic nervous system (Krystal and Neumeister, 2009; Yehuda, 2009). Current theories suggest that increased responsivity of glucocorticoid receptors resulting in reduced cortisol levels at the time of a traumatic exposure, or immediately thereafter, will result in increased noradrenergic transmission associated with a prolonged state of distress (Pervanidou and Chrousos, 2010). This state of arousal will result in the 'hyperconsolidation' of emotional memories, and ultimately, could lead to the development of PTSD. However, since not all persons exposed to trauma develop PTSD, it has also been of interest to identify hormones or signaling molecules that could be responsible for either increasing the probability of PTSD or for promoting resistance to PTSD development. By examining a population-based cohort of individuals exposed to the World Trade Center (WTC) collapse, we have recently identified genetic markers related to glucocorticoid signaling in individuals who developed PTSD (Yehuda et al., 2009; Sarapas et al., 2011). Accordingly, further investigation of systems involved in the regulation and actions of glucocorticoid hormones was undertaken to explore the underlying biological mechanisms specific to the development of PTSD.

The endocannabinoid (eCB) system represents an ideal candidate system to investigate with respect to the pathophysiology of PTSD (Hill and Gorzalka, 2009; Neumeister, 2013). The eCB system is primarily composed of a central CB_1 receptor and two endogenous ligands (*N*-arachidonylethanolamine [anandamide; AEA] and 2-arachidonoylglycerol [2-AG]). In addition, there are also CB_2 receptors, whose expression is primarily restricted to immune cells of macrophage lineage but may also be expressed in the CNS, as well as a family of fatty acid ethanolamides, such as palmitoylethanolamide and oleoylethanolamide, which share biosynthetic and catabolic pathways with AEA, but are not ligands for the CB receptors. The eCB system is known to constrain activation of the stress response through distributed actions in limbic and hypothalamic circuits in the brain (Riebe and Wotjak, 2011; Hill and Tasker, 2012). More so, eCB signaling is responsive to glucocorticoid hormones (Di et al., 2003; Hill et al., 2010a), and the recruitment of eCB signaling by glucocorticoids has been found to mediate many of the physiological actions of these hormones, including negative feedback termination of HPA axis activity (Evanson et al., 2010; Hill et al., 2011) and modulation of emotionally salient cognitive processes (Campolongo et al., 2009; Atsak et al., 2012). In addition to the role of eCBs in mediating the actions of glucocorticoids, eCB signaling is involved in many processes which are dysregulated in PTSD, such as the extinction of emotionally aversive memories (Marsicano et al., 2002; Plendl and Wotjak, 2010; Gunduz-Cinar et al., 2013), habituation and adaptation to stress (Patel et al., 2005b; Hill et al., 2010b) and release of catecholamines from sympathetic nerve terminals (Ishac et al., 1996; Bellocchio et al., 2013).

Based on these converging lines of evidence and the neurobiology of PTSD, we hypothesize that reductions in circulating concentrations of eCB represent a biomarker of stress vulnerability, and that deficient eCB signaling is involved in the biological processes related to PTSD. To examine these hypotheses, we evaluated circulating concentrations of the eCBs AEA and 2-AG in a sample derived from a population-based cohort selected for physical proximity to the WTC at the time of the 9/11 attacks. A sub-sample of this cohort was previously selected for a genome-wide association study of PTSD (Yehuda et al., 2009). This is an ideal cohort in which to search for biological identifiers of PTSD, as risk for exposure was based on proximity to the WTC, and was uncomplicated by the confound that exposure often introduces to genetic association studies (e.g., familial risk for exposure to interpersonal violence). Furthermore, circulating eCB concentrations are elevated in response to acute stress (Hill et al., 2009b; Dlugos et al., 2012), whereas deficient recruitment of eCB signaling in response to acute stress, or low basal eCB contents in the circulation, have been related to excessive stress-induced activation of the HPA axis (Chouker et al., 2010; Dlugos et al., 2012) and negative long-term outcomes following exposure to stressful events, such as cardiac surgery (Hauer et al., 2012). Our data indicate that PTSD is associated with reduced concentrations of 2-AG in the circulation and that both 2-AG and AEA concentrations associate with specific symptom clusters within PTSD.

2. Methods

2.1. Participants

Subjects were 46 participants who comprised a subset of a population based sample ($n = 109$) evaluated at the Mount Sinai School of Medicine (MSSM) four to six years following the 9/11 attacks (Yehuda et al., 2009). The subjects studied at the MSSM were those who responded positively to a mailing asking participants to have an in-person diagnostic

evaluation, complete self-report questionnaires and permit an 8 am blood draw. The study was approved by the Institutional Review Board (IRB) at the Mount Sinai School of Medicine; all subjects provided written informed consent and were subsequently screened to establish eligibility. Subjects were excluded if they met criteria for primary psychotic disorder, bipolar illness, alcohol or substance dependence, or major endocrine, neurological, or other medical illness, including diabetes. Although subjects with PTSD showed greater number of lifetime psychiatric diagnoses than those without PTSD (Table 1), as has been previously described (Breslau et al., 2000), none were receiving psychiatric treatment or taking psychotropic medications at the time of participation. The 46 subjects included in this report were those with remaining frozen samples available for endocannabinoid assay who had previously provided consent for analyses of compounds unrelated to the goals of the initial investigation, i.e., associations with genotype. Selection was based purely on availability of biological sample in conjunction with appropriate consent, and not on any other inclusion or exclusion criteria, clinical or otherwise. Subjects with endocannabinoid determinations ($n = 46$) were similar in age, gender distribution, and lifetime trauma exposure histories to the remaining members of the original cohort ($n = 63$). Of 46 subjects, 22 were deemed to have suffered direct, high magnitude exposure to the events of 9/11 (direct exposure to the events of 9/11), whereas 24 reported indirect exposure. 'Direct exposure' was assigned to participants who were in the vicinity of the World Trade Towers at the time of the attacks with immediate threat to their safety or survival, or had suffered the loss of family members or intimate friends on 9/11. 'Indirect exposure' was attributed to those who witnessed collapse of the Towers from a safe distance, were informed of the attacks while out of town, or observed the events on TV, without enduring direct threat to self or family members.

2.2. Clinical evaluation

Psychologists with established interrater reliability administered the Clinician Administered PTSD Scale (CAPS; Blake et al., 1995) and the Structured Clinical Interview for the DSM-IV (Spitzer et al., 1995) to determine the presence of PTSD and other psychiatric disorders. 24 of the 46 subjects interviewed reached threshold criterion for PTSD in their lifetime, 22 cases did not meet criteria for lifetime presence of this disorder. Participants also completed the self-report Posttraumatic Stress Diagnostic Scale (Foa et al., 1993).

2.2.1. Blood drawing and processing

Fasting blood samples were obtained between 08:00 and 08:30 h, into EDTA containing tubes (BD Vacutainer, K2 EDTA (K2E) Plus Blood Collection Tubes), placed on ice, spun within 40 min of collection, and frozen at −70 °C until analysis. Plasma cortisol levels were determined using commercially available RIA kits (DiaSorin Inc., Stillwater, MN). The intra- and inter-assay coefficients of variation were 2.3% and 6.1% for cortisol, respectively.

Determination of the plasma concentrations of the eCBs 2-AG and AEA, as well as two other fatty acid ethanolamides, palmitoylethanolamide (PEA) and oleoylethanolamide (OEA) was performed using a previously published method (Hill et al., 2008). PEA and OEA are non-cannabinoid fatty acid ethanolamides which share biosynthetic and metabolic pathways with AEA, but do not activate cannabinoid receptors. In brief, all extractions were performed using Bond Elut C18 solid-phase extraction columns (1 ml; Varian Inc, Lake Forest, CA). Plasma samples (0.5 ml each) were thawed and made up to 15% ethanol, to which the internal standards [$^2H_8$]-AEA (16.9 pmol) and [$^2H_8$]-2-AG (46.5 pmol) (Cayman Chemicals, Ann Arbor, MI) were added. Samples were vortexed and centrifuged at 1000 × *g* for 4 min. The supernatant was loaded on C18 columns, which have been conditioned

Table 1 Demographic characteristics of sample population ($n = 46$).

Demographics and clinical characteristics	No PTSD ($n = 22$) Mean ± SD or *n* (%)	Lifetime PTSD ($n = 24$) Mean ± SD or *n* (%)	Group comparisons $F_{1,44}$, *p*- or χ^2, *df* = 1, *p*
Age (years)	57.09 ± 13.44	51.92 ± 15.54	$F = 1.44$, *ns*
Gender (M/F)	10 M/12 F	12 M/12 F	$\chi^2 = .11$, *ns*
Race: White	21 (45.7%)	21 (45.7%)	
African-American	3 (0%)	2 (4.3%)	
Latino	1 (2.2%)	0 (0%)	
Asian/Pacific Islander	0 (0%)	1 (2.2%)	$\chi^2 = 3.92$, df = 3, *ns*
Education (years)	15.3 ± 3.3	15.6 ± 3.7	$F = 0.58$, *ns*
Childhood trauma questionnaire (total)	7.33 ± 2.00	8.71 ± 3.53	$F = 2.38$, *ns*
Directly affected by WTC	8 (36.4%)	14 (58.3%)	$\chi = 2.22$, *ns*
PTSD severity (CAPS[a] total score)	14.67 ± 11.85	59.95 ± 27.57	$F = 41.28$, $p < .0005$
CAPS intrusive subscale	7.22 ± 6.59	19.68 ± 9.11	$F = 22.50$, $p < .0005$
CAPS avoidance subscale	2.22 ± 3.77	20.58 ± 13.28	$F = 31.93$, $p = 0.001$
CAPS hyperarousal subscale	5.22 ± 4.63	19.74 ± 10.55	$F = 28.77$, $p = 0.001$
Current MDD	2 (11.1%)	3 (20.0%)	$\chi^2 = .503$, *ns*
Past MDD	4 (22.2%)	8 (53.3%)	$\chi^2 = 3.42$ ($p = .064$)
Past alcohol abuse	0 (0%)	4 (25.0%)	$\chi^2 = 5.10$, .024
Past drug abuse	2 (11.1%)	1 (6.2%)	$\chi^2 = .244$, *ns*

[a] Clinician Administered PTSD Scale.

with 1 ml redistilled ethanol and 3 ml of double distilled water (ddH_2O). The remaining pellet was washed with 100 μl of 15% ethanol and centrifuged again for 3 min. The resulting supernatant was also loaded onto the C18 column. Columns were washed with 5 ml ddH_2O and eluted with 1 ml of ethyl acetate. The ethyl acetate layer in the resulting elute was removed and dried under N_2. Lipids in the residual ddH_2O phase were extracted by mixing with an additional 1 ml of ethyl acetate, which was added to the original ethyl acetate solution. Once dried, samples were resuspended in 20 μl of methanol and stored at −80 °C. AEA and 2-AG were quantified using isotope-dilution, atmospheric pressure, chemical ionization liquid chromatography/mass spectrometry (LC-APCI-MS) as described previously (Patel et al., 2005a). For analysis, the *m/z* transitions for each molecule were as follows: AEA 348.3, AEA-d8 356.3, 2-AG 379.3, 2-AG-d8 387.3, OEA 326.3 and PEA 300.3. For analysis of 2-AG, given that it isomerizes to 1,3-AG during the extraction process (and since there is virtually no naturally occurring 1,3-AG), both 2-AG and 1,3-AG peaks were integrated and added together to determine levels of 2-AG.

2.3. Statistical analysis

Eight am plasma 2-AG, AEA, OEA and PEA, and cortisol concentrations were compared between subjects with and without PTSD, using univariate analysis of variance (ANOVA), and co-variance (ANCOVA), with PTSD diagnosis as the fixed factor, and gender as the covariate. Severity of exposure ('direct' versus 'indirect' exposure) to WTC related events was compared for both PTSD and non-PTSD groups using chi-square tests, and used as a covariate in comparisons of endocannabinoids between subjects with and without PTSD. There were no significant differences between the PTSD and no-PTSD groups in age, and none of the outcome variables were themselves related to age. Despite this, comparisons were performed with and without covariation for age. Analyses of covariance were additionally conducted for lifetime alcohol abuse and depression diagnoses to evaluate the extent to which results were influenced by these variables. Bivariate correlations were performed to examine relationships of plasma eCBs with cortisol, as well as with CAPS and PDS ratings for overall PTSD symptom severity, and with PTSD symptom clusters (avoidance, hyperarousal, intrusive). Finally intercorrelations among the eCBs and ethanolamides were evaluated for the entire sample, and within the PTSD and no-PTSD groups separately.

3. Results

Table 1 shows the clinical characteristics of the sample. There were no differences in any variables except for CAPS scores of PTSD severity, lifetime diagnosis of major depression and past alcohol abuse, all of which were higher in the PTSD compared to the no-PTSD sample. Interestingly, despite differences in CAPS scores, there were no differences between the PTSD and no-PTSD groups in actual exposures to traumatic events, including childhood adversity or sexual abuse. Single time point analysis of 8am cortisol demonstrated no significant differences in basal cortisol between individuals who did or did not have a diagnosis of PTSD [$F(1, 43) = .001$, *ns*. PTSD, 14.95 ± 4.64 μg/dL; no PTSD, 14.91 ± 5.41 μg/dL ($M \pm SD$); cortisol by PTSD, covaried for gender [$F(1, 42) = .004$, *ns*. PTSD, 14.87 ± 1.04 μg/dL; no PTSD, 15.01 ± 1.11 μg/dL ($M \pm SE$).

Examination of 2-AG concentrations in the circulation, comparing individuals with and without lifetime PTSD, revealed a significant main effect for PTSD diagnosis [$F(1, 43) = 6.94$, $p = 0.012$; 2-AG by PTSD, covaried for gender $F(1, 43) = 7.42$, $p = .009$; Fig. 1], such that PTSD was associated with significantly lower circulating concentrations of 2-AG. There was no significant difference in AEA [$F(1, 43) = 0.16$,

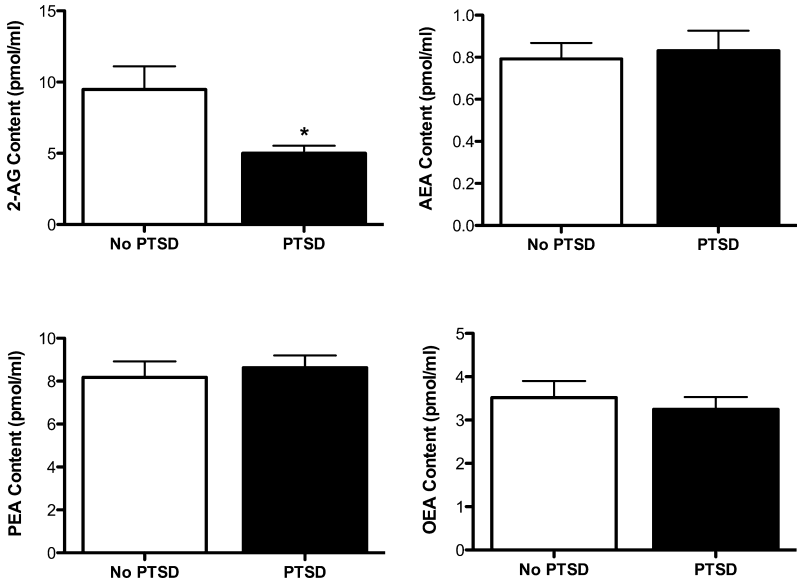


Figure 1 Plasma 2-arachidonoylglycerol (2-AG), anandamide (AEA), palmitoylethanolamide (PEA) and oleoylethanolamide (OEA) concentrations in individuals who were diagnosed with post-traumatic stress disorder (PTSD) or healthy age and gender matched controls (No PTSD). Plasma levels of 2-AG, but not AEA, PEA or OEA, are found to be reduced in PTSD. Data are presented as mean ± SEM. Significant diferences ($p < 0.05$) are denoted by *.

ns; Fig. 1], OEA [$F(1, 43) = 0.44$, ns; Fig. 1] or PEA [$F(1, 43) = 0.27$, ns; Fig. 1] between subjects with and without PTSD. Since individuals with PTSD reported significantly greater lifetime alcohol abuse, we covaried for this diagnosis, which did not affect the significance of the difference in 2-AG levels between subjects with and without PTSD [$F(1, 33) = 4.72$, $p = 0.037$]. Similarly, as a greater proportion of subjects with PTSD also reported lifetime depressive diagnoses (major depression or dysthymia), we covaried for depression [$F(1, 34) = 7.95$, $p = 0.008$] which likewise did not influence the significant reduction in 2-AG seen for those with PTSD.

To further explore the apparent relationship of low concentrations of circulating 2-AG to lifetime PTSD diagnosis, we examined whether severity of exposure to the WTC collapse alone was sufficient to influence circulating 2-AG. A significant effect of intensity of exposure was noted for 2-AG [indirect exposure (9.81 ± 1.01), direct exposure, (4.85 ± 1.08); $F(1, 43) = 11.20$, $p = .005$]. Similar to the result for PTSD, there was no effect of exposure to the WTC collapse on plasma levels of AEA [indirect exposure (0.82 ± 0.09), direct exposure (0.87 ± 0.10); $F(1, 43) = 0.14$, ns], PEA [indirect exposure (8.41 ± 0.67), direct exposure (8.66 ± 0.72); $F(1, 43) = 0.07$, ns] or OEA [indirect exposure (3.03 ± 0.32), direct exposure (3.73 ± 0.34); $F(1, 43) = 2.23$, ns]. Given that the extent of exposure to the WTC collapse was related to reduced levels of 2-AG, an analysis of covariance was performed to determine if the relationship between PTSD and 2-AG was mediated by the exposure. Controlling for intensity of WTC exposure revealed significant effects for both exposure [$F(1, 43) = 6.25$, $p = .016$] and PTSD [$F(1, 43) = 4.71$, $p = .036$]. Thus, even after controlling for exposure to the WTC collapse, the reduction in circulating 2-AG levels in individuals with lifetime PTSD remained significant. These data do suggest, however, that exposure to a traumatic stressor alone may have residual effects to diminish circulating 2-AG levels.

A series of correlation analyses were undertaken to assess associations among the eCBs and ethanolamides, as well as the relationships of these measures to circulating cortisol, and to symptom profiles of the participants. For the entire sample, there was no significant relationship of 2-AG with AEA, PEA or OEA (r's between .05 and .18). However, there were significant intercorrelations among AEA, PEA and OEA (r's between .59 and .80, all p's < 0005), suggesting that 2-AG is regulated independently from AEA, PEA and OEA, which are regulated in concert. These intercorrelation results were similar for the no-PTSD and PTSD subgroups.

We also examined correlations between cortisol and eCB, OEA and PEA concentrations (see Fig. 2). For the entire sample, there was a significant positive relationship between cortisol and OEA ($r = 0.373$, $n = 45$, $p = 0.012$) and AEA ($r = 0.345$, $n = 45$, $p = 0.02$) and positive relationships of cortisol at trend levels of significance with PEA ($r = 0.284$, $n = 45$, $p = 0.059$), indicating that all of the fatty acid ethanolamides exhibit some degree of positive association with circulating cortisol concentrations. In the entire sample, there was no relationship between cortisol and 2-AG concentrations ($r = 0.07$, $n = 45$, ns). Interestingly, when data from the PTSD group alone are analyzed, the correlation between AEA and cortisol concentrations is lost, while a positive and significant correlation between 2-AG and cortisol emerges ($r = 0.434$, $n = 24$, $p = 0.034$). This suggests that relationships between eCB concentrations and glucocorticoids could differ depending on the presence or history of PTSD.

For analysis of how the eCBs and ethanolamides correlated to PTSD symptom clusters, correlations were performed only within the PTSD group. 2-AG concentration was significantly associated with current CAPS subscale

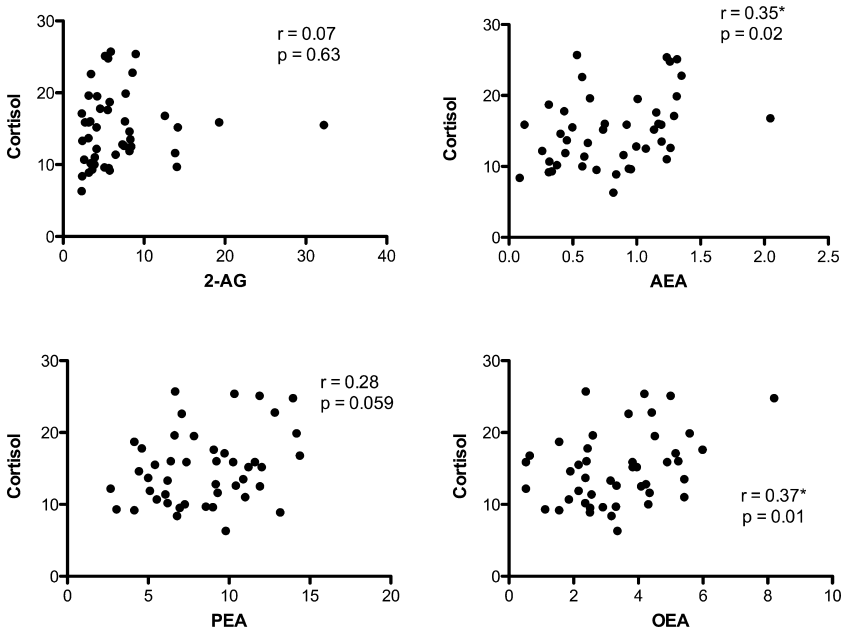


Figure 2 Correlations between plasma cortisol and 2-arachidonoylglycerol (2-AG), anandamide (AEA), palmitoylethanolamide (PEA) or oleoylethanolamide (OEA). All of AEA, PEA and OEA, but not 2-AG, were found to correlate with cortisol in the entire sample (both healthy control and those with post-traumatic stress disorder). Significant correlations ($p < 0.05$) are denoted by *.

scores for avoidance ($r = .553$, $n = 19$, $p = .019$) and with number of CAPS avoidance symptoms ($r = .618$, $n = 19$, $p = .005$). 2-AG was also related to the current PTSD symptom total score, but only at a trend level of significance ($r = .424$, $n = 19$, $p = .070$), and with total number of current PTSD symptoms ($r = .530$, $n = 19$, $p = .020$). AEA concentrations were negatively associated with current CAPS intrusive subscale scores ($r = -.532$, $n = 19$, $p = .019$), and with number of intrusive symptoms ($r = -.527$, $n = 19$, $p = .020$), but was not significantly associated with the current PTSD symptom total score or number. Lifetime CAPS scores were associated with AEA concentration showing a marginally significant association with lifetime intrusive subscale scores ($r = -.455$, $n = 19$, $p = .050$), and with lifetime number of intrusive symptoms ($r = -.472$, $n = 19$, $p = .041$). Thus, among subjects with PTSD, clinician rated CAPS subscale scores for avoidance and intrusions were differentially associated with 2-AG and AEA, respectively.

4. Discussion

These data demonstrate that individuals recruited following the WTC collapse, who met criteria for PTSD in their lifetime, exhibit lower concentrations of the endocannabinoid 2-AG, but not AEA, in the circulation. The relationship between 2-AG and PTSD was also related to the trauma of being exposed to the WTC collapse as even individuals who did not develop PTSD, but had a high degree of exposure to the WTC collapse also exhibited reductions in plasma concentrations of 2-AG. Importantly, despite the fact that individuals with PTSD exhibited higher rates of lifetime depression and alcohol abuse, covariance analyses for each of these comorbidities demonstrated that they did not mediate the significant relationship between PTSD and 2-AG levels. Cigarette smoking was not accounted for in the current study, and might have influenced the results, since smoking rates tend to be elevated among individuals with PTSD (Fu et al., 2007). The recent observation, however, of no significant difference in circulating 2-AG between smokers and non-smokers argues against this possibility (Hauer et al., 2013). Collectively, these data indicate that a complex relationship between stress exposure, PTSD and circulating levels of 2-AG exists.

The reduction in plasma 2-AG concentrations seen in individuals with PTSD is similar in magnitude to that previously reported for individuals with major depression (Hill et al., 2008, 2009b). That both PTSD and depression are stress-related mental illnesses suggests that reduced concentrations of 2-AG could represent a peripheral biomarker of vulnerability to stress-related mood and anxiety disorders. Preclinical research has found that 2-AG signaling is recruited within the brain during acute stress, which contributes to termination of the stress response (Evanson et al., 2010; Hill et al., 2011). Under conditions of repeated stress, an up-regulation of 2-AG signaling is required to facilitate adaptation or habituation to stress exposure (Patel et al., 2005b; Hill et al., 2010b). Similarly, in humans, exposure to an acute social stress has been found to increase circulating levels of 2-AG (Hill et al., 2009b; however, it should be noted that a subsequent study found circulating concentrations of AEA, but not 2-AG, were elevated in response to acute stress; Dlugos et al., 2012), suggesting the possibility of an evolutionarily conserved role for 2-AG signaling in buffering the effects of stress. For instance, exaggerated stress responses were described in humans who did not show elevations in 2-AG following exposure to parabolic flight stress (Chouker et al., 2010). These data suggest that stress-induced increases in 2-AG are an adaptive response that functions to buffer the potential deleterious effects of stress (Hill and Tasker, 2012). Accordingly, one possibility is that a trait deficit in 2-AG signaling might represent a vulnerability factor predisposing an individual to an adverse response following stressful or traumatic exposure. As such, an integral role for 2-AG in stress regulation may explain the finding of reduced 2-AG levels in subjects with PTSD. However, detailed prospective studies will be required to determine if reduced 2-AG is predisposing, or a consequence of PTSD.

It is interesting that despite significant differences in 2-AG levels between those with and without PTSD, analyses of the entire sample did not detect significant relationships between 2-AG and any clinical variable. However, within the PTSD group, 2-AG correlates positively with avoidance and total CAPS scores, as well as with cortisol levels. These associations would suggest that for individuals with PTSD, both higher basal cortisol and greater degree of symptom expression are related to higher levels of circulating 2-AG. As peripheral 2-AG is known to be responsive to stress exposure, this would suggest that elevation in 2-AG within individuals most afflicted by PTSD symptoms may reflect augmented levels of stress. Thus, despite the observation of diminished mean levels of 2-AG in a cohort characterized by lifetime PTSD, 2-AG may be relatively elevated among substantially stressed individuals in a direction to constrain the stress response. Consistent with this formulation for the behavior of the eCB system in PTSD, individuals with major depression exhibit reductions in basal levels of circulating 2-AG but still possess the capacity to mount a 2-AG response following exposure to stress (Hill et al., 2009b). This model is analogous to the results of a recent study that demonstrated sustained elevations of 2-AG (and AEA) levels in a trauma exposed population with PTSD, in which it was suggested that the magnitude of stress was associated with parallel activation of the eCB system (Hauer et al., 2013). While Hauer et al. (2013) found a main effect of elevated, rather than reduced, 2-AG in PTSD, their suggestion that eCBs are engaged to counter the effects of stress is consistent with the current formulation. Future research will be required to understand which factors may be most relevant in determining whether mean 2-AG is elevated or reduced for PTSD subjects; these variables could include duration of stress exposure, acuity of stress exposure relative to measurement of eCB levels and nature of the traumatic stressor. It should be noted though, that while the findings in the study of Hauer et al. (2013) are inconsistent with the current data, our findings of reduced eCB content in PTSD are relatively consistent with another recent report by Neumeister et al. (2013), which found reduced circulating levels of AEA (but not 2-AG) in PTSD.

There were no differences in AEA concentrations between the PTSD and no-PTSD groups, but a robust negative relationship between AEA levels and expression of intrusive symptoms was demonstrated; that is, individuals with lower levels of plasma AEA exhibited the highest rates of intrusive symptoms. Both clinical and preclinical studies suggest an integral role for AEA signaling in the regulation of amygdala reactivity

and the suppression of emotionally aversive memories (Hariri et al., 2009; Hill et al., 2009a; Gunduz-Cinar et al., 2013), which may explain this relationship. Rodent studies indicate that during extinction of aversive memories, there is a recruitment of AEA signaling within the amygdala and that facilitation of this response augments extinction, while blockade of AEA/CB_1 receptor signaling may result in the perseveration of the aversive memory (Marsicano et al., 2002; Chhatwal et al., 2005; Gunduz-Cinar et al., 2013). Further, increased AEA signaling is known to dampen stress responses in rodents (Patel et al., 2004; Hill et al., 2009a), and, in humans, to result in lower levels of trait anxiety and accelerated habituation of the amygdala in response to threat cues (Gunduz-Cinar et al., 2013). More so, it has been demonstrated in both healthy individuals (Dlugos et al., 2012), and those with major depression (Hill et al., 2008), that circulating AEA concentrations negatively correlate with anxiety levels. Since impairment in fear extinction may result in the emergence of intrusive symptoms, the negative association between AEA signaling and aversive memories suggests that reduced AEA signaling may likewise be associated with impaired extinction of aversive memories in the current sample, with an associated increase in intrusive symptoms. Given these findings, we propose that while AEA concentrations are not significantly reduced in association with PTSD in the current cohort, individuals who possess lower concentrations of AEA could be more susceptible to intrusive symptoms.

The relationship between cortisol and concentrations of the NAEs is of interest given the context of an ever-growing body of research demonstrating a functional relationship between glucocorticoids and the eCB system (Hill and McEwen, 2010). Interestingly, we have previously demonstrated in rodents that the administration of glucocorticoids causes a rapid elevation in AEA throughout the limbic system (Hill et al., 2010a). Given the overlap between the biosynthetic and metabolic pathways of AEA, PEA and OEA (Ahn et al., 2008), it is not surprising that circulating concentrations of these molecules correlate with each other. Accordingly, the possibility exists that cortisol positively correlates with concentrations of these molecules through an ability of cortisol either to increase their shared biosynthesis or reduce their metabolism. An earlier study found that PEA concentrations in the circulation positively correlate with cortisol in healthy subjects following exposure to the Trier social stress test (Dlugos et al., 2012). More importantly, a recent study found that circulating AEA concentrations correlated with cortisol levels, and that individuals with PTSD exhibited reductions in both AEA and cortisol concentrations in blood, relative to healthy and trauma exposed controls (Neumeister et al., 2013). As the current PTSD sample did not exhibit reduced levels of cortisol on the basis of a single 8am blood draw, it is perhaps not surprising that we did not detect reduced levels of AEA. Taken together, these data suggest that reduced eCB signaling could be a feature of PTSD, and that relative reductions in AEA or 2-AG may be related to alterations in glucocorticoid signaling. Future studies based on larger samples will be required to examine relationships between eCB levels and cortisol to determine which biological factors predict alterations in AEA versus 2-AG.

One major limitation of this study is that it is based a single time point analysis, both with regard to time of day for biological sampling and the time course of PTSD. However, four studies based on data derived from a single time point have revealed reduced circulating levels of eCB molecules in individuals diagnosed with depression or PTSD (Hill et al., 2008, 2009b; Neumeister et al., 2013; current data; however, see Hauer et al., 2013 discussed above for opposite findings). Second, the current study is based exclusively on peripheral measures, which have been used to support hypotheses that relate to central nervous system function. While it is unlikely that circulating concentrations of eCBs are from the same signaling pool as eCBs in the CNS, there is an interesting functional correlation between stimulae that modulate central and peripheral eCB signaling (Hillard et al., 2012). Studies examining the effects of stress on circulating eCBs in humans have generally reached the same conclusions as those performed in animals (Hill et al., 2009b, 2011; Chouker et al., 2010; Evanson et al., 2010). Further, multiple studies have revealed significant relationships between circulating eCB levels and distinct neurobehavioral domains and/or symptom clusters, particularly those related to anxiety (McPartland et al., 2005; Hill et al., 2008; Mangieri et al., 2009; Gunduz-Cinar et al., 2013). While it is possible that a third variable could contribute to these relationships, the fact that similar associations have held across studies and species suggests that peripheral eCB content is related to eCB signaling in the CNS. The recent finding that low concentrations of circulating AEA in PTSD are significantly correlated with an up-regulation of CB_1 receptor binding sites within forebrain regions associated with stress regulation and emotional processing supports this relationship (Neumeister et al., 2013), suggesting that a deficit in eCB signaling detected at the peripheral level may be associated with a compensatory up-regulation of central CB_1 receptors.

In conclusion, the finding of reduced concentrations of circulating 2-AG in PTSD is consistent with current biological formulations of PTSD. For example, in addition to the relationship between eCBs (AEA and 2-AG), cortisol and the stress response (Hill and Tasker, 2012), there are substantial interactions between eCBs and other systems found to be dysfunctional in PTSD. CB_1 receptors on sympathetic terminals, for instance, regulate noradrenaline release (Ishac et al., 1996), and decrements in eCB signaling at noradrenergic terminals have been shown to result in elevated sympathetic outflow (Srivastava and Lutz, 2012), as is seen in PTSD (Pervanidou and Chrousos, 2010). Furthermore, eCBs (as well as PEA and OEA) possess anti-inflammatory actions (Hansen, 2010), such that reduced eCB signaling could contribute to a basal pro-inflammatory state (Beyer et al., 2010), as has also been documented for PTSD (Spivak et al., 1997; Baker et al., 2001; Plantinga et al., 2013). Finally, eCB signaling promotes the release of NPY (Gamber et al., 2005), which has been demonstrated in association with stress resiliency (Morgan et al., 2000; Yehuda et al., 2006; Sajdyk et al., 2008; Cohen et al., 2012). Reductions in NPY have been found in PTSD (Rasmusson et al., 2000; Sah et al., 2009), which have increased in association with PTSD recovery (Yehuda et al., 2006). Thus, diminished eCB function may additionally result in a deficit in the recruitment of NPY. These biological findings, in the context of known involvement of the eCB system in emotional memory extinction and recall, stress buffering and adaptation, as well as HPA function, suggest the possibility of a pivotal role for the eCB system in PTSD. A deficit in eCB

function is consistent with all of the major symptom dimensions of PTSD, and represents a novel candidate system for further investigation in the pathophysiology and treatment of the disorder. Trials examining the effect of agents that potentiate eCB signaling in PTSD, either alone or in conjunction with treatments that modulate glucocorticoid signaling, will provide proof of principal for the contribution of eCB signaling to PTSD, and will help to elucidate more precisely the respective functional roles of 2-AG and AEA in PTSD development and expression.

Role of funding source

This research was supported by National Institute of Mental Health (NIMH) Innovation Award 1R56MH077321-01 (R.Y.) and NIH Grants DA026996 (C.J.H.) and MH41256 (B.S.M.), operating grants from the Lightfighter Foundation (R.Y. and B.S.M.) and the Canadian Institutes of Health Research (M.N.H.). Partial funding for this study came from the Research and Education Initiative Fund, a component of the Advancing a Healthier Wisconsin endowment at the Medical College of Wisconsin. Funding was also supported, in part, by 5MO1 RR00071 for the Mount Sinai General Clinical Research Center from the National Center for Research Resources. Support for Dr. Yehuda and Dr. Golier is provided by the Department of Veterans Affairs. The funding bodies had no role in the design of this study, the analysis or interpretation of the data or the composition of this manuscript.

Conflict of interest

Dr. Yehuda reported research funding from Eli Lilly and Company and Dr. McEwen reported research funding from Johnson and Johnson. Drs. Hill, Bierer, Makotkine, Golier and Hillard declare no conflicts of interest.

Acknowledgments

The authors would like to thank Kara Stuhr for her technical assistance with the running of these experiments. This research was supported by National Institute of Mental Health (NIMH) Innovation Award 1R56MH077321-01 (RY) and NIH grants DA026996 (CJH) and MH41256 (BSM), operating grants from the Lightfighter Foundation (RY and BSM) and the Canadian Institutes of Health Research (MNH). MNH is the recipient of a Tier II Canada Research Chair in the Neurobiology of Stress. Partial funding for this study came from the Research and Education Initiative Fund, a component of the Advancing a Healthier Wisconsin endowment at the Medical College of Wisconsin. Funding was also supported, in part, by 5MO1 RR00071 for the Mount Sinai General Clinical Research Center from the National Center for Research Resources. Support for Dr. Yehuda and Dr. Golier is provided by the Department of Veterans Affairs. Dr. Yehuda reported research funding from Eli Lilly and Company and Dr. McEwen reported research funding from Johnson and Johnson.

References

Ahn, K., McKinney, M.K., Cravatt, B.F., 2008. Enzymatic pathways that regulate endocannabinoid signaling in the nervous system. Chem. Rev. 108, 1687—1707.

Atsak, P., Hauer, D., Campolongo, P., Schelling, G., McGaugh, J.L., Roozendaal, B., 2012. Glucocorticoids interact with the hippocampal endocannabinoid system in impairing retrieval of contextual fear memory. Proc. Natl. Acad. Sci. U. S. A. 109, 3504—3509.

Baker, D.G., Ekhator, N.N., Kasckow, J.W., Hill, K.K., Zoumakis, E., Dashevsky, B.A., Chrousos, G.P., Geracioti Jr., T.D., 2001. Plasma and cerebrospinal fluid interleukin-6 concentrations in posttraumatic stress disorder. Neuroimmunomodulation 9, 209—217.

Bellocchio, L., Soria-Gomez, E., Quarta, C., Metna-Laurent, M., Cardinal, P., Binder, E., Cannich, A., Delamarre, A., et al., 2013. Activation of the sympathetic nervous system mediates hypophagic and anxiety-like effects of CB(1) receptor blockade. Proc. Natl. Acad. Sci. U. S. A. 110, 4786—4791.

Beyer, C.E., Dwyer, J.M., Piesla, M.J., Platt, B.J., Shen, R., Rahman, Z., Chan, K., Manners, M.T., et al., 2010. Depression-like phenotype following chronic CB1 receptor antagonism. Neurobiol. Dis. 39, 148—155.

Blake, D.D., Weathers, F.W., Nagy, L.M., Kaloupek, D.G., Gusman, F.D., Charney, D.S., Keane, T.M., 1995. The development of a Clinician-Administered PTSD Scale. J. Trauma. Stress 8, 75—90.

Breslau, N., Davis, G.C., Peterson, E.L., Schultz, L.R., 2000. A second look at comorbidity in victims of trauma: the post-traumatic stress disorder-major depression connection. Biol. Psychiatry 48, 902—909.

Campolongo, P., Roozendaal, B., Trezza, V., Hauer, D., Schelling, G., McGaugh, J.L., Cuomo, V., 2009. Endocannabinoids in the rat basolateral amygdala enhance memory consolidation and enable glucocorticoid modulation of memory. Proc. Natl. Acad. Sci. U. S. A. 106, 4888—4893.

Chhatwal, J.P., Davis, M., Maguschak, K.A., Ressler, K.J., 2005. Enhancing cannabinoid neurotransmission augments the extinction of conditioned fear. Neuropsychopharmacology 30, 516—524.

Chouker, A., Kaufmann, I., Kreth, S., Hauer, D., Feuerecker, M., Thieme, D., Vogeser, M., Thiel, M., Schelling, G., 2010. Motion sickness, stress and the endocannabinoid system. PLoS ONE 5, e10752.

Cohen, H., Liu, T., Kozlovsky, N., Kaplan, Z., Zohar, J., Mathe, A.A., 2012. The neuropeptide Y (NPY)-ergic system is associated with behavioral resilience to stress exposure in an animal model of post-traumatic stress disorder. Neuropsychopharmacology 37, 350—363.

Di, S., Malcher-Lopes, R., Halmos, K.C., Tasker, J.G., 2003. Nongenomic glucocorticoid inhibition via endocannabinoid release in the hypothalamus: a fast feedback mechanism. J. Neurosci. 23, 4850—4857.

Dlugos, A., Childs, E., Stuhr, K.L., Hillard, C.J., de Wit, H., 2012. Acute stress increases circulating anandamide and other N-acylethanolamines in healthy humans. Neuropsychopharmacology 37, 2416—2427.

Evanson, N.K., Tasker, J.G., Hill, M.N., Hillard, C.J., Herman, J.P., 2010. Fast feedback inhibition of the HPA axis by glucocorticoids is mediated by endocannabinoid signaling. Endocrinology 151, 4811—4819.

Foa, E.B., Riggs, D.S., Dancu, C.V., Rothbaum, B.O., 1993. Reliability and validity of a brief instrument for assessing post-traumatic stress disorder. J. Trauma. Stress 6, 459—473.

Fu, S.S., McFall, M., Saxon, A.J., Beckham, J.C., Carmody, T.P., Baker, D.G., Joseph, A.M., 2007. Post-traumatic stress disorder and smoking: a systematic review. Nicotine Tob. Res. 9, 1071—1084.

Gamber, K.M., MacArthur, K.M., Westfall, T.C., 2005. Cannabinoids augment the release of neuropeptide Y in the rat hypothalamus. Neuropharmacology 49, 646—652.

Gunduz-Cinar, O., Macpherson, K.P., Cinar, R., Gamble-George, J., Sugden, K., Williams, B., Godlweski, G., Ramikie, T.S., et al., 2013. Convergent translational evidence of a role for anandamide in amygdala-mediated fear extinction, threat processing and stress-reactivity. Mol. Psychiatry 18, 813—823.

Hansen, H.S., 2010. Palmitoylethanolamide and other anandamide congeners. Proposed role in the diseased brain. Exp. Neurol. 224, 48—55.

Hariri, A.R., Gorka, A., Hyde, L.W., Kimak, M., Halder, I., Ducci, F., Ferrell, R.E., Goldman, D., Manuck, S.B., 2009. Divergent effects of genetic variation in endocannabinoid signaling on human threat- and reward-related brain function. Biol. Psychiatry 66, 9—16.

Hauer, D., Weis, F., Campolongo, P., Schopp, M., Beiras-Fernandez, A., Strewe, C., Giehl, M., Toth, R., et al., 2012. Glucocorticoid-endocannabinoid interaction in cardiac surgical patients: relationship to early cognitive dysfunction and late depression. Rev. Neurosci. 23, 681—690.

Hauer, D., Schelling, G., Gola, H., Campolongo, P., Morath, J., Roozendaal, B., Hamuni, G., Karabatsiakis, A., et al., 2013. Plasma concentrations of endocannabinoids and related primary fatty acid amides in patients with post-traumatic stress disorder. PLoS ONE 8, e62741.

Hill, M.N., Gorzalka, B.B., 2009. The endocannabinoid system and the treatment of mood and anxiety disorders. CNS Neurol Disord. Drug Targets 8, 451—458.

Hill, M.N., McEwen, B.S., 2010. Involvement of the endocannabinoid system in the neurobehavioural effects of stress and glucocorticoids. Prog. Neuropsychopharmacol. Biol. Psychiatry 34, 791—797.

Hill, M.N., Tasker, J.G., 2012. Endocannabinoid signaling, glucocorticoid-mediated negative feedback, and regulation of the hypothalamic—pituitary—adrenal axis. Neuroscience 204, 5—16.

Hill, M.N., Miller, G.E., Ho, W.S., Gorzalka, B.B., Hillard, C.J., 2008. Serum endocannabinoid content is altered in females with depressive disorders: a preliminary report. Pharmacopsychiatry 41, 48—53.

Hill, M.N., McLaughlin, R.J., Morrish, A.C., Viau, V., Floresco, S.B., Hillard, C.J., Gorzalka, B.B., 2009a. Suppression of amygdalar endocannabinoid signaling by stress contributes to activation of the hypothalamic—pituitary—adrenal axis. Neuropsychopharmacology 34, 2733—2745.

Hill, M.N., Miller, G.E., Carrier, E.J., Gorzalka, B.B., Hillard, C.J., 2009b. Circulating endocannabinoids and N-acyl ethanolamines are differentially regulated in major depression and following exposure to social stress. Psychoneuroendocrinology 34, 1257—1262.

Hill, M.N., Karatsoreos, I.N., Hillard, C.J., McEwen, B.S., 2010a. Rapid elevations in limbic endocannabinoid content by glucocorticoid hormones in vivo. Psychoneuroendocrinology 35, 1333—1338.

Hill, M.N., McLaughlin, R.J., Bingham, B., Shrestha, L., Lee, T.T., Gray, J.M., Hillard, C.J., et al., 2010b. Endogenous cannabinoid signaling is essential for stress adaptation. Proc. Natl. Acad. Sci. U. S. A. 107, 9406—9411.

Hill, M.N., McLaughlin, R.J., Pan, B., Fitzgerald, M.L., Roberts, C.J., Lee, T.T., Karatsoreos, I.N., Mackie, K., et al., 2011. Recruitment of prefrontal cortical endocannabinoid signaling by glucocorticoids contributes to termination of the stress response. J. Neurosci. 31, 10506—10515.

Hillard, C.J., Weinlander, K.M., Stuhr, K.L., 2012. Contributions of endocannabinoid signaling to psychiatric disorders in humans: genetic and biochemical evidence. Neuroscience 204, 207—229.

Ishac, E.J., Jiang, L., Lake, K.D., Varga, K., Abood, M.E., Kunos, G., 1996. Inhibition of exocytotic noradrenaline release by presynaptic cannabinoid CB1 receptors on peripheral sympathetic nerves. Br. J. Pharmacol. 118, 2023—2028.

Krystal, J.H., Neumeister, A., 2009. Noradrenergic and serotonergic mechanisms in the neurobiology of posttraumatic stress disorder and resilience. Brain Res. 1293, 13—23.

Mangieri, R.A., Hong, K.I., Piomelli, D., Sinha, R., 2009. An endocannabinoid signal associated with desire for alcohol is suppressed in recently abstinent alcoholics. Psychopharmacology (Berl.) 205, 63—72.

Marsicano, G., Wotjak, C.T., Azad, S.C., Bisogno, T., Rammes, G., Cascio, M.G., Hermann, H., Tang, J., et al., 2002. The endogenous cannabinoid system controls extinction of aversive memories. Nature 418, 530—534.

McPartland, J.M., Giuffrida, A., King, J., Skinner, E., Scotter, J., Musty, R.E., 2005. Cannabimimetic effects of osteopathic manipulative treatment. J. Am. Osteopath. Assoc. 105, 283—291.

Morgan 3rd, C.A., Wang, S., Southwick, S.M., Rasmusson, A., Hazlett, G., Hauger, R.L., Charney, D.S., 2000. Plasma neuropeptide-Y concentrations in humans exposed to military survival training. Biol. Psychiatry 47, 902—909.

Neumeister, A., 2013. The endocannabinoid system provides an avenue for evidence-based treatment development for PTSD. Depress. Anxiety 30, 93—96.

Neumeister, A., Normandin, M.D., Pietrzak, R.H., Piomelli, D., Zheng, M.Q., Gujarro-Anton, A., Potenza, M.N., Bailey, C.R., et al., 2013. Elevated brain cannabinoid CB1 receptor availability in post-traumatic stress disorder: a positron emission tomography study. Mol. Psychiatry (in press).

Patel, S., Roelke, C.T., Rademacher, D.J., Cullinan, W.E., Hillard, C.J., 2004. Endocannabinoid signaling negatively modulates stress-induced activation of the hypothalamic—pituitary—adrenal axis. Endocrinology 145, 5431—5438.

Patel, S., Carrier, E.J., Ho, W.S., Rademacher, D.J., Cunningham, S., Reddy, D.S., Falck, J.R., Cravatt, B.F., Hillard, C.J., 2005a. The postmortal accumulation of brain N-arachidonylethanolamine (anandamide) is dependent upon fatty acid amide hydrolase activity. J. Lipid Res. 46, 342—349.

Patel, S., Roelke, C.T., Rademacher, D.J., Hillard, C.J., 2005b. Inhibition of restraint stress-induced neural and behavioural activation by endogenous cannabinoid signalling. Eur. J. Neurosci. 21, 1057—1069.

Pervanidou, P., Chrousos, G.P., 2010. Neuroendocrinology of post-traumatic stress disorder. Prog. Brain Res. 182, 149—160.

Plantinga, L., Bremner, J.D., Miller, A.H., Jones, D.P., Veledar, E., Goldberg, J., Vaccarino, V., 2013. Association between posttraumatic stress disorder and inflammation: a twin study. Brain Behav. Immun. 30, 125—132.

Plendl, W., Wotjak, C.T., 2010. Dissociation of within- and between-session extinction of conditioned fear. J. Neurosci. 30, 4990—4998.

Rasmusson, A.M., Hauger, R.L., Morgan, C.A., Bremner, J.D., Charney, D.S., Southwick, S.M., 2000. Low baseline and yohimbine-stimulated plasma neuropeptide Y (NPY) levels in combat-related PTSD. Biol. Psychiatry 47, 526—539.

Riebe, C.J., Wotjak, C.T., 2011. Endocannabinoids and stress. Stress 14, 384—397.

Sah, R., Ekhator, N.N., Strawn, J.R., Sallee, F.R., Baker, D.G., Horn, P.S., Geracioti Jr., T.D., 2009. Low cerebrospinal fluid neuropeptide Y concentrations in posttraumatic stress disorder. Biol. Psychiatry 66, 705—707.

Sajdyk, T.J., Johnson, P.L., Leitermann, R.J., Fitz, S.D., Dietrich, A., Morin, M., Gehlert, D.R., Urban, J.H., Shekhar, A., 2008. Neuropeptide Y in the amygdala induces long-term resilience to stress-induced reductions in social responses but not hypothalamic—adrenal—pituitary axis activity or hyperthermia. J. Neurosci. 28, 893—903.

Sarapas, C., Cai, G., Bierer, L.M., Golier, J.A., Galea, S., Ising, M., Rein, T., Schmeidler, J., et al., 2011. Genetic markers for PTSD risk and resilience among survivors of the World Trade Center attacks. Dis. Markers 30, 101—110.

Spitzer, R.L., Williams, J.B.W., Gibbon, M., 1995. Structured Clinical Interview for DSM-IV (SCID). New York State Psychiatric Institute Biometrics Research.

Spivak, B., Shohat, B., Mester, R., Avraham, S., Gil-Ad, I., Bleich, A., Valevski, A., Weizman, A., 1997. Elevated levels of serum

interleukin-1 beta in combat-related posttraumatic stress disorder. Biol. Psychiatry 42, 345—348.

Srivastava, R.J., Lutz, B., 2012. Dysregulation of hypothalamic—pituitary—adrenal axis in mice lacking CB1 receptors in adrenergic and noradrenergic neurons. In: P1-28, 22nd Annual Symposium of the International Cannabinoid Research Society.

Yehuda, R., 2009. Status of glucocorticoid alterations in post-traumatic stress disorder. Ann. N.Y. Acad. Sci. 1179, 56—69.

Yehuda, R., Brand, S., Yang, R.K., 2006. Plasma neuropeptide Y concentrations in combat exposed veterans: relationship to trauma exposure, recovery from PTSD, and coping. Biol. Psychiatry 59, 660—663.

Yehuda, R., Cai, G., Golier, J.A., Sarapas, C., Galea, S., Ising, M., Rein, T., Schmeidler, J., et al., 2009. Gene expression patterns associated with posttraumatic stress disorder following exposure to the World Trade Center attacks. Biol. Psychiatry 66, 708—711.

International Journal of Developmental Neuroscience 76 (2019) 95–102

Contents lists available at ScienceDirect

International Journal of Developmental Neuroscience

journal homepage: www.elsevier.com/locate/ijdevneu

Role of the endocannabinoid system in neurological disorders

Mingyang Zou, Dexin Li, Ling Li, Lijie Wu*, Caihong Sun*

Department of Children's and Adolescent Health, Public Health College, Harbin Medical University, Harbin 150081, China

ARTICLE INFO

Keywords:
Autism spectrum disorder
Endocannabinoid system
Metabolic regulation
Neuropsychiatric disorders

ABSTRACT

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder that begins in infancy. Although the etiology and pathogenesis are poorly understood, many studies have shown that ASD is closely related to structural and functional defects in the nervous system, especially synaptic transmission. The endocannabinoid (eCB) system is an important regulatory system of the central nervous system that regulates neurotransmission and synaptic plasticity and plays an important role in emotional and social responses and cognitive function. The relationship between eCB system and ASD has attracted increasing attention from scholars. In this review, we discuss the complex lipid signaling network of the eCB system, intracellular transport pathways, abnormal expression and association with various neurological diseases, and direct and indirect evidence for the link between eCB and ASD. Collectively, the findings to date indicate that the eCB system plays a key role in the pathophysiology of ASD and can provide new insights into potential interventions and rehabilitation strategies for ASD.

1. The eCB system

The eCB system, a unique biological system, affects a wide range of biological processes including brain development and function. It consists of eCB ligands (eCBs), cannabinoid receptors (CBRs), and metabolic enzymes. The main pharmacological component of cannabis, Δ9-tetrahydrocannabinol (Δ9-THC), was identified and isolated in 1964 (Mechoulam and Gaoni, 1965); CB1R was cloned in 1991, and CB2R was identified 2 years later. The two G protein-coupled receptors share 44% homology (Mailleux and Vanderhaeghen, 1992), with a similarity of 68% in the transmembrane domain (Cabral and Griffin-Thomas, 2009); however, their distributions and functions in the body differ. CB1R is located in lipid rafts in the microdomains of the cell membrane mainly in the brain, spinal cord, and peripheral nervous system. The eCBs play a regulatory role in the proliferation, differentiation and migration of neural progenitor (NP) cells by participating in the CB1R (Carr et al., 2013). CB1R activation is important for cognition and memory and modulates other physiological activities by regulating neurohormone levels and signal transduction. CB2R, which mainly has an immunomodulatory role in the body, is distributed in peripheral immune organs or tissues such as the spleen margin and thymus (Pertwee et al., 2010). Although CB2R is usually absent in mature neurons, CB2R combined with CB1R, has functional activity in undifferentiated neurons and may be involved in the regulation of proliferation, cell cycle maintenance and neural differentiation of NP cells fate (Palazuelos et al., 2012). There may also be other CBRs in the brain aside from CB1R and CB2R (Elphick and Egertova, 2001). With the synthesis and application of exogenous cannabinoids, receptor agonists, and antagonists, prior studies have been discovered that N-arachidonylethanolamide (AEA) and 2-arachidonylglycerol (2-AG), are the most active eCBs (Shanker and Aschner, 2003; Sugiura and Waku, 2000). Palmitoylethanolamide (PEA) and oleoylethanolamide (OEA) are also eCBs that share the same ethanolamine group as AEA, but are non-canonical due to their low affinity for CB1R and CB2R (O'Sullivan and

Abbreviations: 2-AG, 2-arachidonylglycerol; AA, arachidonic acid; ABHD, α/β-hydrolase domain-containing protein; AD, Alzheimer's disease; AEA, N-arachidonylethanolamide; ASD, autism spectrum disorder; CBR, cannabinoid receptors; COX, cyclooxygenase; DAG, diacylglycerol; DAGL, diacylglycerol lipas; ECB, endocannabinoid; FAAH, fatty acid amide hydrolase; Fmr1-KO, fragile X mental retardation 1 knockout; GABA, gamma-aminobutyric acid; GPR, G protein-coupled receptor; HPA, hypothalamic–pituitary–adrenal; LTD, long-term depression; MAGL, monoacylglycerol lipase; NAAA, NAE-hydrolyzing acid amidase; NAE, N-acylethanolamine; NAPE, N-acyl-phosphatidylethanolamine; NAPE-PLD, N-acylphosphatidylethanolamine-specific phospholipase D; NLGN, neuroligand; NP, neural progenitor; OEA, oleoylethanolamide; PA, 2-Arachiclonoyl-phosphatidic acid; PAK, P21-activated kinase; PEA, palmitoylethanolamide; PPARs, peroxisome proliferator activated receptors; PtdCho, 2-Arachiclonoyl-phosphatidylcholine; PtdEtn, 2-Arachiclonoyl-phosphatidylethanolamine; PtdIns, 2-arachidonoyl-phosphatidylinositol; PUFAs, polyunsaturated fatty acids; TRPV1, Transient Receptor Potential Vanilloid 1; VPA, valproic acid; Δ9-THC, Δ9-tetrahydrocannabinol

* Corresponding authors.
E-mail addresses: mingyangshine@sina.com (M. Zou), 416736065@qq.com (D. Li), bigmm1993@163.com (L. Li), wulijiehyd@126.com (L. Wu), suncaihong2003@163.com (C. Sun).

https://doi.org/10.1016/j.ijdevneu.2019.03.002
Received 12 October 2018; Received in revised form 6 March 2019; Accepted 7 March 2019
Available online 08 March 2019

Kendall, 2010). AEA, PEA, and OEA are collectively referred to as N-acylethanolamine (NAE). The relevant metabolic enzymes are N-acyl-phosphatidylethanolamine-specific phospholipase D (NAPE-PLD) and fatty acid amide hydrolase (FAAH), which are involved in the synthesis and decomposition of AEA. 2-AG synthetase and catabolic enzymes were diacylglycerol lipas (DAGL) and monoacylglycerol lipase (MAGL), respectively. The former has two isoforms, DAGLα and DAGLβ, that shared 33% sequence identity (Bisogno, 2016). DAGLα appears to be the major 2-AG synthetase and plays a key role in regulating retrograde synaptic plasticity in the nervous system (Gao et al., 2010).

2. Metabolic pathways and intracellular regulation of eCBs

2.1. Biosynthesis and biodegradation of eCBs

Both neurons and glial cells on the surface of spinal dorsal horn can synthesize and release 2-AG and AEA (Hegyi et al., 2012). Both molecules are generated from membrane phospholipid precursors such as 2-arachidonoyl-phosphatidylinositol (PtdIns), 2-arachiclonoyl-phosphatidic acid (PA), 2-arachiclonoyl-phosphatidylcholine (PtdCho), and 2-arachiclonoyl-phosphatidylethanolamine (PtdEtn) on the cell membrane in a stimulus-dependent manner (Muccioli, 2010). The acid-containing 2-AG precursor diacylglycerol (DAG) is mainly synthesized from PtdIns (Fukami et al., 2010), partly by hydrolysis of 2-arachiclonoyl-PA or 2-arachiclonoyl-PtdCho (Ueda et al., 2013). Morever, 2-position hydroxyl group is often esterified with n-3 PUFAs. PLCβ acting on PtdIns generates inositol 3-phosphate and DAG, which is converted to 2-AG by two sn-1-selective DAGLs (Bisogno et al., 2003). Regardless of the synthetic pathway, DAG is produced by neural activity (Stella et al., 1997) or through occupation of membrane receptors (Mechoulam et al., 1998). NAPE, a NAE precursor, is synthesized from PtdCho or PtdEtn derived from the transfer of an acyl chain of glycerophospholipids, which is catalyzed by Ca^{2+}-dependent N-acyltransferase, PLA_2, or lecithin retinol acyltransferase. Arachidonic acid (AA) then generates AEA through the action of NAPE-PLD (Okamoto et al., 2007). FAAH, the main catabolic enzyme of AEA, also plays the reverse role by providing a synthetic route for AEA (Fig. 1).

Excess eCBs are hydrolyzed by the relevant catabolic enzymes and are thus inactivated. Intracellular 2-AG and AEA can be mainly hydrolyzed by FAAH to AA. Other uncharacterized esterases include MAGL (Parkkari et al., 2014), α/β-hydrolase domain-containing protein (ABHD) 6, ABHD12, and NAE-hydrolyzing acid amidase (Blankman et al., 2007). In neurons, ABHD6 accounts for approximately 4% of brain 2-AG hydrolase activity and is similar to MAGL (Savinainen et al., 2012), while FAAH more efficiently hydrolyzes 2-AG (Goparaju et al., 1998). Oxygenation of the eCB moiety of anandamide appears to be another pathway for degradation. These eCBs areoxygenated by cyclooxygenase (COX)-2 and hydroperoxy derivatives produced by lipoxygenases, resultingin the formation of glyceryl prostaglandins and 12-hydroperoxyeicosa-5, 8, 10, 14-tetraenoic acid glycerylester.

2.2. Transport of eCBs and corresponding receptors

In the mature nervous system, neuron produces eCBs on demand. In turn eCBs exert neuromodulation in differentiated neurons (Castillo et al., 2012). CB1R plays an acute/short-term regulation of the growth cone signal at the neurite tip and has a long-term effect on neural gene expression that affect neuronal wiring and overall connectivity (Wu et al., 2010). Activated postsynaptic neurons release eCBs into the synaptic cleft; these eCBs serve as retrograde messengers by acting on presynaptic CB1R and inhibit neurotransmission by one of two mechanisms: by blocking calcium influx in the case of glutamate, gamma-aminobutyric acid (GABA), monoamine, and acetylcholine (Oudin et al., 2011a); and by targeting cyclic (c)AMP-protein kinase A signaling associated with long-term depression (LTD) through inhibition of adenylate cyclase-mediated control of cAMP levels. These processes in turn affect the activation of synapses and the transmission of excitatory and inhibitory signals (Freund et al., 2003), which are closely related to synaptic plasticity (Oudin et al., 2011b). AEA and 2-AG show functional selectivity through activation of a specific receptor—i.e., CB1R and CB2R, thus different receptors are activated resulting in distinct biological effects (Sugiura et al., 2000). AEA is a high-affinity partial agonist of CB1R and is mostly inactive when bound to CB2R, whereas 2-AG is a full agonist of both CBRs (Zou and Kumar, 2018) whose structure is strictly recognized by CB2R. The change in 2-AG affects the distance and rotation frequency among NP cells, which determines the number of contacts between neurons and neurons (Turunen et al., 2018). And by activating CB1R and phosphorylating ERK1/2, 2-AG not only regulates synapse formation, axonal growth, but also controls the fate, migration, and proliferation of NP cells (Keimpema et al., 2013). In addition to the effects exerted through CBR binding, eCBs can directly enter cells to produce the effects of regulating biological activity and balance by three routes. Firstly, eCBs released into the synaptic cleft passively diffuse through the plasma membrane, interact with receptors such as orphan G protein-coupled receptor (GPR) 55 and the transient receptor potential cation channel subfamily V member (TRPV) 1, a nonselective cation channel activated by capsaicin that functions as an amide receptor and is widely expressed in the peripheral and central nervous systems (Toth et al., 2009; Zygmunt et al., 1999). Secondly, extracellular eCB can cross the phospholipid bilayer by active transport through eCB membrane transporters. Lastly, eCBs can be internalized by endocytosis and then targeted to various subcellular locations to influence metabolic and signaling pathways. After entering the cell, eCBs are transported to their target by eCB intracellular transporters such as FAAH-1 cannabinoid transporter, fatty acid-binding protein, and heat shock protein 70, among others. In addition, peroxisome proliferator-activated receptors (PPARs) on the nuclear membrane belonging to the ligand-activated nuclear hormone receptor family can bind eCBs, resulting in conformational changes in the receptor that ultimately lead to repression of eCB-related genes and increased production of intracellular reactive oxygen species such as O_2^- (Iannotti et al., 2016).

3. ECBs and neuropsychiatric disorders

Given its important functions in the development of the nervous system, it is not surprising that the eCB system is associated with a variety of neuropsychiatric diseases such as schizophrenia, depression, anxiety disorder, and epilepsy, which are discussed in the following sections.

3.1. Schizophrenia

Plasma and cerebrospinal fluid AEA levels are higher in patients with schizophrenia than in normal control (Reuter et al., 2017; Potvin et al., 2008). It was reported that AEA concentration in cerebrospinal fluid is negatively correlated with the administered dose of anti-schizophrenia drug (Leweke et al., 2007). The combination of antipsychotics and CB1R antagonists reduced the binding density of CBRs in the prefrontal and cingulate cortices and improved schizophrenia-like symptoms in adolescent rats (Lian and Deng, 2018). Additionally, increased 2-AG metabolism as well as low mRNA and protein levels of CB1 have been detected in the prefrontal cortex of patients with schizophrenia (Volk and Lewis, 2016). These lines of evidence suggest that up regulation of eCBs can exacerbate schizophrenia. However, other studies have arrived at the opposite conclusion: one study suggested that eCBs can be used to treat schizophrenia by alleviating white matter deficits in glial cells as well as damage resulting from glutamate toxicity (de Almeida and Martins-de-Souza, 2018). In addition, in monozygotic twins discordant for schizophrenia, whole-body eCB level was lower in the diseased as compared to the unaffected twin, and a high level of

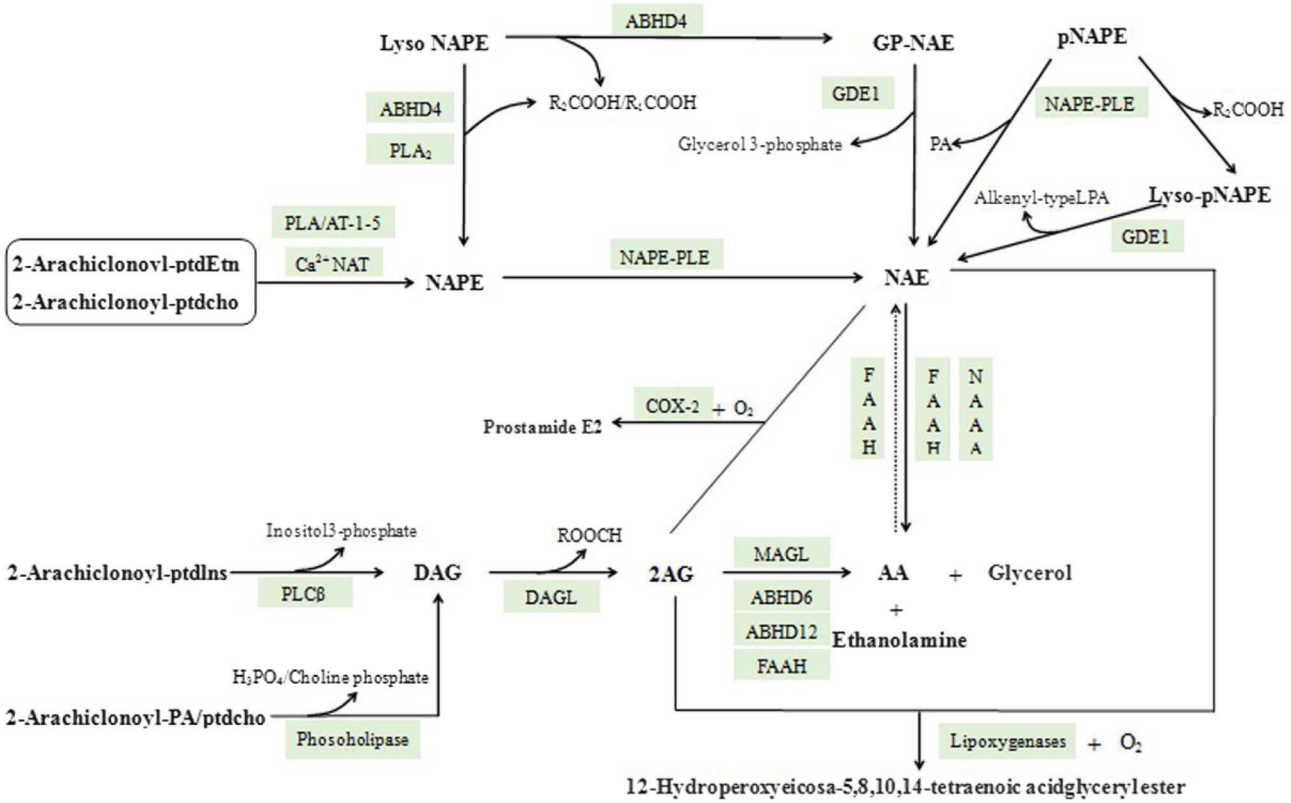


Fig. 1. Metabolic pathways of eCBs. Shadow indicate the enzymes included in the canonical pathway. PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdIns, phosphatidylinositol; PA, phosphatidic acid; PLCβ, phospholipaseC β; PLA_2, phospholipase A_2; ABHD, α/β-hydrolase domain-containing protein; 2-AG, 2-arachidonoylglycerol; COX-2, cyclooxygenase-2; DAG, diacylglycerol; DAGL, diacylglycerol lipase; FAAH, fatty acid amide hydrolase; GDE1, glycerophosphodiester phosphodiesterase 1; GP-NAE, glycerophospho-*N*-acylethanolamine; MAGL, monoacylglycerol lipase; NAAA, N-acyl-phosphatidylethanolamine; pNAPE,*N*-acyl-plasmenylethanolamine; NAPE-PLD, N-acyl-phosphatidylethanolamine-hydrolyzingphospholipase D; PLA/AT, phospholipase A/acyltransferase; AA, arachidonic acid; Ca-NAT, Ca^{2+}-dependent N-acyltransferase.

Table 1
Molecular markers of the eCB system in autism-related models.

First author	Year	Moldel	Case(n)	Control(n)	Biological sample	eCB alterations
Jung KM (Jung et al., 2012)	2012	*fmr1*–/– mouse	11	11	Frontal cortex Striatum	MAGL activity ↑
Denner LA (Denner et al., 2012)	2012	Rat valproic acid	9	9	Hippocampus	PPAR-γmRNA↓ GPR55 mRNA↓
Kerr DM (Kerr et al., 2013)	2013	Rat valproic acid	14	16	Hippocampus Frontal cortex	DAGL mRNA↓
Siniscalco D (Siniscalco et al., 2013)	2013	Human	17	22	PBMCs	CB2R mRNA and protein↑ NAPE–PLD mRNA↓
Siniscalco D (Siniscalco et al., 2014)	2014	Human	22	20	BMDMs	FAAH mRNA↑ NAPE-PLD mRNA↓ CB2R protein↑
Karhson DS (Karhson et al., 2018)	2018	Human	59	53	Plasma	AEA↓

PBMCs = peripheral blood mononuclear cells;BMDMs = bone marrow-derived macrophages; MAGL = monacylglycerol lipase;PPAR:peroxisome proliferator activated receptors; GPR: G protein-coupled receptor; DAGL:Diacylglycerol lipase; CBR:Cannabinoid receptors; NAPE-PLD: N-acylphosphatidylethanolamine-specific phospholipase D; FAAH:Fatty acid amide hydrolase; AEA: N-arachidonylethanolamide.

eCBs was closely related to the reduction of schizophrenia symptoms (Koethe et al., 2018).

3.2. Emotion-related disorders

Circulating plasma levels of eCBs are reduced in patients with mild depression and chronic post-traumatic stress syndrome (Green et al., 2013; Coccaro et al., 2018). Supplementation with eCBs has been shown to mitigate anxiety-like behavior associated with chronic stress; moreover, pharmacological CB1 receptor blockade and eCB in vivo transmission have demonstrated antidepressant effects (Hill and Gorzalka, 2005; Witkin et al., 2005). Hyperactivation of the hypothalamic–pituitary–adrenal (HPA)-axis has been implicated in the pathophysiology of depression. Blockers of FAAH and TRPV1 that increase AEA levels were shown to normalize HPA axis function and improve depression symptoms in Wistar rat (Navarria et al., 2014). These findings provide evidence that eCBs drugs are effective for the treatment of emotion-related disorders.

3.3. Epilepsy

Alterations in epilepsy symptoms via enhanced eCB signaling was demonstrated through administration acute and long-term CBR agonists to epileptic model rats (Vinogradova and van Rijn, 2015), while repeated administration treatment with MAGL inhibitors delayed the onset of epilepsy in a mouse model (Griebel et al., 2015). The combination of ACEA—a highly selective CB1R agonist—and the antiepileptic drug valproic acid (VPA) stimulated the generation of new neurons in

Table 2
Application of eCB intervention in ASD.

First author	Year	Study design	Subject	Intervention	Main findings
Gould GG (Gould et al., 2012)	2012	Case-control	BTBR mice	Intraperitoneal injection WIN 55,212-2	Social engagement↑
Denner LA (Denner et al., 2012)	2012	Case-control	Rat valproic acid	Electronic programmable microinfuser GW9662	Cognition↑
Antonucci N (Antonucci et al., 2015)	2015	Two cases	A 13-year-old male ASD child and a 15-year-old ASD male child	Oral supplementation PEA	Language expression↑ Cognition↑ Behavior↑
Wei D (Wei et al., 2016)	2016	Case-control	BTBR mice	Intraperitoneal injection URB597	Social behavior↑
Servadio M (Servadio et al., 2016)	2016	Case-control	Rat valproic acid	Intraperitoneal injection URB597	Stereotyped behaviors↓ Social deficits↓ Anxiety↓
Khalaj M (Khalaj et al., 2018)	2018	Double-blind Placebo-controlled	4–12 years ASD children	Risperidone + PEA	Hyperactivity↓ Compliance↑
Melancia F (Melancia et al., 2018)	2018	Case-control	Rat valproic acid	Intraperitoneal injection URB597	Cognition↑

mouse hippocampus and influenced the proliferation and differentiation of neural precursor cells; however, VPA alone had no effect on the occurrence and proliferation of neurons. These data indicate that eCBs protect neurons and stimulate neurogenesis (Andres-Mach et al., 2015; Luszczki et al., 2006). Similarly, in a maximal electroshock seizure trial, WIN—a non-selective CB1R and CB2R antagonist—enhanced the anticonvulsant effects of four different drugs (Luszczki et al., 2011). These results provide preliminary evidence that eCBs are an effective auxiliary treatment for epilepsy.

3.4. Fragile X syndrome

The eCBs signaling system in the hippocampus of Fragile X Syndrome model (fragile X mental retardation 1 knockout [Fmr1-KO]) mice has obvious defects; enhancing the 2-AG signal normalized glutaminergic synapses and improved cognitive function and learning and memory (Wang et al., 2017). In Fmr1-KO mice, MAGL activity was increased in the frontal cortex and striatum; administration of the MAGL inhibitor JZL184 enhanced 2-AG signaling and restored synaptic plasticity by modulation of glutamatergic neurotransmission (Jung et al., 2012). Moreover, different CB1R antagonists alleviate behavioral and cognitive abnormalities in Fmr1-KO mice (Busquets-Garcia et al., 2013; Gomis-Gonzalez et al., 2016), and the CB1R blocker rimonabant improved cognitive deficits in Fmr1-KO mice (Servadio et al., 2016). After treatment with FAAH antagonists, which increased AEA level in a mouse model of Fragile X Syndrome, learning and memory performance was significantly improved (Qin et al., 2015). These findings demonstrate that the eCB system is associated with Fragile X Syndrome and can is a promising therapeutic target.

3.5. Alzheimer's disease (AD)

Generally known that the most famous explanation for AD is the dopamine hypothesis (Howes and Kapur, 2009). A recent study reported an interaction between dopamine and eCBs (Kreitzer and Malenka, 2005). Prior to the manifestation of psychiatric symptoms, Δ9-THC has been shown to induce dopaminergic neuron excitation and increase dopamine levels along the margin of the midbrain region, including the ventral tegmentum, nucleus accumbens, and striatum (Ginovart et al., 2012). Similarly, the reduction in eCB levels as a result of CB2R agonists has also been shown to improve AD symptoms (Paez and Campillo, 2018). Interestingly, increased expression of CB1-CB2 was observed in microglia in the hippocampus of a transgenic AD mouse model (Navarro et al., 2018). In mice lacking *CNR1* (which encodes CB1R), amyloid β protein and lipofuscin were not be degraded, leading to autophagy dysfunction, which then affects acceleration of brain aging in AD patients (Piyanova et al., 2013).

In summary, although some of the findings are inconsistent, it is generally acknowledged that eCBs are involved in the development of neuropsychiatric disorders. It is therefore likely that ASD, which has phenotypic similarities to the above neuropsychiatric diseases, may be caused by abnormalities in the eCB system.

4. ECBs and ASD

4.1. Autism spectrum disorder (ASD): an increasingly common disorder of unclear etiology

ASD is a complex neurodevelopmental disorder in children with an onset that is typically before the age of 3 years. According to the fifth edition of the American Psychiatric Association Handbook of Diagnosis and Statistics of Mental Disorders[-5], ASD mainly involves abnormalities in two areas: social communication and interaction, and limited and repetitive behaviors, interests, or activities (American Psychiatric Association, 2013; RdM and Autism, 2015). In the U.S. in 2018, the prevalence of ASD was 1:59 (Baio et al., 2018), which is nearly 1.1

times higher than the rate of 1:125 data reported in 2008 (Baio et al., 2018). However, the etiology and molecular mechanisms underlying ASD are not fully understood. Hundreds of genes to date have been linked to ASD, most of which are closely related to the development of the nervous system, especially synaptogenesis (Lin et al., 2016). For example, neuroligin (NLGN) family members are postsynaptic adhesion molecules that can affect inhibitory neurotransmission in the brain (Nguyen et al., 2016); *SHANK3* reduces synaptic plasticity and further affects triggering abnormal synaptic function (Harony-Nicolas et al., 2017), *ZNF804A* promotes synapse formation and maintains the morphology of synaptic spines (Deans et al., 2017), and *UNC13A* affects the fusion of synaptic vesicles to the presynaptic membrane and blocks synaptic transmission (Lipstein et al., 2017). Thus, abnormalities in synaptic transmission are of particular importance in the etiology of ASD. To this end, the endocannabinoid (eCB) system, which can affect synaptic function, has attracted considerable attention for its potential involvement in the development of ASD. ECB ligands are derived from polyunsaturated fatty acids (PUFAs) in neuronal cell membranes and act on presynaptic membrane receptors to inhibit neurotransmitter release from presynaptic neurons, thereby modulating synaptic plasticity. In the following sections, we present the evidence for the relationship between abnormalities in the eCB system and the pathophysiology of ASD, which can provide valuable insight into possible treatment strategies.

4.2. ECB system possible a hub for ASD onset

The eCB system may be one of the hubs for the association of PUFA metabolism with neuronal development. Recent studies have revealed that defects in n-3 PUFAs inhibit eCB-associated synaptic plasticity in vivo. Mice fed a diet lacking n-3 PUFAs showed a complete absence of LTD induced by eCBs (eCB-LTD) in the prefrontal cortex and nucleus accumbens, while synaptic plasticity induced by other neurotransmitters was unaffected (Lafourcade et al., 2011). At the same time, the deficiency of n-3 PUFAs also decreased the synaptic regulation function of CB1R in mice. The behavior of n-3 PUFAs deficient mice was similar to that of CB1R knockout mice. In the opening experiment, the contact behavior increased and the social behavior decreased (Lafourcade et al., 2011). Moreover, the enhancement of eCB signal could promote the formation of LTD, and the recovery of LTD was found in both brittle X syndrome and mental retardation mice. When female rats were given a diet deficient in n-3 PUFAs from the first gestational day, eCB-LTP was lost in the hippocampus of the offspring (Thomazeau et al., 2017). It is suggested that the lack of n-3 PUFAs diet may lead to synaptic plasticity damage and learning and memory impairment in neurons, which may be due to the decrease in the inhibitory effect of the eCB system on neurotransmitter release. Thus, n-3 PUFAs are required for synaptic plasticity, which could be defective when inhibition of neurotransmitter release is abolished by eCBs. It has been mentioned and speculated that the abnormality of synaptic conduction in the nervous system may be related to the pathogenesis of ASD. Therefore, the low intake of n-3 PUFAs may affect the synthesis of eCBs, or affect the sensitivity of CB1R, resulting in abnormal eCB system, and then damage the synaptic plasticity of neurons. This may be the cause of social and cognitive impairment in children with ASD.

Metabolic abnormalities in PUFAs are common in children with ASD (El-Ansary and Al-Ayadhi, 2014). Some population experiments showed that the decrease of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and AA was related to cognitive impairment, which affected social function (Jiang et al., 2018; Lassek and Gaulin, 2014). A case-control study of a large population showed that AA, DHA, and EPA levels were lower in children with ASD than in normal children; n-3 PUFA supplementation improved social interaction and repetitive, restricted interests and behaviors (Mazahery et al., 2017). Our group previously showed that serum levels of AA, DHA, and docosapentaenoic acid were lower in autistic children than in controls (Zhao et al., 2015). In animal experiments where diet strictly controlled in order to reduce the influence of dietary factors, serum n-3 and n-6 PUFAs levels were still lower in ASD model as compared to control rats. We also observed that the expression of rate-limiting PUFA metabolic enzymes was downregulated in the ASD group (Zhao et al., 2015). The cognitive ability and learning and memory of model mice were significantly improved by PUFA intervention (Gao et al., 2016; Fortunato et al., 2017), and increasing DHA intake during pregnancy promoted the neural development of offspring and alleviated ASD-related behaviors in mice (Weiser et al., 2016). Thus, abnormalities in the eCB system resulting from a lack of n-3 PUFAs possibly can lead to dysregulation of synaptic function and maybe ultimately to ASD cognitive and social function defects.

The *NLGN3* gene encodes a post-synaptic adhesion molecule that inhibits synaptic transmission in the brain (Nguyen et al., 2016); two mutations in this gene are the potential cause of ASD. One is the deletion of the *NLGN3* gene (Jamain et al., 2003), which was found to be a susceptibility gene of ASD by chromosome sequencing, affecting functional synapse formation. The other is the substitution of *R451C*. The *R451C* mutation arising from a C→T transition in the parental *NLGN3* gene resulted in the substitution of the highly conserved arginine residue to cysteine (Jamain et al., 2003). *NLGN3R451C* knock-in (KI) and *NLGN3* KO mice showed ASD-related behavioral phenotypes (Radyushkin et al., 2009; Tabuchi et al., 2007) and impaired eCB signaling (Foldy et al., 2013). Previous studies have reported that eCBs signaling is blocked in the hippocampus of *NLGN3R451C* KI and *NLGN3* KO mouse models, as determined by measuring GABAergic synaptic transmission (Foldy et al., 2013). This is consistent with the observed dysregulation of eCBs in the hippocampus of these two models by inhibitory synaptic transmission between basket cells and pyramidal neurons (Krueger and Brose, 2013). It was recently demonstrate that synaptic plasticity defects in the dorsal striatum of the *NLGN3R451C* model could be alleviated by exogenously activating CB1R or increasing eCB levels (Martella et al., 2018). The link between *NLGN3* and eCBs supports the possibility that changes in eCB signaling contributes to ASD pathophysiology.

It is well known that p21-activated kinase (PAK) 1 inhibitors can significantly improve social and cognitive functioning in animal models of ASD (Dolan et al., 2013). PAK1 is a potent regulator of GABAergic neurotransmission, and its loss leads to impairment of the inhibitory postsynaptic current, which is manifested as a decrease in presynaptic GABA release. PAK1 limits AEA levels by promoting synaptic expression of COX-2 (Xia et al., 2018), and AEA levels are higher in PAK1 KO than in wild-type mice (Xia et al., 2016). It can be speculated that eCBs plays an intermediate role and there is ample evidence that ankylosing AEA signals have been shown to improve social interaction (Trezza et al., 2012).

4.3. Direct evidence for the relationship between eCBs and ASD

ECBs not only serve as a bridge for the onset of ASD; significant changes in the components of the eCB system have been detected in ASD animal models and patients. When BTBR mice—which are frequently used as an ASD model—were given selective FAAH inhibitor to increase the activity of AEA, social deficits associated with ASD were abolished (Wei et al., 2016). Additionally, social engagement was shown to increase AEA levels in the cortex (Gould et al., 2012), amygdala, nucleus accumbens (Trezza et al., 2012), and striatum in BTBR mice (Marco et al., 2011). Prenatal valproic acid (VPA) exposure induced ASD-like symptoms in offspring; this is therefore considered as a preclinical model of ASD that has good construct validity (Servadio et al., 2015). Moreover, social interaction defects in VPA rats were alleviated by AEA treatment (Servadio et al., 2016). Dysfunction of the eCB system may be the basis for some of the ASD-like behavioral changes in mouse models (Kerr et al., 2013). A rat model of VPA-induced ASD, time and frequency in social tests were reduced and DAGL

levels in the hippocampus and frontal cortex were lower than those in normal mice (Kerr et al., 2013). AEA signaling is enhanced by blocking AEA degradation, which reversed ASD-like behavioral deficits in VPA rats (Melancia et al., 2018). Down regulation of PPAR and GRP55 has also been reported in VPA rats. Although it is unclear how this relates to social behavior, recent data suggest that activation of hippocampal PPAR can improve cognitive performance (Denner et al., 2012). Therefore, the down regulation of PPAR may lead to decreased cognitive ability and impaired social behavioral responses in ASD.

Functional magnetic resonance imaging studies have shown that brain connectivity abnormalities in children with ASD may be caused by a lack of CB1 axon guidance (McFadden and Minshew, 2013). Since it has been shown that during brain development, CB1R can drive axon guidance and is responsible for synapse formation (Fride et al., 2009). There are also many case-control studies have revealed abnormal eCB levels in children with ASD from the peripheral immunological level. Plasma concentration of AEA in 59 ASD cases and 53 controls was determined by optimized liquid chromatography-tandem mass spectrometry. The results showed that the AEA concentration in children with ASD was lower than controls (Karhson et al., 2018); meanwhile,*CB2* mRNA and protein expression in peripheral blood mononuclear cells was upregulated whereas NAPE-PLD mRNA expression was downregulated in children with ASD relative to normal children (Siniscalco et al., 2013). Some studies have also shown that NAPE-PLD and CB2R levels in the peripheral blood are increased and that FAAH expression is decreased in autistic children (Siniscalco et al., 2014). Although these findings are somewhat contradictory, they suggest that abnormalities in the eCB system are associated with the occurrence of ASD. Table 1 summarizes the major changes in the eCB system in human patients and autistic animal models.

Glutamate excitotoxicity and inflammation are thought to be involved in the development of ASD, and PEA has been shown to simultaneously prevent glutamate toxicity and inhibit inflammatory responses. When a combination of risperidone and PEA was given to children with ASD aged 4–12 years in a double-blind placebo-controlled trial, the combination therapy improved compliance and hyperactivity in patients as compared to co-administration of risperidone and placebos (Khalaj et al., 2018). In the original report of two ASD cases, it was shown that PEA improved language expression, cognition, and behavior through regulation of mast cells and immune chemistry (Antonucci et al., 2015). The emotional problems associated with ASD and the degree of hyporesponsiveness to social stimulation vary with changes in the *CNR1* genotype (Chakrabarti et al., 2006, 2009). Several atypical phenotypic features of ASD such as sleep disorder, anxiety, and non-normal responses to social rewards are due to the fact that eCB assemble them collectively (Chakrabarti et al., 2015). Table 2 summarizes the application of eCB intervention in ASD.

5. Conclusion

The eCB system is an important regulatory system of the central nervous system that regulates neurotransmission and synaptic plasticity and is not only involved in many aspects of human health and disease, but also plays a key role in emotion, social responses, and cognitive functions. The association between eCB system abnormalities and various neurological diseases such as schizophrenia, depression, anxiety, and epilepsy has been demonstrated, and changes in eCB levels have been observed in these mental illnesses. As such, it is no surprise that ASD also involves the eCB system. In recent years, studies have also suggested that eCB system is associated with important functions in the development of the ASD-related nervous system (learning and memory, cognitive function, etc.). Evidence from human and animal models demonstrates that the main components of the eCB system are perturbed in the brain of ASD patients. Despite these inconsistencies, researchers generally agree that eCB are an important tache in the pathophysiology of various neuropsychiatric disorders. The role of eCB metabolic pathway regulation is complex, eCB can function either directly or through a large amount of metabolites, but our understanding of the eCB's signaling mechanism in ASD is still in its infancy. To date, most of what has been described pertains to AEA and 2-AG and relatively little is known about the roles of PEA and OEA. Further research on eCB signaling and eCB clinical intervention should be conducted in the future. The metabolic enzymes of the eCB system as well as key enzymes in oxidative pathways such as COX appear to be promising targets for ASD therapy. This review not only provides a scientific basis for further targeted nutrition interventions of ASD. Based on this, it also opened the way for the treatment of mental illness by eCB-oriented drugs.

Funding

This work was supported by the National Nature Science Foundation of China (No. 81773458).

Authors' contributions

MZ and LL did the literature review; DL wrote the manuscript. CS and LW assisted with the writing of the manuscript. All authors read, corrected and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

American Psychiatric Association, 2013. Diagnostic and Statistical Manual of Mental Disorders, 5th ed. American Psychiatric Association, Washington,DC, pp. 58–62.
Andres-Mach, M., Haratym-Maj, A., Zagaja, M., et al., 2015. ACEA (a highly selective cannabinoid CB1 receptor agonist) stimulates hippocampal neurogenesis in mice treated with antiepileptic drugs. Brain Res. 1624, 86–94.
Antonucci, N., Cirillo, A., Siniscalco, D., 2015. Beneficial effects of palmitoylethanolamide on expressive language, cognition, and behaviors in autism: a report of two cases. Case Rep. Psychiatry 2015, 325061.
Baio, J., Wiggins, L., Christensen, D.L., et al., 2018. Prevalence of autism spectrum disorder among children aged 8 years – autism and developmental disabilities monitoring network, 11 sites, United States, 2014. Surveill. Summ. 67 (6), 1–23.
Bisogno, T., 2016. Assay of DAGLalpha/beta activity. Methods Mol. Biol. 1412, 149–156.
Bisogno, T., Howell, F., Williams, G., et al., 2003. Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. J. Cell Biol. 163 (3), 463–468.
Blankman, J.L., Simon, G.M., Cravatt, B.F., 2007. A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. Chem. Biol. 14 (12), 1347–1356.
Busquets-Garcia, A., Gomis-Gonzalez, M., Guegan, T., et al., 2013. Targeting the endocannabinoid system in the treatment of fragile X syndrome. Nat. Med. 19 (5), 603–607.
Cabral, G.A., Griffin-Thomas, L., 2009. Emerging role of the cannabinoid receptor CB2 in immune regulation: therapeutic prospects for neuroinflammation. Expert Rev. Mol. Med. 11, e3.
Carr, R.L., Adams, A.L., Kepler, D.R., et al., 2013. Induction of endocannabinoid levels in juvenile rat brain following developmental chlorpyrifos exposure. Toxicol. Sci. 135 (1), 193–201.
Castillo, P.E., Younts, T.J., Chavez, A.E., et al., 2012. Endocannabinoid signaling and synaptic function. Neuron 76 (1), 70–81.
Chakrabarti, B., Kent, L., Suckling, J., et al., 2006. Variations in the human cannabinoid receptor (CNR1) gene modulate striatal responses to happy faces. Eur. J. Neurosci. 23 (7), 1944–1948.
Chakrabarti, B., Dudbridge, F., Kent, L., et al., 2009. Genes related to sex steroids, neural growth, and social-emotional behavior are associated with autistic traits, empathy, and Asperger syndrome. Autism Res. 2 (3), 157–177.

Chakrabarti, B., Persico, A., Battista, N., et al., 2015. Endocannabinoid signaling in autism. Neurotherapeutics 12 (4), 837–847.
Coccaro, E.F., Hill, M.N., Robinson, L., et al., 2018. Circulating endocannabinoids and affect regulation in human subjects. Psychoneuroendocrinology 92, 66–71.
de Almeida, V., Martins-de-Souza, D., 2018. Cannabinoids and glial cells: possible mechanism to understand schizophrenia. Eur. Arch. Psychiatry Clin. Neurosci. 268 (7), 727–737.
Deans, P.J.M., Raval, P., Sellers, K.J., et al., 2017. Psychosis risk candidate ZNF804A localizes to synapses and regulates neurite formation and dendritic spine structure. Biol. Psychiatry 82 (1), 49–61.
Denner, L.A., Rodriguez-Rivera, J., Haidacher, S.J., et al., 2012. Cognitive enhancement with rosiglitazone links the hippocampal PPARgamma and ERK MAPK signaling pathways. J. Neurosci. 32 (47), 16725–16735a.
Dolan, B.M., Duron, S.G., Campbell, D.A., et al., 2013. Rescue of fragile X syndrome phenotypes in Fmr1 KO mice by the small-molecule PAK inhibitor FRAX486. Proc. Natl. Acad. Sci. U. S. A. 110 (14), 5671–5676.
El-Ansary, A., Al-Ayadhi, L., 2014. Relative abundance of short chain and polyunsaturated fatty acids in propionic acid-induced autistic features in rat pups as potential markers in autism. Lipids Health Dis. 13, 140.
Elphick, M.R., Egertova, M., 2001. The neurobiology and evolution of cannabinoid signalling. Philos. Trans. R. Soc. Lond. B Biol. Sci. 356 (1407), 381–408.
Foldy, C., Malenka, R.C., Sudhof, T.C., 2013. Autism-associated neuroligin-3 mutations commonly disrupt tonic endocannabinoid signaling. Neuron 78 (3), 498–509.
Fortunato, J.J., da Rosa, N., Martins Laurentino, A.O., et al., 2017. Effects of omega-3 fatty acids on stereotypical behavior and social interactions in Wistar rats prenatally exposed to lipopolysaccarides. Nutrition 35, 119–127.
Freund, T.F., Katona, I., Piomelli, D., 2003. Role of endogenous cannabinoids in synaptic signaling. Physiol. Rev. 83 (3), 1017–1066.
Fride, E., Gobshtis, N., Dahan, H., et al., 2009. The endocannabinoid system during development: emphasis on perinatal events and delayed effects. Vitam. Horm. 81, 139–158.
Fukami, K., Inanobe, S., Kanemaru, K., et al., 2010. Phospholipase C is a key enzyme regulating intracellular calcium and modulating the phosphoinositide balance. Prog. Lipid Res. 49 (4), 429–437.
Gao, Y., Vasilyev, D.V., Goncalves, M.B., et al., 2010. Loss of retrograde endocannabinoid signaling and reduced adult neurogenesis in diacylglycerol lipase knock-out mice. J. Neurosci. 30 (6), 2017–2024.
Gao, J., Wu, H., Cao, Y., et al., 2016. Maternal DHA supplementation protects rat offspring against impairment of learning and memory following prenatal exposure to valproic acid. J. Nutr. Biochem. 35, 87–95.
Ginovart, N., Tournier, B.B., Moulin-Sallanon, M., et al., 2012. Chronic Delta(9)-tetrahydrocannabinol exposure induces a sensitization of dopamine D(2)/(3) receptors in the mesoaccumbens and nigrostriatal systems. Neuropsychopharmacology 37 (11), 2355–2367.
Gomis-Gonzalez, M., Busquets-Garcia, A., Matute, C., et al., 2016. Possible therapeutic doses of cannabinoid type 1 receptor antagonist reverses key alterations in fragile X syndrome mouse model. Genes (Basel) 7 (9).
Goparaju, S.K., Ueda, N., Yamaguchi, H., et al., 1998. Anandamide amidohydrolase reacting with 2-arachidonoylglycerol, another cannabinoid receptor ligand. FEBS Lett. 422 (1), 69–73.
Gould, G.G., Seillier, A., Weiss, G., et al., 2012. Acetaminophen differentially enhances social behavior and cortical cannabinoid levels in inbred mice. Prog. Neuropsychopharmacol. Biol. Psychiatry 38 (2), 260–269.
Green, C.R., Corsi-Travali, S., Neumeister, A., 2013. The role of BDNF-TrkB signaling in the pathogenesis of PTSD. J. Depress. Anxiety 2013 (S4).
Griebel, G., Pichat, P., Beeske, S., et al., 2015. Selective blockade of the hydrolysis of the endocannabinoid 2-arachidonoylglycerol impairs learning and memory performance while producing antinociceptive activity in rodents. Sci. Rep. 5, 7642.
Harony-Nicolas, H., Kay, M., Hoffmann, J.D., et al., 2017. Oxytocin improves behavioral and electrophysiological deficits in a novel Shank3-deficient rat. Elife 6.
Hegyi, Z., Hollo, K., Kis, G., et al., 2012. Differential distribution of diacylglycerol lipase-alpha and N-acylphosphatidylethanolamine-specific phospholipase d immunoreactivity in the superficial spinal dorsal horn of rats. Glia 60 (9), 1316–1329.
Hill, M.N., Gorzalka, B.B., 2005. Pharmacological enhancement of cannabinoid CB1 receptor activity elicits an antidepressant-like response in the rat forced swim test. Eur. Neuropsychopharmacol. 15 (6), 593–599.
Howes, O.D., Kapur, S., 2009. The dopamine hypothesis of schizophrenia: version III–the final common pathway. Schizophr. Bull. 35 (3), 549–562.
Iannotti, F.A., Di Marzo, V., Petrosino, S., 2016. Endocannabinoids and endocannabinoid-related mediators: targets, metabolism and role in neurological disorders. Prog. Lipid Res. 62, 107–128.
Jamain, S., Quach, H., Betancur, C., et al., 2003. Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. Nat. Genet. 34 (1), 27–29.
Jiang, W., Whellan, D.J., Adams, K.F., et al., 2018. Long-chain omega-3 fatty acid supplements in depressed heart failure patients: results of the OCEAN trial. JACC Heart Fail. 6 (10), 833–843.
Jung, K.M., Sepers, M., Henstridge, C.M., et al., 2012. Uncoupling of the endocannabinoid signalling complex in a mouse model of fragile X syndrome. Nat. Commun. 3, 1080.
Karhson, D.S., Krasinska, K.M., Dallaire, J.A., et al., 2018. Plasma anandamide concentrations are lower in children with autism spectrum disorder. Mol. Autism 9, 18.
Keimpema, E., Calvigioni, D., Harkany, T., 2013. Endocannabinoid signals in the developmental programming of delayed-onset neuropsychiatric and metabolic illnesses. Biochem. Soc. Trans. 41 (6), 1569–1576.
Kerr, D.M., Downey, L., Conboy, M., et al., 2013. Alterations in the endocannabinoid system in the rat valproic acid model of autism. Behav. Brain Res. 249, 124–132.
Khalaj, M., Saghazadeh, A., Shirazi, E., et al., 2018. Palmitoylethanolamide as adjunctive therapy for autism: efficacy and safety results from a randomized controlled trial. J. Psychiatr. Res. 103, 104–111.
Koethe, D., Pahlisch, F., Hellmich, M., et al., 2018. Familial abnormalities of endocannabinoid signaling in schizophrenia. World J. Biol. Psychiatry 1-9.
Kreitzer, A.C., Malenka, R.C., 2005. Dopamine modulation of state-dependent endocannabinoid release and long-term depression in the striatum. J. Neurosci. 25 (45), 10537–10545.
Krueger, D.D., Brose, N., 2013. Evidence for a common endocannabinoid-related pathomechanism in autism spectrum disorders. Neuron 78 (3), 408–410.
Lafourcade, M., Larrieu, T., Mato, S., et al., 2011. Nutritional omega-3 deficiency abolishes endocannabinoid-mediated neuronal functions. Nat. Neurosci. 14 (3), 345–350.
Lassek, W.D., Gaulin, S.J., 2014. Linoleic and docosahexaenoic acids in human milk have opposite relationships with cognitive test performance in a sample of 28 countries. Prostaglandins Leukot. Essent. Fatty Acids 91 (5), 195–201.
Leweke, F.M., Giuffrida, A., Koethe, D., et al., 2007. Anandamide levels in cerebrospinal fluid of first-episode schizophrenic patients: impact of cannabis use. Schizophr. Res. 94 (1–3), 29–36.
Lian, J., Deng, C., 2018. The effects of antipsychotics on the density of cannabinoid receptors in selected brain regions of male and female adolescent juvenile rats. Psychiatry Res. 266, 317–322.
Lin, Y.C., Frei, J.A., Kilander, M.B., et al., 2016. A subset of autism-associated genes regulate the structural stability of neurons. Front. Cell. Neurosci. 10, 263.
Lipstein, N., Verhoeven-Duif, N.M., Michelassi, F.E., et al., 2017. Synaptic UNC13A protein variant causes increased neurotransmission and dyskinetic movement disorder. J. Clin. Invest. 127 (3), 1005–1018.
Luszczki, J.J., Czuczwar, P., Cioczek-Czuczwar, A., et al., 2006. Arachidonyl-2'-chloroethylamide, a highly selective cannabinoid CB1 receptor agonist, enhances the anticonvulsant action of valproate in the mouse maximal electroshock-induced seizure model. Eur. J. Pharmacol. 547 (1–3), 65–74.
Luszczki, J.J., Misiuta-Krzesinska, M., Florek, M., et al., 2011. Synthetic cannabinoid WIN 55,212-2 mesylate enhances the protective action of four classical antiepileptic drugs against maximal electroshock-induced seizures in mice. Pharmacol. Biochem. Behav. 98 (2), 261–267.
Mailleux, P., Vanderhaeghen, J.J., 1992. Distribution of neuronal cannabinoid receptor in the adult rat brain: a comparative receptor binding radioautography and in situ hybridization histochemistry. Neuroscience 48 (3), 655–668.
Marco, E.M., Rapino, C., Caprioli, A., et al., 2011. Social encounter with a novel partner in adolescent rats: activation of the central endocannabinoid system. Behav. Brain Res. 220 (1), 140–145.
Martella, G., Meringolo, M., Trobiani, L., et al., 2018. The neurobiological bases of autism spectrum disorders: the R451C-neuroligin 3 mutation hampers the expression of long-term synaptic depression in the dorsal striatum. Eur. J. Neurosci. 47 (6), 701–708.
Mazahery, H., Stonehouse, W., Delshad, M., et al., 2017. Relationship between long chain n-3 polyunsaturated fatty acids and autism spectrum disorder: systematic review and meta-analysis of case-control and randomised controlled trials. Nutrients 9 (2).
McFadden, K., Minshew, N.J., 2013. Evidence for dysregulation of axonal growth and guidance in the etiology of ASD. Front. Hum. Neurosci. 7, 671.
Mechoulam, R., Gaoni, Y.A., 1965. Total synthesis of DL-delta-1-tetrahydrocannabinol, the active constituent of Hashish. J. Am. Chem. Soc. 87, 3273–3275.
Mechoulam, R., Fride, E., Ben-Shabat, S., et al., 1998. Carbachol, an acetylcholine receptor agonist, enhances production in rat aorta of 2-arachidonoyl glycerol, a hypotensive endocannabinoid. Eur. J. Pharmacol. 362 (1), R1–3.
Melancia, F., Schiavi, S., Servadio, M., et al., 2018. Sex-specific autistic endophenotypes induced by prenatal exposure to valproic acid involve anandamide signalling. Br. J. Pharmacol.
Muccioli, G.G., 2010. Endocannabinoid biosynthesis and inactivation, from simple to complex. Drug Discov. Today 15 (11–12), 474–483.
Navarria, A., Tamburella, A., Iannotti, F.A., et al., 2014. The dual blocker of FAAH/TRPV1 N-arachidonoylserotonin reverses the behavioral despair induced by stress in rats and modulates the HPA-axis. Pharmacol. Res. 87, 151–159.
Navarro, G., Borroto-Escuela, D., Angelats, E., et al., 2018. Receptor-heteromer mediated regulation of endocannabinoid signaling in activated microglia. Role of CB1 and CB2 receptors and relevance for Alzheimer's disease and levodopa-induced dyskinesia. Brain Behav. Immun. 67, 139–151.
Nguyen, Q.A., Horn, M.E., Nicoll, R.A., 2016. Distinct roles for extracellular and intracellular domains in neuroligin function at inhibitory synapses. Elife 5.
O'Sullivan, S.E., Kendall, D.A., 2010. Cannabinoid activation of peroxisome proliferator-activated receptors: potential for modulation of inflammatory disease. Immunobiology 215 (8), 611–616.
Okamoto, Y., Wang, J., Morishita, J., et al., 2007. Biosynthetic pathways of the endocannabinoid anandamide. Chem. Biodivers. 4 (8), 1842–1857.
Oudin, M.J., Hobbs, C., Doherty, P., 2011a. DAGL-dependent endocannabinoid signalling: roles in axonal pathfinding, synaptic plasticity and adult neurogenesis. Eur. J. Neurosci. 34 (10), 1634–1646.
Oudin, M., Hobbs, C., Doherty, P., 2011b. DAGL-dependent endocannabinoid signalling: roles in axonal pathfinding, synaptic plasticity and adult neurogenesis. Eur. J. Neurosci. 34.
Paez, J.A., Campillo, N.E., 2018. Innovative therapeutic potential of cannabinoid receptors as targets in Alzheimer's disease and less well-known diseases. Curr. Med. Chem.
Palazuelos, J., Ortega, Z., Diaz-Alonso, J., et al., 2012. CB2 cannabinoid receptors promote neural progenitor cell proliferation via mTORC1 signaling. J. Biol. Chem. 287 (2), 1198–1209.
Parkkari, T., Haavikko, R., Laitinen, T., et al., 2014. Discovery of triterpenoids as reversible inhibitors of alpha/beta-hydrolase domain containing 12 (ABHD12). PLoS One 9 (5), e98286.

Pertwee, R.G., Howlett, A.C., Abood, M.E., et al., 2010. International union of basic and clinical pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB (1) and CB(2). Pharmacol. Rev. 62 (4), 588–631.
Piyanova, A., Albayram, O., Rossi, C.A., et al., 2013. Loss of CB1 receptors leads to decreased cathepsin D levels and accelerated lipofuscin accumulation in the hippocampus. Mech. Ageing Dev. 134 (9), 391–399.
Potvin, S., Stip, E., Lipp, O., et al., 2008. Anhedonia and social adaptation predict substance abuse evolution in dual diagnosis schizophrenia. Am. J. Drug Alcohol Abuse 34 (1), 75–82.
Qin, M., Zeidler, Z., Moulton, K., et al., 2015. Endocannabinoid-mediated improvement on a test of aversive memory in a mouse model of fragile X syndrome. Behav. Brain Res. 291, 164–171.
Radyushkin, K., Hammerschmidt, K., Boretius, S., et al., 2009. Neuroligin-3-deficient mice: model of a monogenic heritable form of autism with an olfactory deficit. Genes Brain Behav. 8 (4), 416–425.
RdM, Maresca, Autism, L., 2015. In: 1st ed. In: Robinson-Agramonte, M. (Ed.), What Is It? In Translational Approach to Autism Spectrum Disorder Volume 1. Springer International Publishing, Basel, Switzerland, pp. 1–11.
Reuter, A.R., Bumb, J.M., Mueller, J.K., et al., 2017. Association of anandamide with altered binocular depth inversion illusion in schizophrenia. World J. Biol. Psychiatry 18 (6), 483–488.
Savinainen, J.R., Saario, S.M., Laitinen, J.T., 2012. The serine hydrolases MAGL, ABHD6 and ABHD12 as guardians of 2-arachidonoylglycerol signalling through cannabinoid receptors. Acta Physiol. (Oxf) 204 (2), 267–276.
Servadio, M., Vanderschuren, L.J., Trezza, V., 2015. Modeling autism-relevant behavioral phenotypes in rats and mice: do' autistic' rodents exist? Behav. Pharmacol. 26 (6), 522–540.
Servadio, M., Melancia, F., Manduca, A., et al., 2016. Targeting anandamide metabolism rescues core and associated autistic-like symptoms in rats prenatally exposed to valproic acid. Transl. Psychiatry 6 (9), e902.
Shanker, G., Aschner, M., 2003. Methylmercury-induced reactive oxygen species formation in neonatal cerebral astrocytic cultures is attenuated by antioxidants. Brain Res. Mol. Brain Res. 110 (1), 85–91.
Siniscalco, D., Sapone, A., Giordano, C., et al., 2013. Cannabinoid receptor type 2, but not type 1, is up-regulated in peripheral blood mononuclear cells of children affected by autistic disorders. J. Autism Dev. Disord. 43 (11), 2686–2695.
Siniscalco, D., Bradstreet, J.J., Cirillo, A., et al., 2014. The in vitro GcMAF effects on endocannabinoid system transcriptionomics, receptor formation, and cell activity of autism-derived macrophages. J. Neuroinflammation 11, 78.
Stella, N., Schweitzer, P., Piomelli, D., 1997. A second endogenous cannabinoid that modulates long-term potentiation. Nature 388 (6644), 773–778.
Sugiura, T., Waku, K., 2000. 2-Arachidonoylglycerol and the cannabinoid receptors. Chem. Phys. Lipids 108 (1-2), 89–106.
Sugiura, T., Kondo, S., Kishimoto, S., et al., 2000. Evidence that 2-arachidonoylglycerol but not N-palmitoylethanolamine or anandamide is the physiological ligand for the cannabinoid CB2 receptor. Comparison of the agonistic activities of various cannabinoid receptor ligands in HL-60 cells. J. Biol. Chem. 275 (1), 605–612.
Tabuchi, K., Blundell, J., Etherton, M.R., et al., 2007. A neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice. Science 318 (5847), 71–76.
Thomazeau, A., Bosch-Bouju, C., Manzoni, O., et al., 2017. Nutritional n-3 PUFA deficiency abolishes endocannabinoid gating of hippocampal long-term potentiation. Cereb. Cortex 27 (4), 2571–2579.
Toth, A., Blumberg, P.M., Boczan, J., 2009. Anandamide and the vanilloid receptor (TRPV1). Vitam. Horm. 81, 389–419.
Trezza, V., Damsteegt, R., Manduca, A., et al., 2012. Endocannabinoids in amygdala and nucleus accumbens mediate social play reward in adolescent rats. J. Neurosci. 32 (43), 14899–14908.
Turunen, P.M., Louhivuori, L.M., Louhivuori, V., et al., 2018. Endocannabinoid signaling in embryonic neuronal motility and cell–cell contact – role of mGluR5 and TRPC3 channels. Neuroscience 375, 135–148.
Ueda, N., Tsuboi, K., Uyama, T., 2013. Metabolism of endocannabinoids and related N-acylethanolamines: canonical and alternative pathways. FEBS J. 280 (9), 1874–1894.
Vinogradova, L.V., van Rijn, C.M., 2015. Long-term disease-modifying effect of the endocannabinoid agonist WIN55,212-2 in a rat model of audiogenic epilepsy. Pharmacol. Rep. 67 (3), 501–503.
Volk, D.W., Lewis, D.A., 2016. The role of endocannabinoid signaling in cortical inhibitory neuron dysfunction in schizophrenia. Biol. Psychiatry 79 (7), 595–603.
Wang, W., Cox, B.M., Jia, Y., et al., 2017. Treating a novel plasticity defect rescues episodic memory in Fragile X model mice. Mol. Psychiatry 23 (8), 1798–1806.
Wei, D., Dinh, D., Lee, D., et al., 2016. Enhancement of anandamide-mediated endocannabinoid signaling corrects autism-related social impairment. Cannabis Cannabinoid Res. 1 (1), 81–89.
Weiser, M.J., Mucha, B., Denheyer, H., et al., 2016. Dietary docosahexaenoic acid alleviates autistic-like behaviors resulting from maternal immune activation in mice. Prostaglandins Leukot. Essent. Fatty Acids 106, 27–37.
Witkin, J.M., Tzavara, E.T., Nomikos, G.G., 2005. A role for cannabinoid CB1 receptors in mood and anxiety disorders. Behav. Pharmacol. 16 (5–6), 315–331.
Wu, C.S., Zhu, J., Wager-Miller, J., et al., 2010. Requirement of cannabinoid CB(1) receptors in cortical pyramidal neurons for appropriate development of corticothalamic and thalamocortical projections. Eur. J. Neurosci. 32 (5), 693–706.
Xia, S., Zhou, Z., Leung, C., et al., 2016. p21-activated kinase 1 restricts tonic endocannabinoid signaling in the hippocampus. Elife 5.
Xia, S., Zhou, Z., Jia, Z., 2018. PAK1 regulates inhibitory synaptic function via a novel mechanism mediated by endocannabinoids. Small GTPases 9 (4), 322–326.
Zhao, G., Gao, J., Liang, S., et al., 2015. Study of the serum levels of polyunsaturated fatty acids and the expression of related liver metabolic enzymes in a rat valproate-induced autism model. Int. J. Dev. Neurosci. 44, 14–21.
Zou, S., Kumar, U., 2018. Cannabinoid receptors and the endocannabinoid system: signaling and function in the central nervous system. Int. J. Mol. Sci. 19 (3).
Zygmunt, P.M., Petersson, J., Andersson, D.A., et al., 1999. Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. Nature 400 (6743), 452–457.

Journal of Autism and Developmental Disorders (2019) 49:2873–2900
https://doi.org/10.1007/s10803-019-04014-z

ORIGINALPAPER

The Economic Costs of Autism Spectrum Disorder: A Literature Review

Nicky Rogge[1] · **Juliette Janssen**[1]

Published online: 11 April 2019

Abstract
Autism is associated with a range of costs. This paper reviews the literature on estimating the economic costs of autism spectrum disorder (ASD). More or less 50 papers covering multiple countries (US, UK, Australia, Canada, Sweden, the Netherlands, etc.) were analysed. Six types of costs are discussed in depth: (i) medical and healthcare service costs, (ii) therapeutic costs, (iii) (special) education costs, (iv) costs of production loss for adults with ASD, (v) costs of informal care and lost productivity for family/caregivers, and (vi) costs of accommodation, respite care, and out-of-pocket expenses. A general finding is that individuals with ASD and families with children with ASD have higher costs. Education costs appear to be a major cost component for parents with children with ASD.

Keywords Autism · Autism spectrum disorder · Direct costs · Indirect costs · Financial burden

Introduction

Autism spectrum disorder (ASD) is a range of neurodevelopmental disorders that are characterized by the following core deficits: impairments in social interaction and communication, and restricted, repetitive behaviours (DSM-5, American Psychiatric Association 2013). ASD affects people worldwide, irrespective of race, ethnicity or socio-economic status (Sharpe and Baker 2011; Durkin et al. 2010). Studies and empirical evidence also show ASD is related to many potential comorbidities such as epilepsy, attention problems, gastro-intestinal problems, oppositional behaviour, anxiety and depression, sleeping disorder and feeding disorders (Hodgetts et al. 2015; Kogan et al., 2008; Vohra et al. 2017). As to the population prevalence of ASD, estimated figures vary depending on the country of study, the period studied, and the estimation method used. Recent estimates range from 1 per 160, 1 per 100, to 2 per 100 (Baird et al. 2006; Baio et al. 2018; Cidav et al. 2012; Hughes 2009; Knapp et al. 2009). Most recent estimates for the US as collected by the Autism and Developmental Disabilities Monitoring Network (Centers for Disease Control and Prevention, US Department of Health and Human Services), for instance, yielded overall ASD prevalence estimates varying from 13.1 to 29.3 per 1000 children aged 8 years with an average prevalence estimate of 16.8 per 1000 children aged 8 years (Baio et al. 2018). Based on the prevalence estimates of several studies across multiple countries, Lyall et al. (2017) estimated the population prevalence to be around 1.5% in developed countries around the world. As possible reasons for the discrepancy in estimated prevalence figures, studies point out, among other things, that for several countries (e.g., Belgium, Scotland, most of the Arabic countries, etc.) there are no reliable and/or only limited statistics available regarding the prevalence of ASD and that diagnosis in ASD can be difficult or complicated due to no or ineffective screening, and the interactions that occur between development and ASD symptoms. Studies on the prevalence of ASD also show that there is an increasing trend in the percentage of the population that is diagnosed with ASD (Lyall et al. 2017; Jacob et al. 2015; Sharpe and Baker 2007), with more recent studies showing higher estimates of prevalence rates compared to older studies. However, it is unclear whether this increase is due to an actual increase in prevalence of ASD, more broadly defined diagnostic criteria, better public and medical awareness, improved possibilities of diagnosing children at a young age, or a combination of all these factors (Jacob et al. 2015; Kogan et al. 2008; Leslie and Martin 2007). Studies

✉ Nicky Rogge
Nicky.rogge@kuleuven.be

Juliette Janssen
Juliette.janssen@hotmail.com

[1] Faculty of Economics and Business, Research Centre for Economics (ECON), KU Leuven, Warmoesberg 26, 1000 Brussels, Belgium

also showed that a considerable share of the people with ASD have intellectual disabilities. Estimates of individuals with ASD having intellectual disabilities reported by recent studies vary between 30 and 50% (Baio et al. 2018; Buescher et al. 2014; Atladóttir et al. 2007; Baird et al. 2006). For instance, recent estimates for the US show that 31% of children with ASD were classified in the range of intellectual disability, i.e. IQ < 70) (Baio et al. 2018; Centers for Disease Control and Prevention). In a study of the costs of ASD in the US and the UK, based on findings of previous studies, Buescher et al. (2014) reported a 40–60% split (i.e., 40% of individuals with ASD having intellectual disabilities).

The rising number of people being diagnosed with ASD, in combination with the life-long care and support that most individuals with ASD require across multiple domains such as education, healthcare and community services (the need for support depending on the severity of the disorder), makes it a major societal concern involving significant costs for the individual diagnosed with ASD, his/her family, private and/or public health insurance systems, state financial aid programmes, and society, more generally. Having a better understanding of the ASD-related costs is beneficial and informative for several reasons. Firstly, a study of the costs could yield a detailed picture of the size of the costs, the different types of costs, the distribution of the cost burden for the different parties involved, as well as unintended or negative consequences of policies. For example, information about what costs are borne by families and to what extend costs related to ASD are covered by health insurance, the education system or state financial aid programs can reveal the financial burden for families of individuals with ASD. If financial costs are not sufficiently funded by public resources or health insurance programs this might negatively affect the access to certain services for ASD-individuals (or their families) with limited personal financial resources. Secondly, in responding to this challenge of a larger number of people being diagnosed with ASD, those responsible for developing and implementing policies and deciding on resource allocations need to have good knowledge of the consequences of their decisions. A clear overview of the total cost of ASD and all its individual components can help policy makers make informed decisions about public resource allocation and the organization of public services for individuals with ASD. Thirdly, costs related to ASD are not limited to service and healthcare costs. A comprehensive overview of indirect costs, such as informal care and parental lost productivity, might assist policy makers in finding ways to help families through family support systems.

The aim of this paper is to review the literature dealing with estimating the economic costs of ASD. Around 50 papers covering multiple countries (US, UK, Australia, Canada, Sweden, the Netherlands, Egypt and China) are analysed. Six types of ASD-related costs are discussed in depth: (i) medical and healthcare related service costs, (ii) therapeutic costs, (iii) (special) education costs, (iv) costs of production loss for adults with ASD, (v) costs of informal care and lost productivity for family/caregivers, and (vi) costs of accommodation, respite care, and out-of-pocket expenses.

The paper adds to the literature in that it provides a comprehensive overview of the recent literature on ASD costs. To our knowledge, only two studies have actually provided a review of the cost estimation studies on ASD: Amendah et al. (2011) and Sharpe and Baker (2011). Amendah et al. (2011) conducted a literature review on ASD costs thereby following an approach similar to the one used in the present review paper, i.e., screening databases for peer-reviewed literature and additional sources of information on the costs of care for individuals with ASD thereby using a set of key words. However, Amendah et al. (2011) focused primarily on US-based studies (however, due to scarcity of data/studies for the US, also (a limited number of) non-US studies were included for non-medical ASD costs). Though there is some overlap between Amendah et al. (2011) and the present review study (Amendah et al. 2011 reviewed a selection of 40 studies, 13 of which are also reviewed in the present review study), we believe that our paper does add to the literature in that it complements the review study of Amendah et al. (2011) by reviewing the recent literature on ASD costs (period 2011–2017) and also non-US studies on medical costs or expenditures of ASD. Sharpe and Baker (2011) conducted a brief review of a 10 studies on autism-related costs, 5 studies on the US and 5 studies abroad. All 10 studies are also included in our review study.

Literature Review Search Strategy and Structure

The literature search was conducted using multiple databases (Medline, Web of Science, Scopus and ScienceOpen). In the search for studies to include in the literature review, the key terms 'autism', 'ASD', and 'autism spectrum disorder' were combined with the following keywords: 'costs', 'economic costs', 'economic burden', and 'expenditures'. Only papers from 2000 onwards were considered in the search process (i.e., the search period covered January 2000–January 2018). The general terms 'autism spectrum disorder' and 'ASD' were used as an

inclusion criterion. Given the broadness of the ASD-spectrum this implies that different types of pervasive developmental disorders (PDDs), such as autistic disorder, childhood disintegrative disorder, PDD-not otherwise specified (PDD-NOS), and Asperger syndrome were considered (DSM-5, American Psychiatric Association 2013).[1] As a selection criterion, it was decided that studies focusing on costs and cost calculations for both children and adults with ASD were to be included. No language barriers were added to the inclusion criteria, although a large majority of the papers were in English. A first screening of the titles and the abstracts (thereby focusing on whether the key terms appeared in the title and/or abstract) reduced the selection with slightly more than two-thirds. During the readings and reviews, it was decided that some papers should not be further considered in the literature review. It concerned mainly studies that didn't list any cost figures or estimations of ASD costs and/or that didn't elaborate on the costs of ASD. Papers that didn't explicitly calculate/estimate ASD-related costs were retained in the selection if they discussed the concept of costs related to ASD at a more general level as these papers are relevant to papers that do explicitly perform cost calculations, for instance, by identifying the cost categories that should be taken into account in such calculations/estimations (e.g., Murphy et al. 2011). This resulted in a selection of 39 papers remaining. To further expand the literature search, reference lists from selected papers were screened for potentially interesting papers. This resulted in some papers and book chapters (nine in total) being added to the selection (e.g., Amendah et al. 2011; Sharpe and Baker 2011).

The final selection of papers thus includes 48 papers, with most papers from peer-reviewed journals. Over half of the studies were conducted in the US. Other studies were carried out in the UK (e.g. Barrett et al. 2015), Australia (Horlin et al. 2014), Sweden (Järbrink 2007), Canada (Hodgetts et al. 2015), The Netherlands (Peters-Scheffer 2015; Peters-Scheffer et al. 2012), China (Xiong et al. 2011) and Egypt (Mendoza 2010). Note that in the screening of the papers, no strong methodological considerations were imposed as to how cost data were collected, used in cost estimations (i.e. which cost estimation technique was used) and/or reported. As to the sample scale, for instance, there are papers in the final selection which collected national-level data from one or multiple national data sources (e.g., Liptak et al. 2006; Ganz 2007; Leslie and Martin 2007), whereas other papers used data from lower-level (e.g. county-level) administrative sources (e.g., Mandell et al. 2006; Croen et al. 2006), and some papers even used data as collected from small-sample interviews/questionnaires (e.g., Barrett et al. 2012; Järbrink 2007). Data were abstracted from the papers using a standard checks list designating what type of information was to be collected and reported into review tables. In particular, for each paper the following information was abstracted: the author(s) of the study, the year of article publication, the country of study, the data source(s) used, ASD cost components per category, reported/estimated cost figures (eventually with ranges). If available, also the following types of information were to be collected: the share(s) of the cost components to the overall ASD-related costs (or, in case of lifetime costs, the cost share for each age period), the base at which costs were assessed/estimated (annual costs, lifetime costs, etc.), and the currency and year in which cost figures are reported. Depending on the article, costs for individuals with the ASD-diagnosis are compared to typically developing children and adults, or to individuals with other mental disorders or physical impairments. Other papers compare the costs for individuals with ASD across age groups, the costs and benefits of different therapies, or the costs for people with ASD with or without intellectual disabilities. If such comparisons were made, both cost figures were provided in the overview tables.

The literature review is organized into sections that correspond to categories/components of ASD-related costs. Different categorizations of the ASD-related costs have been proposed in the literature. Lavelle et al. (2014), for instance, estimated ASD-related costs in three categories: direct medical, direct non-medical costs (especially special education services and behavioural therapies), and caregiver productivity costs (average wage times increased hours of caregiving). Amendah et al. (2011) distinguished between four domains of ASD costs: medical, nonmedical, productivity, and caregiver time. Buescher et al. (2014) assessed seven ASD-related cost categories: accommodation (residential care), medical services, nonmedical services, special education, employer support, lost parental productivity, and lost individual productivity, with employer support and lost individual productivity cost categories only applying to adults with ASD aged 18 and over. Looking for a balance between cost categories that are too broad or narrow, this paper distinguishes between six types of ASD-related costs: (i) medical and healthcare related service costs, (ii) therapeutic costs, (iii) (special) education costs, (iv) costs of production loss for adults with ASD, (v) costs of informal care and lost productivity for family/caregivers, and (vi) costs of accommodation, respite care, and out-of-pocket expenses. In the review of the cost estimation studies, detailed cost figures are reported in Tables 1, 2, 3, 4, 5 and 6 and general findings are discussed in the text. Note that Tables 1, 2, 3, 4, 5 and 6 list cost figures in the monetary unit and base year

[1] Alternatively, one could opt to include the individual diagnostic labels [autistic disorder, childhood disintegrative disorder, pervasive developmental disorder-not otherwise specified (PDD-NOS), and Asperger syndrome] in the search process.

Table 1 Overview of studies including different types of medical costs

References	Countries	Data	Cost components	Main findings
Liptak et al. (2006)	US	– Cost data collected from three national surveys	In- and outpatient services, Emergency care, Physician/general practitioner, Other healthcare professionals, Pharmacy, Home healthcare	– Average total annual healthcare cost for ASD with ASD of $ 6132 (in 1999 US$) (*$ 9242*) – Outpatient expenditures accounted for roughly 65% of annual healthcare cost
Mandell et al. (2006)	US	– Data from 1994 to 1999 from one large county in Pennsylvania	Medicaid expenditures of children diagnosed with ASD	– Children diagnosed with ASD had on average expenditures 10 times those of other children – Differences in expenditures largely due to differences in inpatient psychiatric care
Croen et al. (2006)	US	– Northern California Kaiser Permanente Medical Care Program (large non-profit healthcare plan) for the period July 2003–June 2004	In- and outpatient services, Emergency care, Other healthcare professionals, Pharmacy, Home healthcare	– Average annual cost of healthcare for children with ASD of $ 2757 (in 2003 US$) (*$ 3763*) (adjusting for age group and gender)
Ganz (2007)	US	– Costs of medical and nonmedical care related to ASD as obtained from Medical Expenditure Panel Survey (MEPS) and the National Health Interview Survey (NHIS)	In- and outpatient services, Emergency care, Physician/general practitioner, Other healthcare professionals, Pharmacy, Home healthcare	– Incremental direct medical lifetime cost for a person with ASD of $ 305,956 (in 2003 US$) (*$ 417,541*) – Total direct medical costs for the average person with ASD decrease with age – 40% of the direct medical costs are incurred before age of 21 (for a typical American, this occurs before the age of 65)
Leslie and Martin (2007)	US	– National US database comprising information on insurance claims from private insurance plans of large employers	In- and outpatient services, Pharmacy	– Average annual healthcare cost for a child with ASD of $ 5979 in 2004 (in 2004 US$) (*$ 7948*) – Increase of 20.4% in healthcare cost estimate for children with ASD (year 2004 vs. 2000)
Shimabukuro et al. (2008)	US	– US nationwide study using the MarketScan® database	In- and outpatient services, Physician/general practitioner, Other healthcare professionals, Pharmacy	– Average incremental medical costs for children with ASD ranged from $ 4110 to $ 6200 (in 2003 US$) (*$ 8461 – $ 5609*) – Medical expenditures 4.1–6.2 times higher for privately insured children with ASD
Cidav et al. (2013)	US	– Medicaid data from all 50 US states and the District of Columbia	In- and outpatient services, Other healthcare professionals, Pharmacy, Home healthcare	– No estimation of cost figure – Expenditures for inpatient care (2%), long-term care (4.4%) and psychotropic medication (9%) increased with each year of age for children with ASD during age period of 3–20 – Largest increase between ages 3–6 and 7–11. Outpatient expenditures increase between age period 7–16 and decline in age period 17–20
Lavelle et al. (2014)	US	– Cost data of medical and nonmedical care related to ASD as obtained from Medical Expenditure Panel Survey (MEPS) and the National Health Interview Survey (NHIS) – Data on non-healthcare utilization and expenditures obtained from a nationally representative survey among parents of children with ASD and parents without a child with ASD (control group)	In- and outpatient services, Emergency care, Physician/general practitioner, Other healthcare professionals, Pharmacy, Home healthcare	– Average annual medical cost for children with ASD of $ 3020 (in 2011 US$) (*$ 4045*) – Healthcare costs constitute 18% of the total annual incremental cost calculated for children with ASD – Out-of-pocket healthcare costs are significantly higher for children with ASD than for children without ASD (on average $ 154 (*$ 206*) higher out-of-pocket expenditures per annum)

Table 1 (continued)

References	Countries	Data	Cost components	Main findings
Lokhandwala et al. (2012)	US	– 2007 Health Care Utilization Project Nationwide Inpatient Sample (HCUP-NIS)	Hospitalization costs, length of stay	– Rates of hospitalizations were the highest among individuals with ASD aged 10–20 years, males – Individuals with ASD had significantly higher length of stay (6.5 vs. 4.2; $p < 0.0001$) and total charges ($ 24,862 (*$ 30,110*) vs. $ 23,225 (*$28,127*); $p < 0.0001$) as compared to those without ASD
Vohra et al. (2017)	US	– Data for adults (22–64 years) with ASD as collected from three state Medicaid Analytic eXtract (period 2000–2008), cost data in 2008 US$	Healthcare utilization and expenditures (outpatient office visits, inpatient hospitalizations, emergency room, and prescription drug use)	– Average annual expenditure for outpatient office visits: $ 4375 (*$ 5103*) for ASD versus $ 824 (*$ 961*) for non-ASD – Average annual expenditure for emergency room: $ 15,929 (*$ 18,577*) for ASD versus $ 2598 (*$ 3030*) for non-ASD – Average annual expenditure for prescription drug use: $ 6067 (*$ 7075*) for ASD versus $ 3144 (*$ 3667*) for non-ASD – Average annual total expenditures: $13,700 (*$ 15,978*) for ASD versus $ 8560 (*$ 9983*) for non-ASD – Presence of psychiatric and non-psychiatric comorbidity increased the annual total expenditures for adults with ASD by $ 4952 (*$ 5778*) and $5084 (*$ 5929*), respectively
Buescher et al. (2014)	US, UK	– Current data on prevalence, level of functioning combined with mean annual costs of services and support of individuals with ASDs with or without intellectual disability – Literature review	In- and outpatient services, Emergency care, Physician/general practitioner, Other healthcare professionals, Other healthcare professionals, Pharmacy, Home healthcare	– Average annual medical cost for children with ASD of $11,453 (in 2011 US$) (*$12,785*) (weighted average for cost estimates stratified by age groups 0–5 and 6–17 years and the presence or absence of co-occurring intellectual disability) – Lifetime cost of supporting an individual with ASD and intellectual disability: in US $2.4 million (*$ 2.7 million*) and £1.5 million (*$ 2.5 million*) in UK (2011 UK £) – Lifetime cost of supporting an individual with ASD, without intellectual disability: in US $1.4 million (*$ 1.6 million*) and £0.92 million (*$ 1.6 million*) in UK

Table 1 (continued)

References	Countries	Data	Cost components	Main findings
Järbrink and Knapp (2001)	UK	– Literature review and previously published studies with cost information related to ASD – Two studies by the Centre for the Economics of Mental Health (CEMH)	In- and outpatient services, Other healthcare professionals, Pharmacy	– Medication costs: £ 50 (*$ 116*) per year for children with ASD and additional learning disabilities, £ 120 (*$279*) per year for children with high functioning ASD (in 1997–1998 £) – Costs for hospital services: £ 360 (*$ 839*) per year for children with ASD and additional learning disabilities, £ 480 (*$ 1120*) per year for children with high functioning ASD (in 1997–1998 £) – Costs for respite care, NHS community services and primary care: £ 1916 (£ 600) [*$4468* (*$1399*)] per year for children (adults) with ASD and additional learning disabilities, £ 750 (£ 300) [*$1748* (*$700*)] per year for children (adults) with high functioning ASD (in 1997–1998 £)
Knapp et al. (2009)	UK	– Multiple sources service use and cost data (e.g., Client Service Receipt Inventory, annual PSSRU compendium of unit costs) as collected from previously published papers as well as own recent studies		– Average annual cost of hospital services: for children with ASD and intellectual disabilities [£862 (*$ 1653*) for age period 4–11 and £ 1587 (*$ 3044*) for age period 12–17], for children with ASD and no intellectual disabilities [£ 777 (*$ 1489*) for age period 4–17] (in 2005–2006 £) – Average annual cost of hospital services: for adults with ASD and intellectual disabilities (£ 4588) (*$8797*), for adults with ASD and no intellectual disabilities (£ 14,004) (in 2005–2006 £) (*$ 26,854*)
Barrett et al. (2012)	UK	– Service use data as obtained from interviews (using CA-SUS) with parents of 152 young children with ASD on their service utilization (using CA-SUS) – Service cost data as collected from various sources such as personal communication with government departments and national surveys, NHS reference costs and mainstream retailers	In- and outpatient services, Emergency care, Physician/general practitioner, Other healthcare professionals, Pharmacy, Home healthcare	– Average total service costs (incl. education costs) were over £ 5000 (in 2006–2007 £) per child per year (*$9194*) – Average annual service cost for community, health, social and voluntary services: £ 2050 (in 2006–2007 £) (*$3769*) – Average annual service cost for hospital based health services: £ 600 (in 2006–2007 £) (*$1104*)
Järbrink (2007)	Sweden	– Postal questionnaire filled out by parents to gather information on the service use of 33 children with ASD living in the Swedish municipality Härryda – Unit cost data as obtained from service providers in the municipality	In- and outpatient services, Physician/general practitioner, Other healthcare professionals, Pharmacy	– Total incremental healthcare costs per child with ASD of € 2361 per year (in 2005 €) (*$3468*) (therapy costs and personal assistance costs not included)

Table 1 (continued)

References	Countries	Data	Cost components	Main findings
Mendoza (2010)	Egypt	– Interviews of 165 Egyptian households representing 174 autistic family members	Direct medical costs of ASD	– No estimation of cost figure – Direct medical costs of ASD in Egypt are low as compared to the costs for their American counterparts (controlling for income and lifestyle differences between Egyptians and Americans)
Xiong et al. (2011)	China	– Survey data as collected from a sample of parents of 227 children (children with ASD, physically disabled children, mentally disabled children and children without disabilities)	Medical costs, caring costs	– Annual medical costs for children with ASD of 3767.38 RMB (base year for the monetary unit not given)

Cost figures in italics concern the cost figures inflated to the year 2018 and conversed into US $ (using conversion rates as on 1 January 2018)

as reported in the reviewed papers as well as in 2018 US$ (in italics). The reason for updating the cost figures to the same base year (i.e., 2018) and the same currency (i.e., US $) is to facilitate comparisons of cost figures.[2]

A Classification of ASD-Related Costs

Medical and Healthcare Related Service Costs

The cost category that is mostly examined in the literature is the ASD-related medical or healthcare costs. It concerns costs that are caused by inpatient and outpatient expenses as well as pharmaceutical expenses. Some studies also consider costs related to emergency care, the use of physicians or other healthcare professionals, and home healthcare services. Table 1 gives an overview of the studies and their cost estimates.

Most of the studies listed in Table 1 calculated and compared the use and costs of medical and healthcare related services for individuals with and without ASD to obtain an idea of how ASD affects the use and costs of such services. Examples include Croen et al. (2006), Liptak et al. (2006), Ganz (2007) and Shimabukuro et al. (2008). Other studies also estimated and compared the medical and healthcare costs of children and/or adults with ASD with intellectual disabilities with the costs of children and/or adults with ASD without intellectual disabilities or children and/or adults with other types of disabilities (e.g., physically disabled children/adults, mentally disabled children/adults, etc.). Examples of such studies are Järbrink and Knapp (2001) and Knapp et al. (2009), with both studies using the IQ score of 70 as a cut-off to distinguish ASD children with and without additional learning disabilities.

Although the studies very likely do not provide a complete identification of the cost differentials, they do reveal several general insights. One general finding is that the medical and healthcare costs are significantly higher for individuals with ASD than for the general population. Croen et al. (2006), for instance, reported that the average annual cost of healthcare for children with ASD was three to two times as large as the healthcare costs for children without ASD (depending on whether there was an adjustment for age and gender or for age, gender and psychiatric comorbidities in the cost computations). A similar cost ratio for medical and healthcare costs for children with ASD versus children without ASD was also found by Ganz (2007). Ganz (2007) reported that, in the US, a person with ASD spends,

[2] In the transformation of the cost figures, reported cost figures were first inflated to the year 2018. Subsequently, inflated cost figures were all converted to US $ thereby adopting the conversion rate as on 1 January 2018 (£1.00 = $1.3491 US, €1.00 = $1.20 US, $1.00 Canadian = $0.800961 US, $1.00 Australian = $0.788955 US).

Table 2 Overview of cost (–benefit) estimates of different types of ASD behavioural therapy

References	Countries	Data	Type of behavioural intervention	Cost estimates
Butter et al. (2003)	US	– Studies about the effects of EIBI or behavioural intervention – Experiences with the Comprehensive Autism Center at Columbus Children's Hospital in Columbus, Ohio	Lovaas therapy	– Annual mean cost exceeds $ 60,000 (in 2003 US$) (*$ 82,000*) – Cost–benefit models estimate possible significant cost savings during the life span of a person with ASD
Sallows and Graupner (2005)	US	– 24 Children with ASD randomly assigned to two groups – Four-year clinical trial (treatment group: clinic-directed treatment services, control group: parent-directed group with consultation treatment services)	Lovaas therapy	– Annual mean cost exceeds $ 50,000 (in 2003 US$) (*$ 68,000*) – Similar outcomes after 4 years of treatment (cognitive, language, adaptive, social, and academic measures)
Chasson et al. (2007)	US	– Previously published papers to make assumptions on therapy outcomes and cost figures	– Discrete Trial Training (DTT) as a proxy for EIBI – Parent-directed model of DTT	– Discrete Trial Training: average yearly cost of $ 40,000 per child (in 2005 US$) (*$ 51,430*) – Parent-directed model: average yearly cost of $ 22,500 per child (*$ 28,929*) – 3 years of EIBI during the first years of life more cost-efficient than special education over an 18-year period (age period 4–22) – Cost savings of $ 84,300–$ 208,500 per child over an 18-year period (age 4–22) (*$108,389–$268,079*)
Amendah et al. (2011)	US	– Peer-reviewed literature and additional sources with cost information of care for individuals with ASD, with a focus on US	Intensive behavioural interventions	Annual cost estimate of $ 40,000–$ 60,000 (in 2003 US$) (*$ 55,000–$ 82,000*) for intensive behavioural interventions for children with ASD prior to school age
Lavelle et al. (2014)	US	– National data from the Medical Expenditure Panel Survey linked to the National Health Interview Survey and a study-specific survey	ASD-related therapies (ABA/DTT and TEACHH method) and other family-coordinated services	– Point estimate of annual mean cost (it concerns non-medical behavioural therapies not included under either medical care or special education services) of $ 350 (in 2011 US$) (*$ 391*), with cost estimations ranging from $ 6 to $ 2143 (*$6.7 to $2392*), depending on the different ASD-subgroups (Asperger's, PDD-NOS, and mild, moderate and severe autism)
Cidav et al. (2017)	US	– Two-year clinical trial (treatment group: ESDM, control group: usual community care) – Parent interviews collected every 6 months on the service use of their ASD child – Long-term follow-up assessments conducted when the child with ASD was 6 years of age	Early Start Denver Model (ESDM)	– The average annual cost of ESDM was estimated at $ 45,580 (in 2015 US$) (*$ 48,611*) – Higher initial costs in the ESDM-group fully offset within a few years after the intervention because of reductions in other service use and associated costs

Table 2 (continued)

References	Countries	Data	Type of behavioural intervention	Cost estimates
Järbrink et al. (2003)	UK	– Personal Social Service Research Unit – Previously published papers – Questionnaire and diary filled out by parents of 17 children with ASD	Early intervention therapy	– Annual mean cost of £ 7508 (in 1999–2000 £) (*$16,748*) – On average 33% of the costs of early intervention therapy paid by parents
Järbrink (2007)	Sweden	– Postal questionnaire filled out by parents to gather information on the service use of 33 children with ASD living in the Swedish municipality Härryda – Unit cost data as obtained from service providers in the municipality	Personal assistance or support worker outside school	– Annual average cost of € 4088 (in 2005 €) (*$6006*) (it concerns the cost of personal assistant or support worker) – Most of the children with ASD the sample were in special education
Peters-Scheffer et al. (2012)	The Netherlands	– Data obtained from reports, websites and studies by Dutch Government and Statistics Office – Rates provided by the Dutch Association for Autism and obtained	EIBI	– Total cost of € 99,967 (*$140,881*) for 33 h per week for 27 months of EIBI – Long term savings of ± € 1,103,067 (*$1,554,532*) from age 3 to 65 years per individual with ASD – For the total Dutch ASD population, cost savings of EIBI are estimated at € 109.2–€ 182 billion (*$128–$214 million*)
Peters-Scheffer (2015)	The Netherlands	– Data obtained from reports, websites and studies by Dutch Government and Statistics Office – Data on intensity, duration, etc. as derived from meta-analytic studies	EIBI	– Total cost of € 101,376 (*$ 142,867*) for 33 h per week for 27 months of EIBI – Long term savings ranging from € 211,821 (*$298,516*) to € 980,650 (*$1,382,012*) per person with ASD (during the age period 3–65 years), with the cost estimate varying depending on the outcome parameters
Motiwala et al. (2006)	Canada	– Government data on the hours and costs of IBI, and costs of educational and respite services – Data on programme efficacy were obtained from the literature	(E)IBI	– Economic evaluation of expansion of IBI programme from current coverage of 1/3 ASD children to all ASD children aged 2–5 in Ontario, Canada – Total cost savings from expansion were $45,133,011 (in 2003 Canadian dollars) (*$46,675,757*) – Sensitivity analyses showed mixed results depending on estimation modelling parameters

Cost figures in italics concern the cost figures inflated to the year 2018 and conversed into US $ (using conversion rates as on 1 January 2018)

Table 3 Overview of cost estimates of ASD-related education costs

References	Countries	Data	Cost estimates
Ganz (2007)	US	– Costs of medical and nonmedical care related to ASD as obtained from Medical Expenditure Panel Survey (MEPS) and the National Health Interview Survey (NHIS)	– Special education costs for children with ASD declined with age from roughly $ 12,000 (*$ 16,377*) per year at age 6, to around $ 6200 (*$ 8461*) per year at ages 18–22 (in 2003 US$)
Chasson et al. (2007)	US	– Data for the cost estimations as obtained through personal communication with the Houston Independent School District	– Distinguish between actual and state-budgeted costs for special education – Annual state-budgeted costs for special education for the State of Texas of $ 11,000 (*$ 14,143*) per child with ASD (in 2005 US$) – For the State of Texas, the actual annual costs for special education more or less $ 20,000 (*$ 25,715*) per child with ASD
Lavelle et al. (2014)	US	– National data from the Medical Expenditure Panel Survey linked to the National Health Interview Survey and a study-specific survey	– Annual education costs ranged from $ 67,819 (*$ 75,709*) for a child with ASD in special education in a residential school to $ 8259 (*$ 9220*) for public regular education for a preschool-aged child with ASD (in 2011 US$) – Regression-adjusted additional education cost for children with ASD compared to children without ASD estimated at $ 8610 (in 2011 US$) (*$ 9612*) per child per year (controlling for multiple control variables such as gender, age, household income, presence of a comorbidity not related to ASD, etc.)
Knapp et al. (2009)	UK	– Multiple sources service use and cost data (e.g., Client Service Receipt Inventory, annual PSSRU compendium of unit costs) as collected from previously published papers as well as own recent studies	– Annual cost education for child with ASD and intellectual disabilities: £ 10,326 (*$ 19,801*) for children aged 4–11 and £ 28,606 (*$ 54,854*) for children aged 12–17 (in 2005–2006 £) – Annual cost education for child with ASD without intellectual disabilities: £ 12,225 (*$ 23,442*) for children aged 4–17 (in 2005–2006 £) – Annual cost education for adult with ASD and intellectual disabilities: £ 2286 (in 2005–2006 £) (*$4383*) – Annual cost education for adult with ASD without intellectual disabilities: £ 2886 (in 2005–2006 £) (*$5534*)
Barrett et al. (2015)	UK	– Data of Special Needs and Autism Project (SNAP) by Baird et al. (2006) – SNAP-project studied (e.g., interviews) large cohort of adolescents with ASD and other special needs as well as typically developing adolescents – SNAP-database: ASD-group split into childhood/core ASD group and broader ASD group	– Adolescents with broader ASD requiring less special education or residential schools: £ 8121 (*$ 14,360*) over 6 months (in 2007–2008 £) – Adolescents with childhood/core ASD requiring more special education or residential schools: £ 10,507 (*$18,578*) over 6 months (in 2007–2008 £) – Education costs account for nearly 90% of the total costs
Barrett et al. (2012)	UK	– Service use data as obtained from interviews (using CA-SUS) with parents of 152 young children with ASD on their service utilization – Service cost data as collected from various sources such as personal communication with government departments and national surveys, NHS reference costs and mainstream retailers	– Education and childcare costs roughly 45% of total service costs for children with ASD aged 2–4 (£ 1152 over 6 months in 2006–2007 £) (*$2118*)

Table 3 (continued)

References	Countries	Data	Cost estimates
Järbrink and Knapp (2001)	UK	– Selection of earlier studies – Personal communication with National Autistic Society	– Average yearly cost of special education for children aged 5–19 with ASD and additional learning disabilities: £ 10,778 (in 1997–1998 £) (*$25,135*) – Special education costs 6.1% of the lifetime cost of an individual with ASD and additional learning disabilities – Average yearly cost of special education for children with high functioning ASD: £ 7216 (in 1997–1998 £) (*$16,829*) – Special education costs 13.8% of the lifetime cost of an individual with high functioning ASD
Järbrink et al. (2003)	UK	– Personal Social Service Research Unit – Previously published papers – Questionnaire and diary filled out by parents of children with ASD, [recruited from an active parental organization (Parents' Autism Campaign for Education)]	– Costs of early intervention programmes, classroom assistance, educational psychologists and additional school fees were included – Average weekly education costs of £ 441 (in 1999–2000 £) (*$ 983*) for children with ASD and learning disability – Average weekly education costs of £ 71 (in 1999–2000 £) (*$ 158*) for children with ASD without learning disability
Järbrink (2007)	Sweden	– Postal questionnaire filled out by parents to gather information on the service use of 33 children with ASD living in the Swedish municipality Härryda – Unit cost data as obtained from service providers in the municipality	– 50% Surveyed sample of children with ASD in some form of special education – Average annual education cost for a child with ASD of € 26,263 (in 2005 €) (*$ 43,375*) (cost of regular education excluded) – Special training schools, residential schools and schooling at treatment home most expensive categories of special education (more than 75% of education costs)
Peters-Scheffer et al. (2012) Peters-Scheffer (2015)	The Netherlands	– Education cost data for 2007, 2008 and 2009 collected from websites, reports and studies by Dutch Government and Statistics Office	– Three types of education for children with ASD – Regular education (29% of children with ASD), less intensive special education (34%), intensive special education (37%) – Total education costs: regular education (€ 114,500) (*$161,363*), less intensive special education (€ 193,200) (*$272,273*), intensive special education (€ 315,600) (*$444,769*)

Cost figures in italics concern the cost figures inflated to the year 2018 and conversed into US $ (using conversion rates as on 1 January 2018)

Table 4 Overview of cost estimates of production loss for individual with ASD

References	Countries	Data	Cost estimates
Ganz (2007)	US	– Previously published studies – Statistical data from the US Department of Commerce	– Lifetime cost (age period 3–66) due to lost productivity for an average individual with ASD of $ 971,072 (in 2003 US$) (*$ 1,325,234*)
Buescher et al. (2014)	US, UK	– Literature review	– For the US: annual cost for lost productivity of $ 10,718 (in 2011 US$) (*$ 11,965*) for average adults with ASD – For the UK: annual cost for lost productivity of £ 25,644 (in 2011 £) (*$ 41,420*) for average ASD adult with intellectual disabilities – For the UK: annual cost for lost productivity of £ 21,797 (in 2011 £) (*$ 35,206*) for average ASD adult without intellectual disabilities
Knapp et al. (2009)	UK	– Empirical evidence of previous studies as well as own recent studies	– Average annual cost for lost productivity for individuals with ASD and with intellectual disabilities £ 22,383 (in 2005–2006 £) (*$ 42,922*) – Average annual cost for lost productivity for individuals with ASD and without intellectual disabilities £ 19,785 (in 2005–2006 £) (*$ 37,939*)
Järbrink and Knapp (2001)	UK	– Findings of previous studies – UK national figures on average gross wages and indirect taxes and subsidies as collected from Office for National Statistics	– Average annual loss in productivity due to early retirement of £1671 (*$ 3896*) between ages of 35 and 65 for people with high functioning ASD (in 1997–1998 £) – Average annual lost earnings due to being employed in unskilled and low-paid work (despite high level of education) of £1932 (*$ 4506*) between ages of 20 and 65 for people with high functioning ASD (in 1997–1998 £, excluding indirect taxes and subsidies) – Average lifetime cost due to lost productivity for individuals with high functioning ASD of approximately £ 137,100 (in 1997–1998 £) (*$ 319,725*)

Cost figures in italics concern the cost figures inflated to the year 2018 and conversed into US $ (using conversion rates as on 1 January 2018)

over the course of his/her lifetime, almost twice as much on direct medical costs as a typical person (without an ASD diagnosis). Croen et al. (2006) also reported that children with ASD and psychiatric comorbidities had higher total healthcare costs than children with just an ASD diagnosis. In their US nationwide study, Shimabukuro et al. (2008) found that total medical expenditures were 4.1–6.2 times higher for privately insured children with ASD when compared to their peers without ASD.

Studies highlight multiple factors for these cost differences of medical and healthcare related services for children with and without ASD. One such factor is that ASD using medical and healthcare services more frequently as compared to the general population. Croen et al. (2006), for instance, found that children with ASD more frequently consulted pediatricians, psychiatrists and neurologists as compared to children without ASD. Children with the ASD diagnosis also had significantly more inpatient hospital days and outpatient (same-day) hospitalisations as compared to children without ASD (the difference between both groups being most significant in the group of 15–18 years old). In their calculations of the medical costs related to ASD in the UK, Järbrink and Knapp (2001) reported similar differences in the use of inpatient psychiatric services, with people with high functioning ASD using inpatient psychiatric services at least four times more frequently as compared to the general population. Croen et al. (2006) also found that more children with ASD (40% more) used prescribed medication (especially psychotherapeutic and gastro-intestinal agents) as compared to children without ASD. These findings correspond to the those of Liptak et al. (2006) and Lavelle et al. (2014), who found that the cost of outpatient visits, physician visits and prescription medications were significantly higher for children with ASD as compared to children without an ASD diagnosis.

A second interesting finding is that most studies showed that the overall medical and healthcare costs for people with ASD steadily increase over the lifetime. For children up to 20 years, Cidav et al. (2013) found that expenditures for

Table 5 Overview of cost estimates of informal care and lost productivity for family/caregivers with an ASD child/adult

References	Countries	Data	Cost estimates
Ganz (2007)	US	– Previously published studies – Statistical data from the US Department of Commerce	– Costs of the productivity loss of parents of a child with ASD of $ 904,595 (in 2003 US$) (*$ 1,234,512*) over the lifetime of an individual with ASD
Montes and Halterman (2008)	US	– National Household Education Survey: after school programs and activities for the year 2005	– Average annual income loss for a household with a child with ASD of $ 6207.70 (in 2005 US$) (*$ 7981*) or roughly 14% of the annual household income
Cidav et al. (2012)	US	– 2002–2008 Medical Expenditure Panel Survey (MEPS) – National representative sample of US households	– Controlling for a multitude of factors (e.g., parent's age, education level, number of children, etc.), analysis of the MEPS-data showed that, on average, mothers of children with ASD earned 56% (or $ 14,468 per year, in 2005 US$) (*$ 18,602*) less as compared to mothers of children with no health limitation – No statistically significant difference in wage earnings of father of children with ASD versus fathers of children with no health limitation – Family earnings of children with ASD are 28% ($17,763 in 2005 US$) (*$22,839*) less than those of children with no health limitation
Buescher et al. (2014)	US, UK	– Review of the literature – Modelling parameters as used by Cidav et al. (2012) for the UK cost estimation	For the UK: – Annual cost of parental loss of productivity of £ 608 (in 2011 £) (*$ 982*) for children with ASD aged 0–3 – Annual cost of parental loss of productivity of £ 5314 (in 2011 £) (*$ 8583*) for children with ASD aged 4–17 – Annual cost of parental loss of productivity of £ 1477 (in 2011 £) (*$ 2385*) for parents with adult children with ASD For the US: – Annual cost of parental loss of productivity of $ 18,720 (in 2011 US$) (*$ 20,898*) for children with ASD aged 0–17 – Annual cost of parental loss of productivity of $ 1896 (in 2011 US$) (*$ 2117*) for parents with adult children with ASD
Järbrink and Knapp (2001)	UK	– Findings of previous studies – UK national figures on national average disposable income by households as collected from Office for National Statistics	– Average annual cost of productivity loss for parents of children with ASD and additional learning disabilities of £ 528 (in 1997–1998 £) (*$ 1230*) – Average annual cost of productivity loss for parents of children with high functioning ASD of £ 192 (in 1997–1998 £) (*$ 448*)
Järbrink et al. (2003)	UK	– Previously published papers – Questionnaire and diary filled out by parents of children with ASD, [recruited from an active parental organization (Parents' Autism Campaign for Education)]	– Average cost for informal care for child with ASD incurred by parents of £ 397 per week (in 1999–2000 £) (*$ 885*) – Average cost of income losses for parents of children with ASD of £ 231 per week per child with ASD (in 1999–2000 £) (*$ 312*)
Knapp et al. (2009)	UK	– Previously published studies – Multiple sources on service use and cost data	– Average annual cost of productivity loss for parents of children with ASD and intellectual disabilities of £ 2059 (in 2005–2006 £) for ages 4–11 (*$ 3949*) – Average annual cost of productivity loss for parents of children with ASD and intellectual disabilities of £ 2015 (in 2005–2006 £) for ages 11–17 (*$ 3864*) – Average annual cost of productivity loss for parents of children with ASD and without intellectual disabilities of £ 216 (in 2005–2006 £) (*$ 414*)

Table 5 (continued)

References	Countries	Data	Cost estimates
Barrett et al. (2012)	UK	– Interviews (using CA-SUS) with parents of young children with ASD – Various sources such as personal communication with government departments and national surveys	– Average cost of productivity loss for parents of young children (ages 2–4) with ASD over 6 month period of £275 (in 2006–2007 £) (*$ 506*)
Horlin et al. (2014)	Australia	– Questionnaire among families with children registered as having an ASD at the Disabilities Services Commission Western Australia	– Median annual income loss for parents or caregivers of children with ASD of $ 29,200 (in 2011 AUD$) (*$ 26,291*) or 29% of the combined household income
Järbrink (2007)	Sweden	– Postal questionnaire filled out by parents to gather information on the service use of 33 children with ASD living in the Swedish municipality Härryda	– Total yearly cost of time losses for parents or caregivers of children with ASD of € 7759 (in 2005 €) (*$ 11,399*)
ASDEU (2018)	EU	– Anonymous online survey for children and adults with the condition, collecting individual data on resources and costs	– The costs of productivity losses among carers, for 6 months, range from € 307.70 (*$ 369*) per carer in Poland to € 4467.40 (*$ 5360*) per carer in Austria

Cost figures in italics concern the cost figures inflated to the year 2018 and conversed into US $ (using conversion rates as on 1 January 2018)

inpatient care (2%), long-term care (4.4%) and psychotropic medication (9%) increased with each year of age for children during the ages of 3–20. The largest increase occurred between the ages of 3–6 and 7–11. Outpatient expenditures were found to increase between the age period 7–11 and 12–16 and decline in the age period 17–20. Shimabukuro et al. (2008) reported a similar result, i.e. as children with ASD get older, inpatient and medications costs make up a bigger part of the total medical cost and the share of outpatient costs decreases. Buescher et al. (2014) compared the medical and healthcare costs of people with ASD in the UK to those in the US and found that the medical costs were much higher for adults with ASD than for children with ASD in both countries. An opposite trend was reported by Ganz (2007), who found that the total direct medical costs for the average person with ASD in the US were decreasing with age. A decreasing trend was also found for most of the different individual components of direct medical costs (exceptions being the physician and dental costs). Note, however, that Ganz (2007) included the expensive cost of behavioural therapies for children in the medical cost category (the cost of behavioural therapies were only included for individuals 19 years of age and younger), whereas most other studies, like Buescher et al. (2014), categorized therapy costs as a nonmedical service cost. This probably explains the finding of an opposite trend.

A third finding is that some studies found that the medical and healthcare expenditures for individuals with ASD are generally higher than for individuals with other mental health conditions. Leslie and Martin (2007), for instance, found that the average annual healthcare cost figure for children with ASD was higher than the average annual healthcare cost figures of children with different diagnoses. Only the average annual healthcare cost for children with mental retardation was found to be higher. Leslie and Martin (2007), however, noted that, considering the lower prevalence rates of ASD as compared to the other mental health conditions examined, total healthcare expenditures for ASD were lower than total healthcare expenditures made for other (more prevalent) mental health conditions. However, it is difficult to say whether this last finding of Leslie and Martin (2007) still holds today, given the increasing trend in the number of people being officially diagnosed with ASD, better public and medical awareness for ASD, and improved possibilities to diagnose children at a young age (or a combination of those factors).

A fourth interesting finding is that several studies indicated that the medical and healthcare costs make up only a small part of the overall cost encountered by individuals with ASD. For instance, for the UK and Sweden, Järbrink and Knapp (2001), Järbrink et al. (2003), Ganz (2007), Järbrink (2007) and Lavelle et al. (2014) concluded that the costs of medication, hospital services and other health and

Table 6 Overview of cost estimates for accommodation, respite care, and out-of-pocket expenses related to ASD

References	Countries	Data	Cost estimates
Ganz (2007)	US	– Previously published studies	– Cost of travel to medical appointments ranging from $ 81 (*$ 111*) for children (aged 3–7) to $ 14 (*$ 19*) yearly for adults aged 63–66 (in 2003 US$) – Home improvement costs ranging from $ 161 (*$ 220*) for young children with ASD (ages 3–7) to $ 120 (*$ 164*) for older children (13–17 years) with ASD – Home improvement costs for adults with ranging from $10 (*$ 13.5*) (for ages 18–22) to $ 3 (*$ 4*) (for ages 63–66) (in 2003 US$) – Respite care costs ranging from $ 1100 (*$ 1501*) for children with ASD (for ages 3–7) to $ 706 (*$ 963*) (for ages 18–22) (both in 2003 US$)
Parish et al. (2012)	US	– Child and family data drawn from the National Survey of Children with Special Health Care Needs (N = 2082 children with autism)	– 78% of families with a child with ASD reported having any out-of-pocket health care expenditures for their child for the prior 12 months – 54% Reported out-of-pocket health care expenditures of more than $500 (in 2005–2006 US$) (*$ 623*), with 34% spending more than 3% of their income
Parish et al. (2015)	US	– Pooled 2000–2009 Medical Expenditure Panel Survey data	– Average out-of-pocket health care (mainly medication, outpatient services, and dental care) expenditures of $9.70 per $1000 of income (*$ 11.35 per $ 1170*)
Buescher et al. (2014)	US, UK	– Cost estimates for residential care from previously published studies	– Additional cost for accommodation for children with ASD ranged from £ 37 to £ 1240 (in 2011 £) (*$59* to *$2003*) per year and increased with age – Annual accommodation cost per adult with ASD and intellectual disabilities of £ 41,512 (in 2011 £) (*$ 67,050*) – Annual accommodation cost for children with ASD without intellectual disabilities of $ 952 (*$ 1434*) (ages 0 – 5) and $ 4758 *($ 7166)* (ages 6 -17) (in 2011 US$) - Annual accommodation cost for children with ASD and intellectual disabilities of $ 1903 *($ 2865)* (ages 0– 5) and $ 9516 (*$ 14,331*) (ages 6–17) (in 2011 US$) – Annual accommodation cost for adults with ASD without intellectual disabilities of $ 18,080 (in 2011 US$) (*$27,229*) – Annual accommodation cost for adults with ASD and intellectual disabilities of $ 36,161 (in 2011 US$) (*$54,460*)
Järbrink and Knapp (2001)	UK	– Findings, estimates and figures reported in previous studies – Information obtained by personal communication with the National Autistic Society	– Annual cost of residential care of £ 29,378 (in 1997–1998 £) (*$ 68,511*) per individual with ASD and intellectual disabilities – Annual cost of living support per individual with high functioning ASD of £ 4302 (in 1997–1998 £) (*$ 10,032*) – Annual cost of day activities and day care provision of £7793 (*$ 18174*) for an individual with ASD and additional learning disabilities – Annual cost of day activities and day care provision of £1375 (*$ 3207*) for an individual high functioning ASD – Annual cost for out-of-pocket expenses for parents of children with ASD of £ 2000 (in 1997–1998 £) (*$ 4664*) for individuals with ASD and additional learning disabilities

Table 6 (continued)

References	Countries	Data	Cost estimates
Järbrink et al. (2003)	UK	– Previously published papers – Questionnaire and diary filled out by parents of children with ASD, [recruited from an active parental organization (Parents' Autism Campaign for Education)]	– Weekly cost for out-of-pocket expenses for parents of children with ASD varying from £ 65.91 to £ 100.15 (in 1999–2000 £) (*$ 147 to $ 224*)
Knapp et al. (2009)	UK	– Data on accommodation placements for children with ASD and intellectual disabilities from Children in Need (CIN) in England – Data on accommodation placements for adults with ASD as obtained from previous studies – Data on placement distribution of individuals with ASD across different accommodation settings as obtained from Professor Emerson	– Annual cost of accommodation ranging from £ 544 (*$1043*) (ages 0–3) to £ 1082 (*$ 2069*) (aged 12–17) (both in 2005–2006 £) for children with ASD and intellectual disabilities living in residential or foster care – Annual cost of accommodation for adult with ASD and intellectual disabilities of £ 36,233 (in 2005–2006 £) (*$69,479*) – Annual family expenses for adults with ASD and intellectual disabilities of £ 762 yearly (in 2005–2006 £) (*$ 1461*) – Annual family expenses for adults with ASD without intellectual disabilities of £ 1494 (in 2005–2006 £) (*$2865*) – Annual cost for respite care for children with ASD and intellectual disabilities of £ 2790 (*$ 5351*) (ages 4–11) and £ 3559 (*$ 6825*) (ages 12–17) (both in 2005–2006 £) – Annual cost for respite care for children with ASD without intellectual disabilities of £ 6510 (*$ 12,483*) (ages 4–17) (in 2005–2006 £) – Annual cost for respite care for adults with ASD and intellectual disabilities of £ 538 (in 2005–2006 £) (*$1032*) – Annual costs for day services for adults with ASD and intellectual disabilities of £ 1998 (in 2005–2006 £) (*$3831*) – Annual costs for day services for adults with ASD without intellectual disabilities of £ 2226 (in 2005–2006£) (*$ 4269*)
Barrett et al. (2012)	UK	– Interviews (using CA-SUS) with parents of young children with ASD – Various sources such as personal communication with government departments and national surveys	– Out-of-pocket expenses for children with ASD (aged 2–4) of £ 227 (*$ 417*) for a 6 months period (in 2006–2007 £)
Järbrink (2007)	Sweden	– Postal questionnaire filled out by parents to gather information on the service use of 33 children with ASD living in the Swedish municipality Härryda	– Annual cost of home placement of € 913 (in 2005 €) (*$1342*) per child with ASD – Annual cost of respite care (€ 6843) (*$ 10,052*), camp (€ 1839) (*$ 2701*), domestic support worker (€ 463) (*$680*), day outings (€ 78) (*$ 114*), personal assistant or support worker (€ 4088) (*$ 6006*), and befriending services (€ 77) (*$ 113*) (all in 2005 €)
Raz et al. (2013)	Israel	– A survey among parents of 178 children with ASD aged 4–10 years (of which 87% agreed to participate)	– Average annual out-of-pocket costs (mainly for health services and hours of therapy) of $8288 (*$ 9252*), with a median of $4473 (*$ 4993*) and a range of $0–89,754 (in 2011 US$) (*$ 0–$ 100,195*) – Higher severity of ASD associated with higher out-of-pocket expenditures

Cost figures in italics concern the cost figures inflated to the year 2018 and conversed into US $ (using conversion rates as on 1 January 2018)

social services taken together only account for a small fraction of the total incremental cost for individuals with ASD. Järbrink (2007) estimated that the total medical and healthcare cost constitutes only a very small part, less than 5%, of the total societal cost for children with ASD in Sweden.

However, it is important to point out some reasons to be cautious about comparing the ASD-related medical and healthcare cost estimates of the different studies. One reason is that cost estimations are typically limited by data availability and/or imperfections in data sources and analyses. Another reason is that studies utilize different research designs and/or data sources in the cost estimations. As noted by Amendah et al. (2011), differences in ASD-related medical cost estimates across studies are linked to methodological choices made by the authors (using parental surveys vs. administrative data, using a medically diagnosed ASD population vs. a study population that was diagnosed with ASD outside the medical system, the type of insurance plan included in the study, etc.). While some studies use service use and cost data as collected from official databases, other studies use survey data to estimate the user frequency and costs (e.g., Ganz 2007; Liptak et al. 2006). Some studies also include more cost components than others. For instance, in the estimation of the ASD-related medical and healthcare service costs, Liptak et al. (2006) considered the costs of in- and outpatient services, emergency care, physician/general practitioner, other healthcare professionals, pharmacy, and home healthcare in the cost computations, whereas Leslie and Martin (2007) only included costs of in- and outpatient services and pharmacy. Other complicating issues are the different healthcare systems and financial aid programmes in countries and the fact that costs and prices can vary both between countries and within a country (e.g. across states in the US) as well as over time. As an illustration, Mendoza (2010) estimated direct medical costs of ASD in Egypt to be comparatively low as compared to the costs for their American counterparts (controlling for income and lifestyle differences between Egyptians and Americans). To explain this large difference, Mendoza referred to the trade-offs between household care and institutional sources of healthcare in Egypt. Mendoza also pointed to the restricted provision and financing of healthcare by the Egyptian state which results in a large part of the care for family members with ASD taking place within the household. All the above probably explains the recurrent finding of remarkable variance in the cost estimations across studies.

Before concluding the review of the studies about the ASD-related medical or healthcare costs, mention should be made of some other medical and healthcare expenses which were less discussed (or less explicitly so) in previous studies. One such expenditure is the cost of the ASD diagnosis. Anecdotical evidence obtained from families or individuals with ASD shows that the diagnosis process can be a real struggle, with, among other things, long waiting lists and high upfront costs. Murphy et al. (2011) explained that the length of the diagnostic process, the need for expert clinicians and the high involvement of caregivers make ASD diagnosis a costly matter. This is nicely illustrated by Shimabukuro et al. (2008), who found higher levels of healthcare utilization and healthcare costs in the year in which children receive their medical ASD diagnosis. For Australia, Taylor et al. (2016) reported that the median cost of an ASD assessment for families was $580 (SD = $599.47), as computed across all services. However, data revealed considerable variability in the assessment costs across states and service settings, with the cost of ASD assessment to families ranging from $ 0 in the public sector to $ 200–$ 2750 in the private sector. Another example of an ASD-related medical and healthcare cost which was not included in most of the previous studies (notable exception is Järbrink 2007) is the expenses of healthcare services for relatives, such as counselling and medication costs, which are caused by the child or family member with ASD. Sharpe and Baker (2007), for instance, noted that the increase in stress due to having a child or family member with ASD can result in higher medical costs for relatives and/or other family members.

Therapeutic Costs

With more people being diagnosed with ASD and the subsequent expansion of (public and private) resources that are directed to research on ASD and treatment and care for ASD individuals, there has been an increasing interest in studying the use, the outcomes and/or the cost(-effectiveness) of behavioural treatments/therapies for individuals with ASD. Currently, (early) intensive behavioural intervention/treatment (EIBI) based on applied behaviour analysis principles is considered the treatment of choice for children with ASD. Despite a plethora of research projects and studies having examined the use and the effectiveness of such interventions and treatments (with the focus on the clinical outcomes such as behavioural improvements, increased cognitive skills, language and speech improvements, better social and emotional development, and improved fine and gross motor development, see e.g. Eldevik et al. 2009; Cidav et al. 2017; Peters-Scheffer et al. 2012; Reichow 2012), only a limited number of studies have estimated and evaluated the costs of ASD-related behavioural interventions and therapies (although, this number has been building up over the past years). Of the studies that did examine the costs of treatment services for ASD, the large majority were performed in the US.[3] The

[3] For North America, Payakachat et al. (2018) found an increase in the use of treatment services for individuals with ASD, with the top three of the most frequently received services including speech therapy (67%), occupational therapy (50%) and behavioural therapies/services (28%) such as ABA, Lovaas therapy, (Early Start) Denver Model, and Discrete Trial Training (DTT).

detailed estimates of the ASD-related therapeutic costs made by previous studies are listed in Table 2. In what follows, we discuss some general findings obtained from the review of these studies.

A first general finding is that the costs of EIBI-programmes for ASD are substantial. Most US studies indicate that the annual cost for such programmes range between $40,000 and $60,000. Similar estimates were reported by Peters-Scheffer et al. (2012, 2015) for the Netherlands. However, there are some studies such as Lavelle et al. (2014) for the US, Järbrink et al. (2003) for the UK and Järbrink (2007) for Sweden, which reported cost estimates for EIBI-programmes for ASD that are considerably lower. One possible explanation for these lower cost estimates are the differences in how studies define and classify costs of ASD-related therapy programmes. Some studies classify costs related to the organization and implementation of ASD therapies as medical costs (Cidav et al. 2013, 2017; Ganz 2007; Horlin et al. 2014; Liptak et al. 2006), whereas other studies categorize these costs as non-medical costs or under community support (Barrett et al. 2012; Järbrink 2007). Other studies categorize therapy-related costs as (special) education costs (Järbrink 2007; Järbrink et al. 2003; Lavelle et al. 2014). In their estimations of the ASD-related costs, Lavelle et al. (2014), for instance, considered costs for speech/language therapy and physical/occupational therapy to be education costs. One possible explanation for the classification of ASD therapy costs as (special) education costs could be that, in some countries, legislation incorporates ASD therapy into education. In the US, for instance, the federal Individuals with Disabilities Education Act (IDEA) guarantees free appropriate public education (FAPE) for every child with a disability. Note, however, that this explanation is not conclusive as even under IDEA, ABA services are not always offered in the school context due to economic considerations and/or because the IDEA only requires schools to provide disabled children with the tools to achieve a minimum level of accomplishment (Holland 2010, p. 1270). As such, speech and language therapy are most often offered to children with ASD in the public-school system (Sharpe and Baker 2007, 2011). However, additional therapy for children with ASD to help them achieve their maximum (or at least a higher) level of personal accomplishment may well be organized in a non-school context.

A second main finding is that cost-effectiveness studies of EIBI-programmes (Jacobson and Mulick 2000; Butter et al. 2003; Chasson et al. 2007; Cidav et al. 2017; Peters-Scheffer et al. 2012; Peters-Scheffer 2015; Motiwala et al. 2006) reported possibly significant cost savings that can be generated by such programmes for individuals with ASD, although with the exact estimate for cost savings differing across studies. To draw conclusions, however, regarding these cost-saving estimates, the review indicates some factors that have to be kept in mind. A first factor is that studies differ remarkably in the model parameters set in the cost–benefit computations (e.g., the intensity of the therapy, the success rate of the therapy, the duration of the therapy, the hourly cost of therapy, etc.) which makes comparing cost–benefit estimates across studies difficult. As an illustration, in cost–benefit analysis of EIBI-therapy, Chasson et al. (2007) and Peters-Scheffer (2015) set different duration periods (after 36 months vs. after 27 months of EIBI therapy) and different rates of children with ASD stream into regular or less intensive special education after such therapy (72% vs. 63%). Marcus et al. (2000) illustrated the importance of the method and the parameters set in the cost–benefit calculations. In particular, in a study of the parameters set by Jacobson and Mulick (2000), they raised several questions about the assumptions made by Jacobson and Mulick (2000) and discussed how Jacobson and Mulick's modelling choices may have impacted the results of their study. A second factor is that, as indicated by Peters-Scheffer et al. (2012 p. 1764), most studies used high effectiveness rates in the modelling of the outcomes/effectiveness of the EIBI-programmes. Peters-Scheffer et al. (2012) give as an example the study of Chasson et al. (2007) which estimated costs and benefits for the State of Texas across 18 years of education with EIBI, using effectiveness rates for EIBI as obtained from studies with the most positive EIBI outcomes (i.e., 72% of children who receive EIBI eventually mainstreaming into regular education). Peters-Scheffer et al. (2012) discussed that the study of Motiwala et al. (2006) is an exception as it used somewhat more conservative effectiveness rates in the cost-effectiveness analysis (CEA) of IBI to all autistic children in Ontario (respectively 30, 50 and 20% streaming into regular education, less intensive special education, intensive special education after intensive behavioral intervention vs. 25, 25 and 50% after receiving no such treatment).[4] Notwithstanding this exception, Peters-Scheffer et al. (2012) noted that the effectiveness rates used by most studies are probably too positive. Therefore, in order to obtain more realistic cost–benefit estimates of EIBI programmes, they noted that it would be interesting for future studies to consider both optimistic and pessimistic effectiveness rates of such programmes in the computation models.

[4] In a sensitivity analysis carried out to address uncertainty and lack of good evidence for IBI efficacy, Motiwala et al. (2006) also tested more optimistic and pessimistic efficacy rates for IBI. Based on these parameter settings, Motiwala et al. (2006) obtained estimates of savings that are lower than the figures reported by Chasson et al. (2007) (i.e., between 34.479 and 53.720 Canadian $ per individual vs. 208,500 US $ per child as in Chasson et al. 2007).

A third key finding relates to the variation in the use and costs of treatment and therapy services across the lifetime of individuals with ASD. Generally, studies showed that the ASD therapy costs are high during early childhood, with the costs gradually decreasing as the child with ASD gets older. For the US, for instance, Cidav et al. (2013) estimated that the expenditures, measured as Medicaid reimbursements (Medicaid being a US government funded programme that helps people with low income and resources with medical costs), for mental health, social skills and behaviour modification services for people with ASD increased with age, whereas expenditures for occupational, physical, speech and family therapy tended to decrease with age. A more detailed picture of the evolution of the expenditures/costs demonstrated that the largest changes in expenditures and use of these ASD-related services occurred between the age groups 3–6 and the age group 7–11. As noted before, one possible explanation for this change could be that, in the US, the education system organizes and finances some therapies for disabled children (and, thus, also children with ASD). This would mean that the costs for these therapies and services are no longer funded by Medicaid but by the education system or "other public or private programmes" (Cidav et al. 2013, p. 929). Similar results for the US were found by Ganz (2007), who estimated that behavioural therapies are a large cost component within the incremental costs of individuals with ASD, with costs for behavioural therapies being significantly higher for the children with ASD aged 3–6. As an explanation for the high therapy costs during early childhood, Ganz (2007) referred to the growing body of evidence in the literature showing that the initiation of behavioural therapies at a young age is associated with positive outcomes for children with ASD, which has led to EIBI becoming often the therapy of choice, with in several countries an increasing number of young children with ASD receiving EIBI-therapies.[5]

We conclude this section with an important general remark about the cost–benefit studies on EIBI-programmes for ASD. Several of these studies performed a CEA to examine the cost-effectiveness of such programmes (e.g., Penner et al. 2015; Motiwala et al. 2006). In CEA-studies, typically, an incremental cost-effectiveness ratio (ICER) is used as a summary measure representing the cost-effectiveness of the respective ASD intervention or therapy programme(s). It concerns a ratio of the difference in costs (incremental cost) between the evaluated intervention programme and the comparison intervention programme, divided by the difference in the effect (incremental effect) of both intervention programmes. As such, it indicates the average incremental cost for each additional unit of the measure of effect.[6] Per definition, ICERs depend on several parameters such as the time horizon that is selected for measuring the costs and the effects of the intervention programmes, the effectiveness rates for the assessed intervention programmes and the costs categories that are included in the computation of the incremental cost difference between the respective programmes (Garber and Phelps 1997). As to the time horizon, Cohen and Reynolds (2008) and Meltzer (1997) discussed that the outcomes of CEA-studies can be sensitive to the time horizon of the analysis. Ideally, the time horizon should be defined such that it covers the entire period in which intervention-related costs and/or effects can occur. Studies with too short or too long time horizons may very well misestimate ICERs. For ASD intervention programmes, in particular, it is not always clear-cut about what is a proper time horizon to compute the costs and effects of such programmes. Motiwala et al. (2006) and Penner et al. (2015), for instance, set an upper age limit of 65 in their CEA-studies of EIBI, the reasoning being that after the age of 65, costs typically increase for all individuals (ASD and non-ASD) making it difficult to attribute costs solely to the effects of ASD. The definition of the effectiveness rates of the assessed intervention programmes is a second intricate matter (see also above). As indicated above, typically, effectiveness rates are defined based on rates reported in previous literature, with most CEA-studies adopting the more optimistic effectiveness rates in the evaluation of EIBI-programmes. Moreover, it is not always straightforward to compare effectiveness rates as intervention programmes can differ in terms of intensity, duration, format, etc. A third important parameter is the selection of cost categories that are to be considered in the computation of the incremental cost differences. ICERs can differ depending on whether costs and effects are considered from government perspective, societal perspective, or both (Bambha and Kim 2004). In addition, ICERs of ASD intervention programmes as computed by CEA-studies always depend on the comparison intervention/scenario. By result, it makes little sense for other ASD cost studies to use ICERs of previous studies if examined ASD intervention programmes (and comparison programmes) are not sufficiently similar. Given all the intricacies involved in the assessment and the measurement of the incremental costs and the incremental effects, it is clear that both the adopted ICERs as well as the outcomes of the CEA-studies

[5] Several cost-effectiveness analysis studies showed that offering EIBI during the first years of childhood results, on average, in cost savings during a lifetime (e.g., Butter et al. 2003; Chasson et al. 2007; Cidav et al. 2017; Peters-Scheffer et al. 2012).

[6] It is discussed in the literature (e.g., Garber and Phelps 1997) that ICERs can be useful as a decision rule in resource allocation particularly for making decision about (relatively new) intervention and/or therapy programmes that are costly but generate improved effects over time. ICERs can be compared with an a priori established cost-effectiveness threshold (i.e., willingness-to-pay value per unit of effect) in order to decide whether the new intervention is an efficient use of resources.

of ASD intervention programmes should be interpreted with caution (and expertise).

Education Costs

Whilst some children with ASD can attend regular education without much further assistance, most children with ASD require special education or at least extra special education services in regular education. A minority of children with ASD attend residential schools.[7] Several studies have estimated the annual and/or the total education costs for children or adolescents with ASD. Most of the studies focused on the US and the UK. Only a few studies have estimated education costs in other countries. Exceptions include Järbrink (2007) for Sweden and Peters-Scheffer et al. (2012) and Peters-Scheffer (2015) for the Netherlands. The details on the data sources and the education costs estimates for the reviewed studies can be found in Table 3. Below we focus on key findings and (dis)similarities between the different studies.

A first observation is that it is difficult to compare the calculations and estimations of ASD-related education costs that are done by US studies (i.e., Ganz 2007; Chasson et al. 2007; Lavelle et al. 2014), the main reasons being the differences between these papers about what costs are considered as ASD-related education costs as well as the level of detail in the provided cost estimates. Studies like Lavelle et al. (2014) included a very broad set of services and activities in the education services category, such as speech/language therapy, physical/occupational therapy, vision therapy, social worker services, personal health aid support, community-based training services and summer school services. Similarly, Chasson et al. (2007) estimated the special education costs of ASD, thereby accounting for the costs of a large assortment of educational and therapeutic techniques for children with ASD that are used in various ways across school districts in the US. In the estimations, the authors distinguished between actual and state-budgeted costs for special education. The actual costs of special education include both the state-budgeted costs as well as local, federal and private funds for special education. Ganz (2007), on the other hand, estimated the education costs for children with ASD by averaging multiple cost estimates found in the literature. As to the second reason, Lavelle et al. (2014) reported estimates for ASD-related education costs for different types of special education for children with ASD whereas other studies like Ganz (2007) estimated the education costs for children with ASD more generally.[8]

A second observation is that it is more straightforward to compare the estimates of ASD-related education costs that are provided by UK studies such as Knapp et al. (2009), Järbrink and Knapp (2001), Järbrink et al. (2003), and Barrett et al. (2015), the reason being that these studies focus on the education costs that are directly related to ASD, thus, ignoring non-direct costs such as the costs of regular education (which is also used by typically developing children and, thus, cannot be attributed to ASD directly). Notable exception is the study of Barrett et al. (2012) which included both the costs of regular and specialised education as well as childcare costs in their calculations of education costs for very young children with ASD (ages 2–4). Most of the UK-studies also focused on calculating the ASD-related education costs for children with ASD (only Barrett et al. 2015 examined the ASD-related education cost for adolescents with ASD). A general finding for the UK-studies is that education costs that are directly related to ASD are higher for children with ASD and intellectual disabilities as compared to children with ASD without intellectual disabilities and adolescents/adults with ASD. Estimation results also showed that for children with ASD, both with and without intellectual disabilities, special education cost was the highest cost element analysed in the study. As a possible explanation, Järbrink et al. (2003) pointed out that (young) children with ASD are more frequently enrolled in EIBI-programmes (which are often intensive and, thus, expensive programmes, see previous section). For adults with ASD living in a private household, day care and respite services were amongst the highest cost elements. Another interesting finding was that education costs account for roughly 90% of the total service costs for adults with ASD (Barrett et al. 2015) whereas this cost share is only 45% for children with ASD. As discussed by Barrett et al. (2015), the reason for this difference in estimated cost share is that young children with ASD require much more community health and social/community services (e.g., general practitioner visits, community pediatrician visits, speech and language therapy and social worker visits) as compared to adults with ASD.

For Sweden, Järbrink (2007) included different types of special education, speech therapy, personal assistance

[7] Amendah et al. (2011) found that residential schools are more common for children with ASD in Sweden compared to children with ASD in the US.

[8] Lavelle et al. (2014) provided detailed estimations of ASD-related education costs by distinguishing between eleven mutually exclusive school placement categories for children with ASD based on type of school (public, private day, residential, home and other), type of classroom (special or general education) and age [preschool age (3- to 4-year olds) or school age (5- to 17-year olds)]. The (regression-based) analysis revealed large different estimates of education costs for children with ASD depending on the type of school placement category the child with ASD attended. Based on the cost estimates, Lavelle et al. (2014) concluded that education was the biggest cost category in the total cost for children with ASD.

within the school, school transport, aid and special diet in the estimates of the estimates of ASD-related education costs. This study found that education costs as well as community support costs contributed most to the total additional cost for children with ASD. Peters-Scheffer (2015) and Peters-Scheffer et al. (2012) estimated the education costs for the three types of education provided to children with ASD (depending on the severity of the ASD) in the Netherlands: regular education, less intensive special education, and intensive special education. Total education costs for less intensive special and intensive special education for children with ASD were estimated to be approximately 70% and 175% more expensive respectively in comparison to regular education.

Costs of Production Loss for Individuals with ASD

In 2015, the United Nations estimated that more than 80% of adults with ASD are underemployed or not employed at all (United Nations Department of Public Information 2015). Jacob et al. (2015) stated that 50–75% of adults with ASD are unemployed. As the main reason for this high number, the authors pointed out that individuals with ASD experience difficulties in obtaining and maintaining competitive employment due to, among other things, issues of social interaction and communication with supervisors and colleagues. Jacob et al. (2015) discussed several costs of people with ASD being unemployed. This involves costs for the government, society, employers as well as individuals with ASD themselves. Examples include societal cost of adult care for unemployed adults with ASD and a lower life quality of individuals with ASD as a result of being less independent, not having a sense of purpose and decreased cognitive performance.

The few studies that have focused on estimating the costs of having a high number of individuals with ASD being unemployed, focused predominantly on the cost of production loss. Ganz (2007) estimated the economic value of lost or impaired wages, benefits and household services for individuals with ASD in the US. Järbrink and Knapp (2001) and Knapp et al. (2009) estimated the costs of lost productivity due to ASD for the UK. Buescher et al. (2014) estimated the cost of lost productivity for people with ASD in both the US and the UK. For the US, the assumption was made that 40% of adults with ASD were either in full- or part-time employment. For the UK, in line with Knapp et al. (2009), Buescher et al. (2014) assumed that 15% of the individuals with ASD without intellectual disabilities were employed full-time. As for adults with ASDs and intellectual disabilities, the assumption was made that no adults were in open employment. A general finding in all these studies was that the cost of lost employment/productivity is one of the highest cost elements for an adult with ASD. Ganz (2007) computed that this cost component accounts for 30.7% of the total discounted lifetime costs for adults with ASD.[9] For individuals with high functioning ASD, Järbrink and Knapp (2001) computed that the cost of lost productivity accounts for 17.5% of the total lifetime cost, a cost share that is lower than the one found for the average adult with ASD, yet that is still substantial.[10]

Costs of Informal Care and Lost Productivity for Family/Caregivers

Several studies have shown that parents, family members and caregivers of individuals with ASD sustain several financial consequences and income-related losses. Using data collected from a national telephone survey in the US, Kogan et al. (2008) showed that most of the American families of children with ASD were impacted by their child's disorder, with 57.1% of these families stating that "family members had to reduce or stop employment because of the child's condition". The survey data also revealed that 34.9% of the families with children with ASD said that they "needed additional income to cover the child's medical expenses" (Kogan et al. 2008, p. e1153). While some papers tried to estimate the cost of the lost employment for parents and caregivers, other papers focused more on the estimation of the costs of informal care that was provided to individuals with ASD. As with the previous cost components, most of the studies were conducted for the UK and the US. Table 5 gives an overview of the studies and the detailed cost estimates.

Most of the studies found that the costs of informal care and lost productivity for family/caregivers are substantial and that this cost component makes up a large part of the overall (lifetime) costs related to ASD. For instance, for both the UK and the US, Buescher et al. (2014) found that the cost of lost productivity for parents of children with ASD was one of the biggest contributors to total costs for children with ASD. For Australia, Horlin et al. (2014) even estimated that this cost component made up 89% of the total family cost of ASD. For the US, Ganz (2007) concluded that the costs of the productivity loss of parents of a child with ASD were 28.6% of the total discounted lifetime costs of ASD. A few studies, like Järbrink and Knapp (2001), Barrett et al.

[9] It is important to note that Ganz (2007) assumed that the cost of lost productivity for individuals with ASD and additional learning disabilities was assumed to be zero. The authors emphasized that this cost figure of zero did not correspond to the real cost figure but was merely due to a lack of available information.

[10] Based on findings of previous studies, Järbrink and Knapp (2001) assumed that individuals with high functioning ASD work in low-skilled, low-paid jobs, often despite a high level of education. For this group, the cost of lost productivity was estimated based on gross wages according to the human capital method (Järbrink and Knapp 2001, p. 9).

(2012) and Järbrink (2007), found relatively small cost estimations for lost productivity and informal care and concluded that these costs constitute only a small portion of the overall total lifetime cost for ASD. Barrett et al. (2012), for example, calculated that this cost made up only 8.92% of the total costs related to ASD. However, both Järbrink and Knapp (2001) and Järbrink (2007) pointed out that their cost estimates for productivity loss and/or loss of leisure time are probably underestimating the real costs of these losses, the reasons being the use of conservative parameters for time losses and the exclusion of other types of productivity losses (e.g. diminished promotion opportunities, early retirement, etc.) in the cost estimations

The figures in Table 5, however, also show large variation in the estimated costs across studies. A review of the papers pointed out a number of reasons which explain for this variation. Firstly, no doubt some of this variation is explained by the different dates on which the studies were conducted as well as by the fact that studies have been performed in different countries. Secondly, it is very likely that some of this variation is due to different cost estimation method being used across studies. As to the former, different methods were used in the literature to estimate the costs of income losses due to lost productivity for the parents, family members or caregivers of children with ASD. Some studies (Montes and Halterman 2008; Cidav et al. 2012) used regression-type analyses to estimate these costs. As an example, Montes and Halterman (2008) employed ordinal logistic regression analysis to estimate the expected household income of families with children with ASD and families without a child with ASD, thereby controlling for a set of independent variables such as average parent age, type of family (2-parent family or other type), race, level of parental education, urban or rural living area, ASD status, and other disability status. The logistic regression results were used to estimate the loss of income associated with having a child with ASD. Cidav et al. (2012) combined logit and Tobit models to estimate the effect of having children with ASD on parental labour force participation and parental earnings. Other studies such as Barrett et al. (2012), Horlin et al. (2014), Järbrink et al. (2003) and Järbrink (2007) used questionnaire and/or interview data as collected among parents or families with ASD children to estimate the time they had lost on paid work, unpaid work and leisure due to their child's ASD diagnosis, etc. In such questionnaires/interviews parents, family members or caregivers were asked whether their employment status was affected by their child's ASD diagnosis. Although the use of questionnaires and interviews surely has some advantages (e.g., collection of quantitative and/or qualitative data among the target group), Järbrink et al. (2003), for instance, noted that generally parents of children with ASD found it difficult to give an accurate indication of time spent on different informal care activities or to identify the number of hours spent on informal care activities due to the ASD of their child. Illustrative of this was the large standard deviation for time lost on unpaid work as indicated by parents. Obviously, the use of less accurate time loss data will lower the accuracy of the cost estimates. A third factor is that the modelling parameters being set in the cost estimations, differ across studies. The review showed that studies used different parameters for the labour force participation rate of parents of children with ASD, for the time loss incurred by the parents due to the ASD-condition of their child, the value of 1 h of paid work and 1 h of unpaid work and leisure, etc. As an illustration, using questionnaire and diary data as collected among parents with a child with ASD in the UK, Järbrink et al. (2003) found that parents estimated time loss due to their child's ASD-condition on paid work to be on average 22 h per week and on leisure activities to be on average 17 h per week.[11] However, much lower time loss figures were reported by Buescher et al. (2014) who, following insights of Cidav et al., (2012), assumed that parents of children with ASD ($\leq$ 18 years) in the UK worked on average 7 h less per week as compared to parents of children without ASD.

In sum, although the resulting costs estimates vary among the studies, overall cost estimations show that parents, family members and caregivers of individuals with ASD sustain high costs due to productivity loss, loss of labour income and loss of leisure time related to ASD of their child. In addition, a general finding is that these costs are one of the biggest contributors to total ASD-related costs for families with children with ASD. Studies highlighted multiple reasons for the high income loss and productivity loss suffered by parents, family or caregivers with an ASD child. Montes and Halterman (2008), for instance, referred to the following three issues: (1) poorer-than-expected labour market performance, (2) lower-than-expected labour participation, and (3) lower-than-expected savings and investment (Montes and Halterman 2008, p. e824). Cidav et al. (2012) identified the diversity of care that children with ASD require and how this makes it difficult for parents and particularly mothers of children with ASD to balance the management of this care and their professional career.

Costs of Accommodation, Respite Care, and Out-of-Pocket Expenses

The cost of accommodation and residential care for individuals with ASD is rarely estimated in the literature. Nonetheless, for individuals with ASD who do not live at home

[11] Similar time loss figures were found by Järbrink (2007) for Sweden, with parents reporting that they spend on average 977 h per year on caring for their child with ASD.

with their family, the accommodation cost can represent a large element in the total costs related to ASD. In this section, we look at the few studies that have estimated the ASD-related accommodation and residential care costs. We also briefly look at estimates given for some other costs related to ASD. This concerns, among other things, costs for respite care and out-of-pocket expenses made by parents (families/caregivers) for children/adults with ASD. Table 6 presents a summary overview of the studies, their data sources and cost estimates for accommodation, respite care, and out-of-pocket expenses related to ASD.

A first general finding is that the cost estimates vary depending on whether it concerns accommodation and residential care for children with ASD or adults with ASD, and whether it concerns accommodation and residential care for individuals with ASD and intellectual disabilities or individuals with ASD without intellectual disabilities. Overall cost estimates show that the costs for accommodation and residential care are higher for adults with ASD as compared to children with ASD, the reason being that a large majority of the children with ASD are living at home. Buescher et al. (2014), for instance, assumed that only 1% of the American children with ASD aged 0–5, and 5% of the American children with ASD aged 6–17 used residential care (Buescher et al. 2014, p. 723). For Sweden, Järbrink (2007) found similar low percentages, with only 6% of the children with ASD in their sample living in home placement settings. In addition, cost estimates for accommodation and residential care are higher for individuals with more severe ASD, the main reason being that these individuals have a higher need to live in supported accommodation and residential care. For adults with ASD in the UK, for instance, based on findings of previous studies, Buescher et al. (2014) modelled that of the adults with ASD without intellectual disabilities, 16% lived in residential care, 5% in supported accommodation and 79% at home, whereas for adults with ASD and intellectual disabilities, these percentages were respectively 24% (residential care), 27% (supported accommodation), 1% (hospital), and 48% (at home) (Buescher et al. 2014, p. 723).[12]

As for the cost estimations of respite care and out-of-pocket expenses related to ASD, it is not straightforward to compare and interpret the cost estimates of previous studies due to differences in the selections of cost elements. For the estimation of the family out-of-pocket expenses related to ASD, for instance, Järbrink and Knapp (2001) included home improvements or adaptations, consumer durables, special nursing equipment, extra household items, cleaning, repairs, and transports (Järbrink and Knapp 2001, p. 16). In another study, Järbrink et al. (2003) included, amongst other things, damage, special dietary requirements, clothes, extra laundry, extra help, transport, special activities, additional costs for therapy/education, extra costs for siblings, and court cases/solicitor (Järbrink et al. 2003, p. 399). In their study of parents' out-of-pocket expenses related to ASD, Barrett et al. (2012) included adaptations and security for the home and garden, replacement and repair of damage to house and contents, specialist equipment such as push-chairs and toys, attendance at seminars and training courses, travel to receive health services and assessments abroad, and smaller items such as nappies, bedding, education materials, additional clothing and specialist diets (Barrett et al. 2012, p. 800). Another issue is that cost estimates of ASD-related out-of-pocket expenses may not always be accurate, the reason being that parents forget certain expenses when estimating the expenses retrospectively. As an illustration, Järbrink et al. (2003) found that when parents were asked to estimate the cost of these expenses on a weekly basis in a questionnaire, the average cost was £ 65.91. However, when looking at the out-of-pocket expenses filled out by parents in a diary, the average weekly out-of-pocket expenses turned out to be £ 100.15 (both in 1999–2000 £).

To summarize, only a few studies have estimated the costs of (supported) accommodation and residential care and other out-of-pocket expenses. Despite the difficulties in estimating these costs (as reported by some of these studies) and the limited comparability of these cost estimates, studies do show that, generally, for individuals with ASD who do not live at home or with family, the cost of accommodation or residential care can represent a large cost element in the total costs related to ASD. As an example, for the UK, Järbrink and Knapp (2001) concluded that for individuals with ASD and intellectual disabilities as well as for individuals with high functioning ASD, the cost of (supported) accommodation and/or residential care was the biggest cost element in the total lifetime costs for an individual with ASD, accounting for 72.6% and 39.8%, respectively. As for the cost estimates of ASD-related out-of-pocket expenses, studies show that these expenses can become a financial burden for individuals with ASD and their family and, therefore, should not be ignored when reviewing costs related to ASD.

Conclusions

About 50 papers were reviewed in this literature review about costs related to ASD. Even though most papers regarding ASD-related costs originated in the US and the UK, this literature review tried to take a broader view, by including papers from The Netherlands, Sweden, Australia, Canada, Egypt, and China. The review was organized into

[12] In another cost estimation study for the UK, Knapp et al. (2009) reported similar percentages, i.e. a higher percentage of adults with ASD and intellectual disabilities living in supported living accommodation (7% vs. 5%), in residential care (52% vs. 16%), and in a hospital (6% vs. 0%) as compared to adults with ASD, yet, without intellectual disabilities (Knapp et al. 2009, p. 320).

six categories of ASD-related costs: (i) medical and healthcare related service costs, (ii) therapeutic costs, (iii) (special) education costs, (iv) costs of production loss for adults with ASD, (v) costs of informal care and lost productivity for family/caregivers, and (vi) costs of accommodation, respite care, and out-of-pocket expenses.

A recurrent finding was that it is not straightforward to compare the cost estimates across studies. No doubt, one of the reasons for this is that the cost estimation studies differ in the use of data sources, the categorization of cost components, the inclusion and/or exclusion of cost elements, the research method and/or modelling parameters, the period of study, etc. In the selection of the papers for this review, only limited selection criteria were imposed as to how cost data were collected, which cost estimation technique was used, and/or how cost estimates or figures were reported. The advantage of imposing limited selection criteria is that the selection of papers presented in the paper is broad and comprehensively covering the literature on costs of ASD. The disadvantage is that it is not always straightforward to compare findings across papers and/or countries. However, a second and very likely more important reason for why it is difficult and complex to compare and interpret estimations of ASD-related costs across studies, are the differences in how countries organize (or, have organized, given that changes may have occurred during the period 2000–2018) the health care system as well as the financing of that system.[13] These organizational differences typically translate into different ASD policy plans and intervention programmes being provided, different financial support mechanisms being available for ASD individuals, etc. Illustrative is the US where typically a higher percentage of GDP is spend on health care as compared to any other country, which of course influences the national absolute ASD cost values. In a study of the aggregated national costs for ASD for the UK and the US, Buescher et al. (2014) nicely illustrated the impact of the different approaches to organizing and financing the health care system and the education system on the estimates of total ASD costs as well as the contribution of the different cost components. Even though the lifetime cost of supporting an individual with ASD for the US and the UK were largely similar (i.e., $2.4 million and $2.2 million for an individual with ASD and intellectual disability in the US and the UK, and $1.4 million and $1.4 million for an individual with ASD without intellectual disability), it was found that the relative contributions of the costs components to the total lifetime cost of supporting an individual with ASD differed considerably across both countries, with, for the US, 79% of the ASD costs being accounted for by services, 12% by the productivity costs for the ASD-individual, and 9% by caregiver time costs and, for the UK, 56% of the total ASD-costs being accounted for by services, followed by lost employment for the individual with ASD (42%) and caregiver time costs (2%).

The observation that countries differ in the organization of the health care and education system as well as the financing of those systems, and that such differences complicates comparisons of ASD cost figures across countries, also holds for the EU. In a recent study, Roleska et al. (2018) found that in the EU, under the subsidiarity principle, education and disability policies remain within the competence of EU Member States, with educational standards and provisions for individuals with ASD being determined and implemented at the national level (and, in several countries, even at the sub-national, regional level). Looking at the country policies in the field of education, special education needs and disability, they found that Poland, for example, does not have an autism specific strategy whereas other countries such as the UK and Spain have tailored policies and plans for ASD individuals to promote, among other things, inclusive education for ASD children. But also within countries, at the local level, systems can vary considerably in policies regarding services for which children with ASD are eligible, the efficiency and the effectiveness of these services, and hence the ASD-related costs.[14] As an example, Mandell and Palmer (2005) explored the variation among the 50 US states in the administrative prevalence of ASD and factors associated with that variation. Using data for the year 2000–2001, they found that the proportion of children being diagnosed with ASD ranged significantly across the US states (from 0.6 per 1000 to 4.6 per 1000).[15]

The comparison issues notwithstanding, this review study revealed some interesting and important insights. Firstly, one key finding was that ASD is associated with a high financial burden in a multitude of domains, resulting in overall lifetime costs of ASD for the average individual with ASD (or

[13] Another possible reason for the cross-country differences in ASD-related costs are the differences in ethno-racial and demographical diversity across countries. For instance, Shattuck et al. (2012) provided empirical evidence which suggests the presence of racial and/or demographical disparities in access to health services across a wide range of health conditions and service systems.

[14] With *efficiency* we refer to the link between inputs and outputs of ASD services (e.g., how do the resources invested in the organization and implementation of EIBI programmes relate to the number of young children with ASD effectively being able to enter in such programmes), whereas with *effectiveness* we refer to the link between the outputs and the outcomes of ASD services [e.g., how does the number of people being provided with the organized EIBI programme relate to the total number of dependency-free life years (DFLYs) generated by this programme].

[15] A linear regression analysis revealed that the cross-state variation in the administrative prevalence of ASD relate to characteristics of the education and health system with the administrative prevalence of ASD being positively associated with education-related spending as well as the number of paediatricians and the number of school-based health centers in the state.

family with a child with ASD) that are substantial. Based on the studies reviewed, the overall lifetime costs for individuals with ASD are estimated to be situated somewhere between $ 2.4 million (in 2011 US$) (Buescher et al. 2014) to $ 3.2 million (in 2003 US$) (Ganz 2007) for the US and from £ 1.5 million (in 2011 £) (Buescher et al. 2014) to £ 2.4 million (in 1997–1998 £) (Järbrink and Knapp 2001) for the UK. As a total figure for the US, Leigh and Du (2015) estimated annual direct medical, direct non-medical, and productivity costs combined to be $268 billion (range $162–$367 billion; 0.884–2.009% of GDP) for 2015 and forecast this cost to be $461 billion (range $276–$1011 billion; 0.982–3.600% of GDP) for 2025. Moreover, as pointed out by some studies, reported cost estimation figures are likely to underestimate true ASD-related costs due to omitted health impacts, omitted economic impacts, omitted impact on social life, and the costs of health actions in other sectors.

A second key finding is that, for the six cost categories studied in the review, estimated costs are higher for individuals with ASD and/or families with children with ASD than other individuals/families. In addition, the estimates of ASD-related costs generally show that costs are higher for individuals with more severe ASD. For instance, costs across the different categories have been found to be higher for individuals with ASD and intellectual disabilities than individuals with ASD without intellectual disabilities (or for families or caregivers with such individuals). Related to this, a recent EU-wide survey of the costs of ASD (ASDEU 2018) found that type of ASD, age, and comorbidities—intellectual disability especially—are important drivers of the costs of ASD. One reason for this is that individuals with more severe ASD require more medical and healthcare, intensive (early) behavioural treatment, therapy and special education during their childhood. Another reason is that individuals with more severe ASD have a higher need to live in supported accommodation and residential care during adulthood. Parents of children or adults with more severe ASD also sustain higher costs due to productivity loss, loss of labour income and loss of leisure time related to the ASD of their child.

A third general finding was that the cost of (special) education, EIBI and therapy, individual productivity loss, parental productivity loss, and (supported) accommodation and residential care are among the largest contributors to total lifetime costs for an individual with ASD. The recent anonymous online survey for children and adults with ASD organized across multiple EU-countries in a large-scale project (ASDEU 2018), found similar results, with the cost of special education services being the highest cost component, followed by the costs of tutorial support, especially among younger people with ASD. Medical and healthcare costs related to ASD have been found to constitute only a small part of the total costs for individuals with ASD, with medical costs being higher for adults with ASD than for children with ASD. Estimates also show that smaller out-of-pocket expenses related to ASD, such as travel costs, cost related to making the house more ASD-friendly, purchase of specialised tools or equipment, etc., cannot be ignored when analysing the costs related to ASD. Summed together, all these out-of-pocket expenses can place a significant financial burden on the family budget. As to the costs of ASD-related therapies, due to the differences in therapy categorization and widely divergent cost estimates for ASD therapies, it is difficult to get a clear picture of the costs of therapy and/or EIBI programmes for individuals with ASD. Nevertheless, the studies that assessed the cost-effectiveness of EIBI for (young) children with ASD found that such therapy programmes are cost-effective and can result in cost savings throughout the lifetime of individuals with ASD.

We conclude the paper with three important remarks. A first important remark is that the present review paper does not discuss all cost elements of ASD. Other ASD-related costs include the costs of vocational rehabilitation programmes (Cimera and Cowan 2009, 2011), the costs of universal or high-risk screening for ASD (Yuen et al. 2018) and private insurance premiums for individuals with ASD. For instance, Cimera and Cowan (2009) estimated the costs of vocational rehabilitation programmes for individuals with ASD as well as for non-ASD individuals with similar disabilities using data collected from the federal US Rehabilitation Services Administration "911" database for the period 2002–2006. Estimates showed that compared to all other disability groups in the study, individuals with ASD were among the most expensive in terms of cost per capita for vocational rehabilitation ($ 2992 per capita, in 2006 US$). In fact, only the per capita cost for individuals with sensory impairments was higher. Among the individuals with ASD, individuals with ASD and learning disabilities or sensory impairments had higher costs for vocational rehabilitation services.[16] In a related study, Cimera and Burgess (2011) found that individuals with ASD who had their cases closed by government-operated vocational rehabilitation agencies and worked in the community, generated more monetary benefits than monetary costs (i.e. average benefit–cost ratio of 5.28 and monthly net benefit of $643.20). Another cost element is the impact of ASD coverage on private insurance premiums. Using the Pennsylvania legislation as an example, Bouder et al. (2009) estimated this impact to be

[16] As a possible explanation for this higher cost figure, Cimera and Cowan (2009) pointed out that the proportion of individuals with ASD is small (0.55% in 2006) in the overall vocational rehabilitation population. Vocational rehabilitation counsellors might thus be unfamiliar with the ASD population and their unique needs. This could result in a 'trial and error' method of providing services" (Cimera and Cowan 2009, p. 298), which increases the cost of vocational rehabilitation.

approximately 1%, with a lower bound of 0.19% and an upper bound of 2.31%. Also interesting is the link between insurance type (private vs. public funded) and costs, accessibility, and use of health care services, medication costs, and therapy services for children/adolescents/adults with ASD (e.g., Young et al. 2009; Wang et al. 2013).

A second remark concerns how costing estimates changed over the years and what has driven these changes. We note that it is difficult to say whether estimates of ASD costs made by studies in first decade of the twenty first century (even when the cost figures are inflated to the present) are still comparable with and/or representative for the ASD costs today, given the increasing trend in the number of people being officially diagnosed with ASD, better public and medical awareness for ASD, improved possibilities to diagnose children at a young age, changes in the organization and the financing of the health care system and the education system (with, e.g., in a lot of countries more provision of (special) education and EIBI for (young) children with ASD), or a combination of those factors. In that perspective, the literature would benefit from longitudinal study designs that investigate how costs of ASD (as well as the relative contributions of the different cost elements) have evolved over time and look at (and explain) how changes in national and/or local policies for services for ASD have affected these cost estimates, etc.

As a final remark, even though the between- and within-country differences as well as changes over time in the organization of insurance policy, education system, healthcare system, etc., make it difficult to give general policy advice in terms of funding for ASD-related costs, two categories of costs related to ASD are worth mentioning when discussing policy recommendations. Firstly, generally, cost-effectiveness studies of EIBI suggests that such programmes are cost-effective and might reduce certain costs that would occur in the future without these programmes. However, the up-front cost of such programmes is very large and, as demonstrated by multiple articles, this might result in parents not being able to offer their child with ASD such a treatment or might result in using EIBI programmes only for a limited number of hours per week. Making EIBI programmes available for as many children with ASD as possible might save governments and society at large other costs. Further research on different types of EIBI programmes and how to best deliver these programmes in order to make them as effective as possible can guide policy makers in resource allocation decisions. Another cost category that is worth mentioning is the cost of lost employment for individuals with ASD. Unemployed individuals with ASD incur high costs, such as costs for day activities and day care provision. Finding suitable employment and providing long-term support for employed individuals with ASD might reduce or eliminate other costs. However, further studies concerning vocational rehabilitation programmes for individuals with ASD might be useful in order to make these services more efficient and effective.

Author Contributions The search and the filtering of the papers for the review article was performed by JJ (under supervision and with help from NR). JJ performed the abstraction of the data from the papers. Both NR and JJ worked on the writing of the paper manuscript. JJ mainly worked on the writing of the first draft. NR considerably reworked this draft and designed the tables for the figure. The revision of the paper was done by NR.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval The authors declare that the writing of the paper was done in compliance with all ethical standards.

References

Amendah, D., Grosse, S. D., Peacock, G., & Mandell, D. S. (2011). The economic costs of autism: A review. In D. Amaral, D. Geschwind, & G. Dawson (Eds.), *Autism spectrum disorders* (pp. 1347–1360). Oxford: Oxford University Press.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders (DSM-5®)*. American Psychiatric Publ.

ASDEU. (2018). *Autism spectrum disorders in the European Union—ASDEU*. Summary of Report WP1. Task 1.2 Burden of ASD and Task 1.3 Cost of ASD screening. http://asdeu.eu/.

Atladóttir, H. Ó., Parner, E. T., Schendel, D., Dalsgaard, S., Thomsen, P. H., & Thorsen, P. (2007). Variation in incidence of neurodevelopmental disorders with season of birth. *Epidemiology, 18*(2), 240–245.

Baio, J., Wiggins, L., Christensen, D. L., et al. (2018). Prevalence of autism spectrum disorder among children aged 8 years—Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2014. *MMWR Surveillance Summary, 67*(No. SS-6), 1–23. https://doi.org/10.15585/mmwr.ss6706a1.

Baird, G., Simonoff, E., Pickles, A., Chandler, S., Loucas, T., Meldrum, D., et al. (2006). Prevalence of disorders of the autism spectrum in a population cohort of children in South Thames: The Special Needs and Autism Project (SNAP). *The Lancet, 368*(9531), 210–215.

Bambha, K., & Kim, W. R. (2004). Cost-effectiveness analysis and incremental cost-effectiveness ratios: Uses and pitfalls. *European Journal of Gastroenterology and Hepatology, 16*(6), 519–526.

Barrett, B., Byford, S., Sharac, J., Hudry, K., Leadbitter, K., Temple, K., et al. (2012). Service and wider societal costs of very young children with autism in the UK. *Journal of Autism and Developmental Disorders, 42*(5), 797–804.

Barrett, B., Mosweu, I., Jones, C. R. G., Charman, T., Baird, G., Simonoff, E., et al. (2015). Comparing service use and costs among adolescents with autism spectrum disorders, special needs and typical development. *Autism: The International Journal of Research and Practice, 19*(5), 562–569.

Bouder, J. N., Spielman, S., & Mandell, D. S. (2009). Brief report: Quantifying the impact of autism coverage on private insurance premiums. *Journal of Autism and Developmental Disorders, 39*(6), 953–957.

Buescher, A. V. S., Cidav, Z., Knapp, M., & Mandell, D. S. (2014). Costs of autism spectrum disorders in the United Kingdom and the United States. *JAMA Pediatrics, 168*(8), 721–728.

Butter, E. M., Wynn, J., & Mulick, J. A. (2003). Early intervention critical to autism treatment. *Pediatric Annals*, *32*(10), 677–684.

Chasson, G., Harris, G., & Neely, W. (2007). Cost comparison of early intensive behavioral intervention and special education for children with autism. *Journal of Child and Family Studies, 16*(3), 401–413.

Cidav, Z., Lawer, L., Marcus, S. C., & Mandell, D. S. (2013). Age-related variation in health service use and associated expenditures among children with autism. *Journal of Autism and Developmental Disorders, 43*(4), 924–931.

Cidav, Z., Marcus, S. C., & Mandell, D. S. (2012). Implications of childhood autism for parental employment and earnings. *Pediatrics, 129*(4), 617–623.

Cidav, Z., Munson, J., Estes, A., Dawson, G., Rogers, S., & Mandell, D. S. (2017). Cost offset associated with early start denver model for children with autism. *Journal of the American Academy of Child and Adolescent Psychiatry, 56*(9), 777–783.

Cimera, R. E., & Burgess, S. (2011). Do adults with autism benefit monetarily from working in their communities? *Journal of Vocational Rehabilitation, 34*(3), 173–180.

Cimera, R. E., & Cowan, R. J. (2009). The costs of services and employment outcomes achieved by adults with autism in the US. *Autism: The International Journal of Research and Practice, 13*(3), 285–302.

Cohen, D. J., & Reynolds, M. R. (2008). Interpreting the results of cost-effectiveness studies. *Journal of the American College of Cardiology, 52*(25), 2119–2126.

Croen, L. A., Najjar, D. V., Ray, T., Lotspeich, L., & Bernal, P. (2006). A comparison of health care utilization and costs of children with and without autism spectrum disorders in a large group-model health plan. *Pediatrics, 118*(4), e1203–e1211.

Durkin, M. S., Maenner, M. J., Meaney, F. J., Levy, S. E., DiGuiseppi, C., Nicholas, J. S., et al. (2010). Socioeconomic inequality in the prevalence of autism spectrum disorder: Evidence from a US cross-sectional study. *PLoS ONE, 5*(7), e11551.

Eldevik, S., Hastings, R. P., Hughes, J. C., Jahr, E., Eikeseth, S., & Cross, S. (2009). Meta-analysis of early intensive behavioral intervention for children with autism. *Journal of Clinical Child and Adolescent Psychology, 38,* 439–450.

Ganz, M. L. (2007). The lifetime distribution of the incremental societal costs of autism. *Archives of Pediatrics and Adolescent Medicine, 161*(4), 343–349.

Garber, A. M., & Phelps, C. E. (1997). Economic foundations of cost-effectiveness analysis. *Journal of Health Economics, 16*(1), 1–31.

Hodgetts, S., Zwaigenbaum, L., & Nicholas, D. (2015). Profile and predictors of service needs for families of children with autism spectrum disorders. *Autism: The International Journal of Research and Practice, 19*(6), 673–683.

Holland, C. D. (2010). Autism, insurance, and the idea: Providing a comprehensive legal framework. *Cornell Law Review, 96*(6), 1253–1282.

Horlin, C., Falkmer, M., Parsons, R., Albrecht, M. A., & Falkmer, T. (2014). The cost of autism spectrum disorders. *PLoS ONE, 9*(9), e106552.

Hughes, J. R. (2009). Update on autism: A review of 1300 reports published in 2008. *Epilepsy and Behaviour, 16*(4), 569–589.

Jacob, A., Scott, M., Falkmer, M., & Falkmer, T. (2015). The costs and benefits of employing an adult with autism spectrum disorder: A systematic review. *PLoS ONE, 10*(10), e0139896.

Jacobson, J. W., & Mulick, J. A. (2000). System and cost research issues in treatments for people with autistic disorders. *Journal of Autism and Developmental Disorders, 30*(6), 585–593.

Järbrink, K. (2007). The economic consequences of autism spectrum disorder among children in a Swedish municipality. *Autism: The International Journal of Research and Practice, 11*(5), 453–463.

Järbrink, K., Fombonne, E., & Knapp, M. (2003). Measuring the parental service and cost impacts of children with autistic spectrum disorder: A pilot study. *Journal of Autism and Developmental Disorders, 33*(4), 395–402.

Järbrink, K., & Knapp, M. (2001). The economic impact of autism on Britain. *Autism: The International Journal of Research and Practice, 5*(1), 7–22.

Knapp, M., Romeo, R., & Beecham, J. (2009). Economic cost of autism in the UK. *Autism: The International Journal of Research and Practice, 13*(3), 317–336.

Kogan, M. D., Strickland, B. B., Blumberg, S. J., Singh, G. K., Perrin, J. M., & van Dyck, P. C. (2008). A national profile of the health care experiences and family impact of autism spectrum disorder among children in the United States, 2005–2006. *Pediatrics, 122*(6), e1149–e1158.

Lavelle, T. A., Weinstein, M. C., Newhouse, J. P., Munir, K., Kuhlthau, K. A., & Prosser, L. A. (2014). Economic burden of childhood autism spectrum disorders. *Pediatrics, 133*(3), e520–e529.

Leigh, J. P., & Du, J. (2015). Brief report: Forecasting the economic burden of autism in 2015 and 2025 in the United States. *Journal of Autism and Developmental Disorders, 45*(12), 4135–4139.

Leslie, D. L., & Martin, A. (2007). Health care expenditures associated with autism spectrum disorders. *Archives of Pediatrics and Adolescent Medicine, 161*(4), 350–355.

Liptak, G. S., Stuart, T., & Auinger, P. (2006). Health care utilization and expenditures for children with autism: Data from U.S. national samples. *Journal of Autism and Developmental Disorders, 36*(7), 871–879.

Lokhandwala, T., Khanna, R., & West-Strum, D. (2012). Hospitalization burden among individuals with autism. *Journal of Autism and Developmental Disorders, 42*(1), 95–104.

Lyall, K., Croen, L., Daniels, J., Fallin, M. D., Ladd-Acosta, C., Lee, B. K., et al. (2017). The changing epidemiology of autism spectrum disorders. *Annual Review of Public Health, 38,* 81–102.

Mandell, D. S., Cao, J., Ittenbach, R., & Pinto-Martin, J. (2006). Medicaid expenditures for children with autistic spectrum disorders: 1994 to 1999. *Journal of Autism and Developmental Disorders, 36*(4), 475–485.

Mandell, D. S., & Palmer, R. (2005). Differences among states in the identification of autistic spectrum disorders. *Archives of Pediatrics and Adolescent Medicine, 159*(3), 266–269.

Marcus, L. M., Rubin, J. S., & Rubin, M. A. (2000). Benefit–cost analysis and autism services: A response to Jacobson and Mulick. *Journal of Autism and Developmental Disorders, 30*(6), 595–598.

Meltzer, D. (1997). Accounting for future costs in medical cost-effectiveness analysis. *Journal of Health Economics, 16*(1), 33–64.

Mendoza, R. L. (2010). The economics of autism in Egypt. *American Journal of Economics and Business Administration, 2*(1), 12–19.

Montes, G., & Halterman, J. S. (2008). Association of childhood autism spectrum disorders and loss of family income. *Pediatrics, 121*(4), e821–e826.

Motiwala, S. S., Gupta, S., Lilly, M. B., Ungar, W. J., & Coyte, P. C. (2006). The cost-effectiveness of expanding intensive behavioural intervention to all autistic children in Ontario. *Healthcare Policy, 1*(2), 135.

Murphy, D. G. M., Beecham, J., Craig, M., & Ecker, C. (2011). Autism in adults. New biological findings and their translation implications to the cost of clinical service. *Brain Research, 1380,* 22–33.

Parish, S., Thomas, K., Rose, R., Kilany, M., & McConville, R. (2012). State insurance parity legislation for autism services and family financial burden. *Intellectual and Developmental Disabilities, 50*(3), 190–198.

Parish, S. L., Thomas, K. C., Williams, C. S., & Crossman, M. K. (2015). Autism and families' financial burden: The association with health insurance coverage. *American Journal on Intellectual and Developmental Disabilities, 120*(2), 166–175.

Payakachat, N., Tilford, J. M., & Kuhlthau, K. A. (2018). Parent-reported use of interventions by toddlers and preschoolers with autism spectrum disorder. *Psychiatric Services, 69*(2), 186–194.

Penner, M., Rayar, M., Bashir, N., Roberts, S. W., Hancock-Howard, R. L., & Coyte, P. C. (2015). Cost-effectiveness analysis comparing pre-diagnosis autism spectrum disorder (ASD)-targeted intervention with Ontario's autism intervention program. *Journal of Autism and Developmental Disorders, 45*(9), 2833–2847.

Peters-Scheffer N. (2015). Vroegtijdige gedragstherapie: een kostenplaatje. In B. E. B. M. Huskens & H. C. M. Didden (Eds.), *Applied Behavior Analysis bij kinderen en adolescenten met autisme* (pp. 133–147).

Peters-Scheffer, N., Didden, R., Korzilius, H., & Matson, J. (2012). Cost comparison of early intensive behavioral intervention and treatment as usual for children with autism spectrum disorder in the Netherlands. *Research in Developmental Disabilities, 33*(6), 1763–1772.

Raz, R., Lerner-Geva, L., Leon, O., Chodick, G., & Gabis, L. V. (2013). A survey of out-of-pocket expenditures for children with autism spectrum disorder in Israel. *Journal of Autism and Developmental Disorders, 43*(10), 2295–2302.

Reichow, B. (2012). Overview of meta-analyses on early intensive behavioral intervention for young children with autism spectrum disorders. *Journal of Autism and Developmental Disorders*, *42*(4), 512–520.

Roleska, M., Roman-Urrestarazu, A., Griffiths, S., Ruigrok, A. N., Holt, R., Van Kessel, R., et al. (2018). Autism and the right to education in the EU: Policy mapping and scoping review of the United Kingdom, France, Poland and Spain. *PLoS ONE, 13*(8), e0202336.

Sallows, G. O., & Graupner, T. D. (2005). Intensive behavioral treatment for children with autism: Four-year outcome and predictors. *American Journal on Mental Retardation*, *110*(6), 417–438.

Sharpe, D. L., & Baker, D. L. (2007). Financial issues associated with having a child with autism. *Journal of Family and Economic Issues, 28*(2), 247–264.

Sharpe, D. L., & Baker, D. L. (2011). The financial side of autism private and public costs. In M.-R. Mohammadi (Ed.), *A comprehensive book on autism spectrum disorders* (pp. 275–296). Rijeka: InTech.

Shattuck, P. T., Roux, A. M., Hudson, L. E., Taylor, J. L., Maenner, M. J., & Trani, J. F. (2012). Services for adults with an autism spectrum disorder. *The Canadian Journal of Psychiatry, 57*(5), 284–291.

Shimabukuro, T. T., Grosse, S. D., & Rice, C. (2008). Medical expenditures for children with an autism spectrum disorder in a privately insured population. *Journal of Autism and Developmental Disorders, 38*(3), 546–552.

Taylor, L., Brown, P., Eapen, V., Midford, S., Paynter, J., Quarmby, L., et al. (2016). *Autism spectrum disorder diagnosis in Australia: Are we meeting best practice standards?*. Brisbane: Autism Cooperative Research Centre.

United Nations Department of Public Information. (2015). "Employment: The Autism Advantage" in observance of World Autism Awareness Day on Thursday, 2 April 2015. https://www.un.org/en/events/autismday/events2015.shtml.

Vohra, R., Madhavan, S., & Sambamoorthi, U. (2017). Comorbidity prevalence, healthcare utilization, and expenditures of Medicaid enrolled adults with autism spectrum disorders. *Autism, 21*(8), 995–1009.

Wang, L., Mandell, D. S., Lawer, L., Cidav, Z., & Leslie, D. L. (2013). Healthcare service use and costs for autism spectrum disorder: A comparison between Medicaid and private insurance. *Journal of Autism and Developmental Disorders, 43*(5), 1057–1064.

Xiong, N., Yang, L., Yu, Y., Hou, J., Li, J., Li, Y., et al. (2011). Investigation of raising burden of children with autism, physical disability and mental disability in China. *Research in Developmental Disabilities, 32*(1), 306–311.

Young, A., Ruble, L., & McGrew, J. (2009). Public vs. private insurance: Cost, use, accessibility, and outcomes of services for children with autism spectrum disorders. *Research in Autism Spectrum Disorders, 3*(4), 1023–1033.

Yuen, T., Carter, M. T., Szatmari, P., & Ungar, W. J. (2018). Cost-effectiveness of universal or high-risk screening compared to surveillance monitoring in autism spectrum disorder. *Journal of Autism and Developmental Disorders*, *48*(9), 2968–2979.

Received: 17 March 2022 | Accepted: 11 December 2022

DOI: 10.1002/aur.2882

RESEARCH ARTICLE

The impact of autism spectrum disorder on parent employment: Results from the r-Kids study

Frances L. Lynch[1,2] | **Joanna E. Bulkley**[1] | **Alexandra Varga**[1] | **Phillip Crawford**[1] | **Lisa A. Croen**[3] | **Yihe G. Daida**[4] | **Eric Fombonne**[5] | **Brigit Hatch**[2,5] | **Maria Massolo**[3] | **John F. Dickerson**[1]

[1]Kaiser Permanente Center for Health Research, Portland, Oregon, USA

[2]OCHIN, Inc., Portland, Oregon, USA

[3]Kaiser Permanente Autism Research Program, Kaiser Permanente Northern California, Oakland, California, USA

[4]Center for Integrated Health Care Research, Kaiser Permanente, Honolulu, Hawaii, USA

[5]School of Medicine, Oregon Health & Science University, Portland, Oregon, USA

Correspondence
Frances L. Lynch, Kaiser Permanente Center for Health Research, 3800 North Interstate Avenue, Portland, OR 97227-1110, USA.
Email: frances.lynch@kpchr.org

Funding information
National Institute of Mental Health (NIMH), Grant/Award Number: 1R01MH106680

Abstract

Parents of children with autism spectrum disorder (ASD) and other chronic health conditions often face exceptional caregiving demands that can lead to challenges related to maintaining and succeeding in employment. Detailed information on the specific ways in which these health conditions impact parent employment could aid in designing equitable, effective policies to support families. The r-Kids study used electronic health records to identify three groups of children: those with ASD, asthma, or neither condition (control), from several health care systems. We oversampled racial and ethnic minorities and matched the asthma and control groups to the age and sex distribution of the ASD group. Parents completed three online surveys over the course of a year to measure annual employment outcomes. Surveys included the Family Economic Impact Inventory (measuring employment impacts) and measures of quality of life and symptom severity. All materials were provided in English and Spanish. The study enrolled 1461 families (564 ASD, 468 asthma, 429 control). Youth were 3–16.5 years old and predominantly male (79%). The sample was diverse (43% non-Hispanic White; 35% non-Hispanic Asian, Black, Native Hawaiian, or Other; and 21% Hispanic ethnicity). Parents of children with ASD were significantly less likely to be employed than parents of youth with asthma and control combined (OR: 14.2, $p < 0.001$), and were more likely to have other difficulties with employment and productivity while at work. Public and employer policies to help mitigate these impacts could aid families in managing care for youth with ASD.

Lay Summary

Caring for a child with a chronic health condition such as autism spectrum disorder (ASD) can interfere with parental employment in a number of ways. We found that parents of children with ASD are less likely to be employed, and when they are employed, many face challenges in managing productivity and caregiving. A detailed understanding of these impacts can guide clinical and employment policies to help families navigate the financial implications of a child's health condition.

KEYWORDS
asthma, autism, employment, special health care needs

INTRODUCTION

Autism spectrum disorder (ASD) is a lifelong developmental condition that affects social interaction and communication skills and may impact cognitive function (APA, 2013). Early intervention and treatment can significantly improve symptoms and functioning in youth with ASD. In recent studies, 1 in 44 youth were identified with

ASD in the USA (Maenner et al., 2021). ASD is about 4.2 times more common in boys than girls and has been reported in all racial, ethnic, and socioeconomic groups.

ASD can have a significant effect on parents and families across multiple domains. Because ASD is a lifelong neurodevelopmental disorder, the caregiving burden for parents is often very high with increased parental stress and decreased parenting efficacy for both mothers and fathers (Baker-Ericzén et al., 2005; Bonis, 2016; Dabrowska & Pisula, 2010; Karst & Van Hecke, 2012; Picardi et al., 2018). In addition, the severity of a child's ASD has been shown to be a significant predictor of parental stress (Pastor-Cerezuela et al., 2016). Children with ASD typically need significant services related to health, education, and the acquisition of communication and social interaction skills, and many of these services are not provided through insurance or at school. Thus, the care of a child with ASD requires substantial time, effort, and persistence on the part of parents. Exceptional caregiving responsibilities (Brennan & Brannan, 2005; Stewart et al., 2018) also affect parents of children with other physical and mental health conditions (Ambler et al., 2018; Everhart et al., 2018; Ketelaar et al., 2008; Silva et al., 2015), although the level and areas of caregiving needed for different conditions vary considerably. In addition, caregiving, and other parental responsibilities for youth with ASD and some other health conditions, can continue longer into childhood and young adulthood and can entail significant ongoing physical, emotional, and financial demands on families that are not experienced by families of youth who do not have a chronic health condition.

One important area of impact for families caring for a youth with ASD is parental employment. The combined burden of exceptional caregiving and caregiver strain can negatively impact parents' work life in multiple ways. Recent studies have documented that demands related to exceptional care can lead to parents working reduced hours, bringing in less income, or leaving the workforce altogether (Brennan & Brannan, 2005; Cidav et al., 2012; Porterfield, 2002). The demands can also have a dramatic impact on parents' career paths, especially since parents are often responding to their children's needs during a formative time in their working lives (Leiter et al., 2004)—thus affecting not only current income but future employability or career progression.

This report provides an update on and extension of the literature on the employment challenges of parents with children with ASD by providing greater detail and specificity about the issue. We used data from the r-Kids study to examine whether having a child with ASD affects labor force participation, changes in employment, missed time from work, and work productivity. Furthermore, we estimated the incremental employment-related impact of having a child with ASD compared with having a child with asthma or with no significant chronic health condition. We also examined whether these effects change as a child gets older. Our findings may help employers and government policymakers in developing programs and policies to ease employment challenges for parents of youth with ASD.

METHODS

The focus of this analysis was to examine how employment outcomes for parents of children with ASD are impacted relative to parents of children with another chronic health condition, asthma, or no significant chronic condition. The conceptual framework for the analysis combines insights from two economic models. The first model, labor supply theory (McCall & Starr, 2016; Richard et al., 2014), describes an individual's employment choices, including hours of work, and suggests these employment choices are shaped by a multitude of factors, including age, education level, sex, race/ethnicity, cultural norms, and family characteristics (e.g., marital status). The second model, household production theory (Becker, 1981; Bergstrom, 1996; Berman et al., 1994; Harkness & Super, 1994; Snow Jones et al., 1999), describes how families use available resources within the household. Families may have several resources—money, availability of family members' time, and access to services such as health care (e.g., employment-based health insurance)—that families utilize to meet the needs of family members, such as provision of meals or helping children with schoolwork. However, families are also subject to limitations. In particular, parents have a limited amount of time to provide for the different needs of family members and most families have limited financial resources based on parent income.

By bringing these two models together, we intend to explicitly acknowledge how a child's health impacts parents employment decisions. Specifically, when a household includes a child with a chronic health condition, parents will meet medical and nonhousehold needs related to the child's condition, as constrained by available resources (e.g., income, time). Prior studies have suggested several implications of the combined models (labor supply and household production). The amount of household resources needed to ensure children's well-being (e.g., parent time, medical care, other services) will be greater for families whose children have chronic health conditions such as ASD, and as severity increases, more resources are needed (Croen et al., 2006; Lavelle et al., 2014). An additional implication is that parents of children with ASD might choose to increase paid work time for added income to pay for services (Cidav et al., 2012). However, parents might also decrease paid work in order to have time to provide care for the child at home (Brannan et al., 2018; Cidav et al., 2012). If parents are better able to provide caregiving related to the child's chronic condition (e.g., parent is knowledgeable

about the child's specific needs) compared with available alternative childcare providers, parents may reduce their work time to provide caregiving (Houser et al., 2014). In addition, if childcare providers who have the ability to care for a child with a chronic condition are not available, parents may have no choice but to care for their children at home (Brannan et al., 2018). Thus, the impact of having a child with ASD or another health condition on employment choices may result in increased paid work time or decreased paid work time depending on the family's available resources (money, parent time), available community resources (e.g., health, childcare), and parental knowledge and skill in caregiving related to the child's condition.

Several studies have found that the likelihood of parents being employed is less when they have children with ASD or another health condition (Cidav et al., 2012; McCall & Starr, 2016; Powers, 2003), and that when employed, they work fewer hours. However, some studies have found that after controlling for important demographic characteristics, there is no significant impact on parental employment in such households (Loprest & Davidoff, 2004). In addition, most prior research regarding the impact of child disability on parental employment has focused on the decision to work outside the home (Cidav et al., 2012; Loprest & Davidoff, 2004; Powers, 2003) or on hours of employment (Cidav et al., 2012; Loprest & Davidoff, 2004). However, there may be other ways that a child's health could impact parental employment, including missing time from work, a shift from full-time to part-time employment, choosing a less-demanding job, or reduced productivity while on the job, all of which likely have a long-term impact on a parents' employment opportunities and on family income (Brannan et al., 2018; Brennan & Brannan, 2005). To date, few studies have included these additional outcomes. The current analysis addresses these gaps in the literature by examining whether ASD impacts employment on five levels: (1) the choice to work at all, (2) total hours worked, (3) missed time from work, (4) job changes to accommodate the child's health needs, and (5) productivity while at work.

Participants and setting

The Understanding Family Economic Impact of Chronic Child Health Conditions study (r-Kids) was a prospective observational study to assess family financial, time, and employment costs for three groups of children: those with ASD, asthma, or neither condition. The study included families of children between 3 and 16.5 years of age from three Kaiser Permanente regions (Northwest [Oregon and southwest Washington State], Hawaii, and Northern California) and health clinics in the OCHIN, Inc., community health center network.

We searched the electronic health records (EHRs) of all children from 3 to16.5 years of age at each site, with at least one face-to-face encounter (inpatient or outpatient visit) during a 2-year lookback period, for clinical diagnoses made at >1 year of age. To be eligible for the ASD group the child must have met one of the following criteria: (1) two or more diagnoses of ASD (ICD-9 code 299.0) separated by >30 days in the EHR; (2) an active diagnosis on the EHR "ongoing problem list"; or (3) one diagnosis of ASD from a specialty ASD clinic/provider identified by each site separately. Children in the asthma group were required to have two or more encounter- or medication-based diagnoses of asthma (ICD-9 code 493.0, 493.1, 493.9) in the previous 2 years (separated by >30 days). Children who fit the criteria for both ASD and asthma were included in the ASD group. Children eligible for the control group had no diagnosis of ASD or asthma in the EHR. We excluded children with a cancer diagnosis within the previous 36 months, and families were ineligible at screening if the participant was a foster parent or if the child did not live with the parent ≥50% of the time. We oversampled children from historically underrepresented racial/ethnic groups. Within each group of children who were eligible as described above, we identified children who were from racial and/or ethnic minority groups as self-reported to their health system, we prioritized recruiting these children. The Kaiser Permanente Northwest Institutional Review Board (IRB) was the single IRB for all collaborating sites and approved this study.

Parents were sent a letter inviting them to participate in the r-Kids study. The letter included the study web address and a child-specific enrollment code. Parents could go to the website and consent and join the study on their own. We followed up with nonresponders by reaching out by phone and/or email up to three times. Participants could complete a survey online or over the phone with a trained interviewer. All recruitment and survey materials, including letters and patient phone contacts, were provided in English and Spanish.

Participants were invited three times, 4 months apart, to complete three surveys. Data were collected between November 2017 and January 2020. Gift cards were provided for participation: $30 for the first survey, $40 for the second, $50 for the third. Participants who completed all three surveys were provided an additional $15.

Survey content

The r-Kids survey comprised the Family Economic Impact Inventory (FEII; Lynch & Dickerson, 2012; Lynch et al., 2007), which measures financial, time, and employment costs of caring for a child's health, additional information on this measure is available in the supplemental material provided. In addition the survey included existing validated instruments measuring the

child's quality of life (PEDSQL; Varni et al., 2001), behavior (SDQ; Goodman, 2001), and symptom severity of those with ASD using the Social Responsiveness Scale (SRS; Constantino et al., 2003) or asthma using the Pediatric Asthma Impact Scale (Yeatts et al., 2010). We also assessed parenting stress (PSS; Berry & Jones, 1995) in addition to collecting family and household demographic variables. Measures included in the analyses presented here are a subset of the r-Kids study survey. Additional information on outcome measures used in these analyses are provided in Table S7 and additional details of the r-Kids study and survey are presented elsewhere (Bulkley et al., 2022).

Employment outcomes included the impact of a child's health on the following employment outcomes: the decision to work at all, hours worked per week, any type of job change, number of job changes, any missed time from work, number of hours missed from work per week, and whether the child's health ever impacted how the parent spent their time or how well they focused or performed tasks during the workday (any impact on productivity while at work) and how frequently the parent experienced an impact on productivity while at work (frequency of impact on work productivity).

STATISTICAL ANALYSIS

We included study participants who completed one or more items of the FEII component of the survey at any time during the study ($N = 1420$). We imputed missing data via multiple imputation methods, using employment information spanning all assessment waves in addition to information on the parent or guardian (race, family composition, education) and the child (study site, age, health condition, and sex). In developing the imputation model we considered measures outside the scope of employment (e.g., parenting stress) that were collected at the first assessment as potential additional auxiliary variables (parent or guardian's age, sex, self-reported mental health, and parental stress summary score; and child's SDQ and PedsQL scores) if those measures were strongly correlated ($r > 0.4$) with employment outcomes (Enders, 2010). The social function subscale of the PedsQL met this standard for labor market participation outcomes. We imputed 10 datasets and used the combination rules developed by Rubin to appropriately adjust coefficients and standard errors of all estimates. In addition, we estimated models using only study participants with complete data to evaluate any influence of the imputation process. In all cases significant results are similar in both imputed data and complete data samples. We also examined whether missing data was related to our oversampling of children from historically underrepresented racial/ethnic groups. Neither child's race/ethnicity or parent race/ethnicity was a significant predictor of missing data.

We compared demographic characteristics for the ASD, asthma, and control groups using Chi-squared tests. We calculated employment outcomes as annual measures and compared them among groups using a variety of multivariable regression methods. Dichotomous outcomes were modeled via logistic regression, and results were calculated as odds ratios (OR). We modeled number or hours worked, number of employment changes, and number of hours missed from work using count regression methods; specifically, we considered both Poisson and negative binomial regression and chose the specification with optimal fit. Estimates from these models were presented as incidence rate ratios (IRR). Finally, we fit an ordered logistic model to the ordinal scale measuring frequency of impact on productivity to estimate an ordered OR. All study participants for whom we imputed data were included in analyses of labor market outcomes, whereas only those who reported employment at any point during the study were included in analyses of work productivity outcomes. Models were fit separately for each outcome. We applied a two-sided $\alpha = 0.05$ for all inferential tests, and analyses were conducted using Stata version 16.1. We examined whether our results would change if we adjusted for multiple comparisons using Holm–Bonferroni adjustments, in all cases interpretation of results remained the same. Full results of the regression models for our primary analyses are reported in the supplemental materials (see Tables S3 and S4). We also conducted exploratory analyses for each outcome by age group, sex of child, and within the ASD group only, by severity of ASD.

RESULTS

Participant characteristics

Of the 1461 families enrolled in the study ($n = 564$ ASD, 468 asthma, 429 control), children in the study were predominantly male (78.9%). The sample was well distributed across age groups: 3- to 5-year-olds comprised 22.3%, 6- to 11-year-olds comprised 45.6%, and 12- to 17-year-olds comprised 32.1% of the sample. The sample was racially and ethnically diverse, with 43% of children categorized as non-Hispanic White. There were no differences among health groups by sex or age group (Table 1). Children in the asthma group were less likely to be white (38.8%) than those in the ASD and control groups (44.3% and 45.9%, respectively; $p = 0.002$). Families of children in the ASD and asthma groups were less likely to be privately insured (65.4% and 64.8%, respectively) than those in the control group (73.1%; $p \leq 0.001$). Along similar lines, families of children in the ASD and asthma groups were less likely to report high incomes (24.4% and 25.8%, respectively) than families in the control group (34.6%; $p = 0.006$). Analyses of employment outcomes include all

TABLE 1 Demographic characteristics at baseline by health group

	Overall	Health group			
		ASD[a,b]	Asthma	Control	
	N (%)	*N* (%)	*N* (%)	*N* (%)	*p*-value[d]
Consented and eligible	1420	544	457	419	
Child characteristics[c]					
Sex: Male (yes)	1116 (78.6%)	413 (75.9%)	366 (80.1%)	337 (80.4%)	0.153
Age					0.175
Early childhood (3–5 years)	314 (22.1%)	120 (22.1%)	87 (19.0%)	107 (25.5%)	
Middle childhood (6–11 years)	651 (45.8%)	242 (44.5%)	220 (48.1%)	189 (45.1%)	
Adolescent (12–17 years)	455 (32.0%)	182 (33.5%)	150 (32.8%)	123 (29.4%)	
Race/Ethnicity					0.004
Non-Hispanic White	607 (43%)	238 (44.6%)	178 (39.1%)	191 (46.0%)	
Non-Hispanic Native Hawaiian or other Pacific Islander	122 (8.9%)	20 (5.1%)	54 (11.9%)	41 (9.9%)	
Non-Hispanic Asian	134 (9.8%)	51 (9.6%)	45 (9.9%)	39 (9.2%)	
Non-Hispanic Black	55 (3.9%)	20 (3.7%)	24 (5.3%)	11 (2.7%)	
Other or multiple race/ethnicity	183 (12.7%)	69 (12.9%)	55 (12.1%)	59 (14.2%)	
Hispanic	303 (21.7%)	129 (24.2%)	99 (21.8%)	75 (18.1%)	
Insurance type					<0.001
Private	883 (67.6)	322 (65.4)	273 (64.8)	288 (73.1)	
Public	340 (26.0)	154 (31.3)	123 (29.2)	63 (16.0)	
Uninsured	84 (6.4)	16 (3.3)	25 (5.9)	43 (10.9)	
Respondent characteristics					
Sex: Female	1086 (86.0)	398 (85.2)	360 (88.0)	328 (84.8)	0.341
Biological parent	1207 (95.6)	438 (93.8)	393 (96.1)	376 (97.2)	0.048
Education level					0.555
High school or less	171 (13.6)	56 (12.1)	62 (15.3)	53 (13.7)	
Some college	301 (24.0)	116 (25.1)	101 (25.0)	84 (21.8)	
College degree	436 (34.8)	170 (36.7)	131 (32.4)	135 (35.0)	
Graduate degree	345 (27.5)	121 (26.1)	110 (27.2)	114 (29.5)	
Married or living with partner	988 (78.5)	375 (80.6)	311 (76.2)	302 (78.2)	0.282
Household characteristics					
Household monthly income					0.006
≤$4000	448 (36.8)	169 (37.5)	163 (40.8)	116 (31.6)	
$4001–$8000	430 (35.3)	172 (38.1)	134 (33.5)	124 (33.8)	
>$8000	340 (27.9)	110 (24.4)	103 (25.8)	127 (34.6)	

[a]ASD is autism spectrum disorders.
[b]The ASD group includes 50 children with both ASD and Asthma.
[c]Demographic data for this table come primarily from respondent-reported survey responses. Missing data was supplemented for some characteristics using data from each health systems EHR data.
[d]All comparisons of demographic characteristics (categorical) were analyzed using Chi-square tests.

respondents who provided data on employment outcomes ($n = 1420$).

Workforce participation

Parents of youth with ASD were significantly more likely to report not working because of their children's health compared with parents of youth in the asthma or control groups. The percent not working was ASD: 8.9%; asthma: 1.4%, control: 0%. Odds of not working: ASD vs. asthma/control combined: (OR = 14.2, 95% CI = 5.95–34.03, $p < 0.001$). Because no members of the control group reported not working due to their child's health, we compared the ASD group to the combined asthma and control group. For all other employment outcomes we compare the ASD group to each comparison group (asthma, control) separately.

Employed parents of youth with ASD also reported working significantly fewer hours per week than those

TABLE 2 Labor market participation and changes to employment over 1 year ($N = 1420$)

	Not working due to Child's health		Average number of hours worked per week		At least one change to employment		Average number of changes to employment	
Study group	% (n)[a]		Mean (standard error)[a]		% (n)[a]		Mean (standard error)[a]	
ASD	8.8% (48)		24.9 (0.77)		41.0% (223)		1.1 (0.07)	
Asthma	1.3% (6)		27.0 (0.78)		24.9% (114)		0.5 (0.05)	
Control	0% (0)		27.3 (0.82)		16.0% (67)		0.4 (0.05)	
Study group comparisons	IRR	p-value	IRR	p-value	OR	p-value	IRR	p-value
ASD vs. Control	NE	NE	0.94 (0.90–0.97)	<0.001***	4.01 (2.83–5.68)	<0.001***	3.25 (2.67–3.97)	<0.001***
ASD vs. Asthma	NE	NE	0.94 (0.90–0.97)	0.001**	2.23 (1.66–3.00)	<0.001***	2.28 (1.91–2.73)	<0.001***
Asthma vs. Control	NE	NE	1.00 (0.96–1.03)	0.929	1.80 (1.23–2.62)	0.002**	1.43 (1.10–1.85)	0.009**

Abbreviations: ASD, autism spectrum disorder; IRR, incident rate ratio; NE, model not estimable due to zero cell size; OR, odds ratio.
[a]Mean (standard error [SE]) or % (n) calculated using imputed data.
**$p < 0.01$; ***$p < 0.001$.

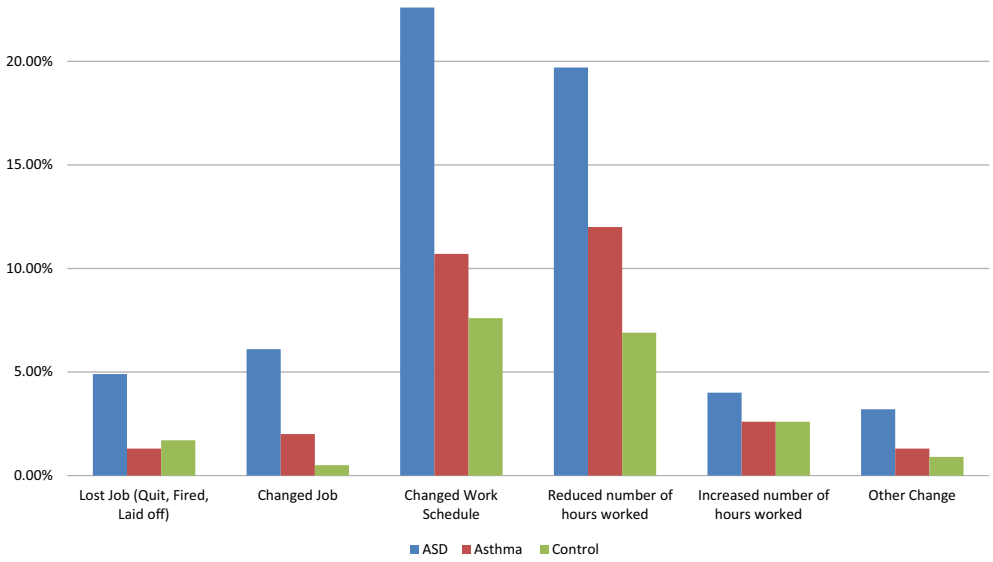


FIGURE 1 Type of job changes due to child's health by group over 1 year

with youth in the asthma or control group. Parents of youth with ASD also reported significantly greater likelihood of job changes due to their children's health. Table 2 presents the means and related OR or IRR for these outcomes. Parents of youth with ASD worked fewer hours per week (IRR ASD vs. control: 0.94; $p < 0.001$; ASD vs. asthma: 0.94; $p = 0.001$) and were significantly more likely to have at least one job change related to their child's health (ASD vs. control OR: 4.01; $p < 0.001$; ASD vs. asthma OR: 2.23; $p < 0.001$) and multiple job impacts (ASD vs. control OR: 3.25; $p < 0.001$; ASD vs. asthma OR: 2.28; $p < 0.001$). Figure 1 describes the types of job changes experienced by families during the study period.

Work productivity

We examined the impact of children's health conditions on the productivity of parents during the time they were working. Table 3 presents the means and related OR or IRR for the following outcomes related to child health: hours missed from work, any negative impact on work productivity (e.g., interruptions while working), and frequency of negative impact on work productivity. Parents of youth with ASD were significantly more likely to ever miss time from work due to their children's health (OR: ASD vs. control: 2.11; $p < 0.001$; ASD vs. asthma: 1.59; $p < 0.001$) and to miss work for a greater number of hours (IRR: ASD vs. control: 1.89; $p < 0.001$; ASD vs. asthma: 1.50; $p < 0.001$). Parents of youth with ASD were also significantly more likely to report any negative impact on their productivity due to their children's health (ASD vs. control OR: 7.43; $p < 0.001$; ASD vs. asthma OR: 4.10; $p < 0.001$) and they also reported having more frequent negative productivity impacts (ASD vs. control OR: 7.69; $p < 0.001$; ASD vs. asthma OR: 4.12; $p < 0.001$). Figure 2 describes the types of productivity impacts experienced by families during the study period.

TABLE 3 Impact of child health on productivity while at work among employed participants for 1 year ($N = 1124$)

	Ever reported missing work due to Child's health		Average hours missed from work per week due to Child's health		Ever experience reduced productivity at work due to Child's health		Frequency of impact on productivity	
Study group	% (*n*)[a]		Mean (SE)[a]		% (*n*)[a]		Mean (SE)[a]	
ASD	83.3% (339)		4.0 (0.30)		79.6% (324)		2.7 (0.08)	
Asthma	76.2% (282)		2.8 (0.21)		49.7% (184)		1.5 (0.09)	
Control	71.4% (247)		2.2 (0.19)		35.8% (124)		1.0 (0.08)	
Study group comparisons	OR	*p*-value	IRR	*p*-value	OR	*p*-value	Ordered OR	*p*-value
ASD vs. Control	2.11 (1.45–3.08)	<0.001***	1.89 (1.63–2.20)	<0.001***	7.43 (5.00–11.04)	<0.001***	7.69 (5.61–10.56)	<0.001***
ASD vs. Asthma	1.59 (1.00–2.52)	0.050*	1.50 (1.36–1.66)	<0.001***	4.10 (2.86–5.88)	<0.001***	4.12 (3.11–5.46)	<0.001***
Asthma vs. Control	1.33 (0.89–2.00)	0.165	1.26 (1.09–1.46)	0.003**	1.81 (1.29–2.55)	0.002**	1.87 (1.38–2.53)	<0.001***

Abbreviations: ASD, autism spectrum disorder; OR, odds ratio; Order OR, OR from ordered logistic regression, IRR, incident rate ratio.
[a]Mean (standard error [SE]) or % (*n*) calculated using imputed data.
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Supplemental analyses

We examined whether our results were moderated according to age group (3–5 years, 6–11 years, 12–17 years) or sex (see Tables S1, S2, S5, S6). In most cases we did not find moderation of our results. For one outcome, mean hours that parents were working, we found moderation by age group with increased hours worked for all three health groups as the children got older. Parents of youth with ASD in the two youngest age categories worked fewer hours than those with children in the other two health groups. In the age 12–17 category there were no statistically significant differences in the number of hours parents worked. We found that the analysis of mean hours of work for parents of male children was very similar to the overall analysis, with parents of youth with ASD working less than parents in either of the other groups. For female children, the pattern was different, with no difference between ASD and control groups and significantly lower hours worked for ASD compared with asthma groups. Analyses of the ASD group only found that in almost all analyses parents of children with more severe ASD (based on SRS score) experienced significantly greater impact on employment outcomes than parents of children with less severe ASD (see Tables S5 and S6).

DISCUSSION

Previous research has documented that having a child with ASD is associated with lower likelihood of parents being in the workforce (Cidav et al., 2012; McCall & Starr, 2016), reduced hours of work (Cidav et al., 2012; McCall & Starr, 2016), and decreased income compared with parents of children without ASD (Cidav et al., 2012; McCall & Starr, 2016). However, few studies have had data to directly link these differences with children's health or to provide detailed information on the specific ways in which ASD creates employment difficulties for parents. The r-Kids study was designed to add to this literature through prospectively collected data on a large sample of families during the same time period and the provision of additional detail about the specific ways in which employment is impacted over a 1-year period.

Similar to previous work, we found that parents of youth with ASD were less likely to work at all (Cidav et al., 2012; McCall & Starr, 2016) and tended to work fewer hours (Cidav et al., 2012; McCall & Starr, 2016) than parents of children with other health conditions or those without significant health concerns. We report a similar pattern of results to previous research, but our marginal estimates are modestly larger. Leaving the workforce or reducing work hours can impact families significantly. In the short run, dropping out of the workforce reduces income for families (Cidav et al., 2012; Earle & Heymann, 2014; McCall & Starr, 2016;

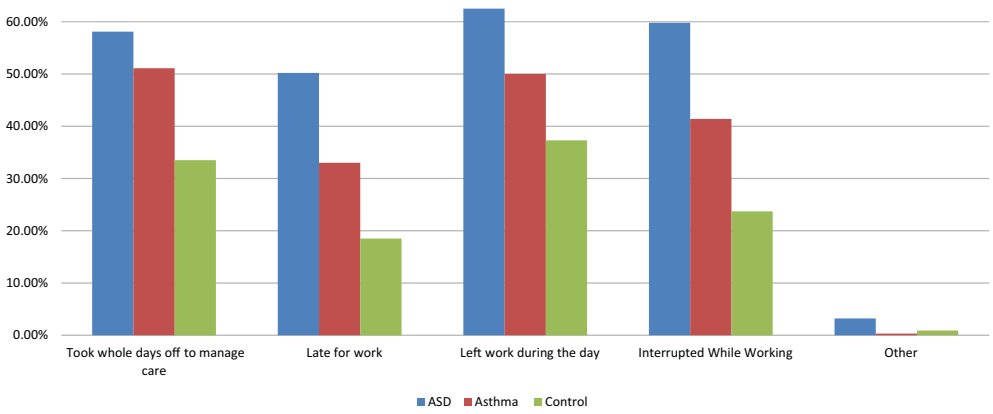


FIGURE 2 Types of productivity impacts related to child's health over 1 year

Montes & Halterman, 2008), while at the same time families of children with ASD or other chronic health conditions face higher than normal medical and other costs (Croen et al., 2006; Cummings et al., 2016; Kogan et al., 2008; Lavelle et al., 2014; Liptak et al., 2006), a combination that can increase the risk of financial difficulties for families. In the longer run, parents of children with ASD or other chronic conditions who remain out of the workforce for long periods due to heavy caregiving burdens risk a more limited career trajectory because this often occurs while the parent is in early adulthood, a critical time for career development and advancement. Thus, leaving the workforce and/or reducing hours of work can affect parents' future employability or career advancement, impacting family's long-term financial well-being.

In addition to leaving the workforce or reducing work hours, parents of children with special health care needs report other impacts on work such as missed time from work, interruptions while at work, or reduced ability to focus due to caregiving-related stress (Brannan et al., 2018; Brennan & Brannan, 2005). A few studies have documented the process by which parents may exit the labor force, in particular showing that parents of children with special needs are more likely to quit their jobs due to caregiving responsibilities or to have lost their jobs because of these responsibilities (Brannan et al., 2018; Houser et al., 2014; Rosenzweig et al., 2008). In the current study, we estimated the impact of parenting a child with ASD on job changes, missed time from work, and productivity while working, finding that across all outcomes there are greater negative impacts on employment for parents of children with ASD than for parents of children with asthma or controls.

These specific impacts could indicate places where workplace or public policy could help to support families of youth with ASD. Improving employment outcomes for parents of children with ASD, or other health conditions, will likely require policy changes at multiple levels. Some private employers are beginning to craft benefits programs that support parents with children with special needs, such as offering flexible time off or paid childcare (Adams, 2021; Schomer, 2021). Encouraging expansion of these programs could help parents of children with ASD or other special needs to cope with additional care demands. However, these types of private policies typically support higher income or professional employees which companies have a strong incentive to retain. Private employer policies are much less likely to improve outcomes for lower wage workers, as employers are less concerned with their recruitment and retention (Winston, 2014). Thus it is critical to also support and expand broad public policies such as mandatory paid time off, expansion of health insurance coverage and minimum benefits, greater access to inclusive childcare, and ASD responsive programs in education (Chau, 2010). These types of policies could provide support for a broader range of families by helping children to function better and could help parents of children with ASD function more easily at work and experience less work-related stress, potentially helping them to maintain employment and advance their careers.

In addition to examining whether employment impacts were significantly different for parents of youth with ASD compared with parents of youth who did not have ASD, we also wanted to determine whether these impacts were different for families of youth with another common chronic health condition, asthma. Among almost all outcomes analyzed, there was a significantly greater negative impact on employment outcomes for parents of youth with ASD. Furthermore, while the impacts were greater for families of children with asthma than for families in the control group, results were more closely aligned for these two groups. Thus, it is important to consider the impact of specific conditions as different health conditions may lead to very different parental employment outcomes.

All data for the r-Kids study were collected prior to the onset of the COVID-19 pandemic. Early reports indicate that families of children with ASD and other developmental disabilities have been particularly stressed during the pandemic (Friesen et al., 2021; Kalb et al., 2021). Working at home and managing education for children with ASD has been especially arduous for families (Friesen et al., 2021; Kalb et al., 2021), and these

difficulties likely also affected employment outcomes for working parents. Understanding a broad range of employment impacts during such crises is critical to development of equitable policies.

We followed families for 1 year to obtain employment outcomes for multiple seasons, across which these outcomes might differ due to seasonal issues (e.g., school related constraints). Most previous studies have depended on parent report of ASD, which could lead to misclassification of children. By contrast, we recruited r-Kids participants through health care systems, so we were able to confirm clinical diagnoses of ASD. We used an instrument, the FEII, that was specifically developed to measure the impact of a child's health on employment and other family costs; as a result, the survey focused more specifically on the ways in which child health impacts employment outcomes than measurements used in previous studies.

The r-Kids study has several limitations. We recruited participants from health care systems in five states and we oversampled children from historically underrepresented racial/ethnic groups. Because of these design features the sample may not be generalizable beyond these populations. Our study includes some employment outcomes not included in previous studies (e.g., impact of child health on parent work productivity) and more research is needed on the measurement of these outcomes. Because of the study design, we cannot demonstrate causation. Although we confirmed youth health conditions through clinical diagnoses and parental report, employment outcomes were based on parents' self-reports only.

CONCLUSIONS

Parents of children with ASD are vulnerable to multiple negative impacts on employment. Without intervention, these negative impacts likely place families of children with ASD at risk for financial strain, long-term loss of income, poorer career development, and difficulty balancing work and family. Understanding in greater detail how having a child with ASD impacts parental employment could aid in crafting more effective public and workplace policy. These findings underscore the need to design both health care and workplace policies that address the comprehensive burden of ASD.

AUTHOR CONTRIBUTIONS

Frances L. Lynch conceived the study; designed the survey; supervised the implementation of survey, recruitment and data collection, design of analyses, and interpretation of statistical analyses; and finalized the manuscript. John F. Dickerson helped to design the survey, oversaw data collection, design of analyses, conduct of analyses, and interpretation of statistical analyses, and contributed to the final manuscript. Joanna E. Bulkley helped to design the survey; supervised the implementation of survey, recruitment, and data collection; helped to design and interpret analyses and contributed to the final manuscript. Alexandra Varga helped to design the survey; helped to manage data for recruitment and survey data collection; helped to design, conduct, and interpret analyses; and contributed to the final manuscript. Phillip Crawford helped to design the survey, developed survey data collection process, and contributed to the final manuscript. Lisa A. Croen contributed to finalizing the survey, helped to manage data for recruitment, contributed to design and interpretation of analyses, and contributed to the final manuscript. Yihe G. Daida contributed to finalizing the survey, helped to manage data for recruitment, contributed to design and interpretation of analyses, and contributed to the final manuscript. Eric Fombonne contributed to finalizing the survey, contributed to the design of recruitment and survey data collection, contributed to design and interpretation of analyses, and contributed to the final manuscript. Brigit Hatch contributed to finalizing the survey, helped to manage data for recruitment and survey data collection, contributed to design and interpretation of analyses, and contributed to the final manuscript. Maria Massolo contributed to finalizing the survey, helped to manage data for recruitment, contributed to design and interpretation of analyses, and contributed to the final manuscript.

ACKNOWLEDGMENTS

This work was supported by the National Institute of Mental Health (NIMH) 1R01MH106680. The authors express their gratitude to Jill Pope for her editorial support as well as Jan Van Marter, Cassie Angus, and Mary Harper for their administrative support. We also thank the families who participated in the r-Kids study.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in NDAR at https://nda.nih.gov/, reference number C2468.

ETHICS STATEMENT

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all study participants prior to inclusion in the study.

ORCID

Frances L. Lynch https://orcid.org/0000-0002-0547-6812

Lisa A. Croen https://orcid.org/0000-0001-7849-9428

REFERENCES

Adams, T. (2021, November 21). Mental Health America (MHA). https://mhanational.org/blog/5-company-policies-support-working-parents

Ambler, O., Medford, E., & Hare, D. J. (2018). Parenting a child with phenylketonuria: An investigation into the factors that contribute to parental distress. *JIMD reports*, *41*, 91–100. https://doi.org/10.1007/8904_2018_105

American Psychiatric Association (APA). (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). Author.

Baker-Ericzén, M. J., Brookman-Frazee, L., & Stahmer, A. (2005). Stress levels and adaptability in parents of toddlers with and without autism spectrum disorders. *Research and practice for persons with severe disabilities*, *30*(4), 194–204.

Becker, G. S. (1981). *A treatise on the family*. Harvard University Press.

Bergstrom, T. (1996). Economics in a family way. *Journal of Economic Literature*, *34*, 1903–1934. http://www.jstor.org.ezproxy.uwindsor.ca/stable/2729597

Berman, P., Kendall, C., & Bhattacharyya, K. (1994). The household production of health: Integrating social science perspectives on micro-level health determinants. *Social Science and Medicine.*, *38*(2), 205–216 PMID: 8140447.

Berry, J. D., & Jones, W. H. (1995). The parental stress scale: Initial psychometric evidence. *Journal of Social and Personal Relationships.*, *12*, 463–472.

Bonis, S. (2016). Stress and Parents of Children with Autism: A Review of Literature. *Issues in mental health nursing*, *37*(3), 153–163. https://doi.org/10.3109/01612840.2015.1116030

Brannan, A. M., Brennan, E. M., Sellmaier, C., & Rosenzweig, J. M. (2018). Employed parents of children receiving mental health services: Caregiver strain and work-life integration. *Families in Society*, *99*(1), 29–44.

Brennan, E. M., & Brannan, A. M. (2005). Participation in the paid labor force by caregivers of children with emotional and behavioral disorders. *Journal of Emotional and Behavioral Disorders*, *13*, 237–246.

Bulkley, J. E., Varga, A. M., Dickerson, J. F., Crawford, P., Croen, L. A., Daida, Y. G., Fombonne, E., Hatch, B., Lee, A., Massolo, M., Vaugh, K., & Lynch, F. L. L. (2022). Measuring the cost of caring for children with autism Spectrum disorder: Design, methodology, and study population of the r-kids study. *Under review*, February.

Chau, M. (2010, February). Making Work Supports *Work*. National Center for Children in Poverty. https://www.nccp.org/publication/making-work-supports-work-tools-for-policy-analysis/

Cidav, Z., Marcus, S. C., & Mandell, D. S. (2012). Implications of childhood autism for parental employment and earnings. *Pediatrics*, *129*, 617–623. https://doi.org/10.1542/peds.2011-2700

Constantino, J. N., Davis, S. A., Todd, R. D., Schindler, M. K., Gross, M. M., Brophy, S. L., Metzger, L. M., Shoushtari, C. S., Splinter, R., & Reich, W. (2003). Validation of a brief quantitative measure of autistic traits: Comparison of the Social Responsiveness Scale with the autism diagnostic interview-revised. *Journal of autism and developmental disorders.*, *33*(4), 427–433.

Croen, L. A., Najjar, D. V., Ray, G. T., Lotspeich, L., & Bernal, P. (2006). A comparison of health care utilization and costs of children with and without autism spectrum disorders in a large group-model health plan. *Pediatrics*, *118*(4), e1203–e1211. https://doi.org/10.1542/peds.2006-0127

Cummings, J. R., Lynch, F. L., Rust, K. C., Coleman, K. J., Madden, J. M., Owen-Smith, A. A., Yau, V. M., Qian, Y., Pearson, K. A., Crawford, P. M., Massolo, M. L., Quinn, V. P., & Croen, L. A. (2016). Health services utilization among children with and without autism Spectrum disorders. *Journal of Autism and Developmental Disorders*, *46*(3), 910–920. https://doi.org/10.1007/s10803-015-2634-z

Dabrowska, A., & Pisula, E. (2010). Parenting stress and coping styles in mothers and fathers of pre-school children with autism and down syndrome. *Journal of Intellectual Disability Research*, *54*(3), 266–280.

Earle, A., & Heymann, J. (2014). Working conditions and parents' ability to care for children's preventive health needs. *Journal of Primary Care and Community Health*, *5*(2), 122–127. https://doi.org/10.1177/2150131913504590

Enders, C.K. (2010) *Applied Missing Data Analysis*. The Guilford Press, New York.

Everhart, R. S., Miller, S., Leibach, G. G., Dahl, A. L., & Koinis-Mitchell, D. (2018). Caregiver asthma in urban families: Implications for school absenteeism. *The Journal of School Nursing: The Official Publication of the National Association of School Nurses*, *34*(2), 108–113. https://doi.org/10.1177/1059840516689326

Friesen, K. A., Weiss, J. A., Howe, S. J., Kerns, C. M., & McMorris, C. A. (2021). Mental health and resilient coping in caregivers of autistic individuals during the COVID-19 pandemic: Findings from the families facing COVID study [published online ahead of print, 2021 Jul 8]. *Journal of Autism and Developmental Disorders*, *52*(7), 3027–3037. https://doi.org/10.1007/s10803-021-05177-4

Goodman, R. (2001). Psychometric properties of the strengths and difficulties questionnaire. *Journal of the American Academy of Child and Adolescent Psychiatry*, *40*(11), 1337–1345.

Harkness, S., & Super, C. M. (1994). The developmental niche: A theoretical framework for analyzing the household production of health. *Social Science & Medicine*, *38*(2), 217–226. https://doi.org/10.1016/0277-9536(94)90391-3

Houser, L., McCarthy, M., Lawer, L., & Mandell, D. S. (2014). A challenging fit: Employment, childcare, and therapeutic support in families of children with autism spectrum disorders. *Journal of Social Service Research*, *40*, 681–698. https://doi.org/10.1080/01488376.2014.930944

Kalb, L. G., Badillo-Goicoechea, E., Holingue, C., Riehm, K. E., Thrul, J., Stuart, E. A., Smail, E. J., Law, K., White-Lehman, C., & Fallin, D. (2021). Psychological distress among caregivers raising a child with autism spectrum disorder during the COVID-19 pandemic [published online ahead of print, 2021 Aug 7]. *Autism Research*, *14*, 2188.

Karst, J. S., & Van Hecke, A. V. (2012). Parent, and family impact of autism spectrum disorders: A review and proposed model for intervention evaluation. *Clinical Child and Family Psychology Review*, *15*(3), 247–277. https://doi.org/10.1007/s10567-012-0119-6

Ketelaar, M., Volman, M. J., Gorter, J. W., & Vermeer, A. (2008). Stress in parents of children with cerebral palsy: what sources of stress are we talking about?. *Child: care, health and development*, *34*(6), 825–829. https://doi.org/10.1111/j.1365-2214.2008.00876.x

Kogan, M. D., Strickland, B. B., Blumberg, S. J., & Singh, G. K. (2008). A national profile of the health care experiences and family impact of autism spectrum disorder among children in the United States, 2005–2006. *Pediatrics*, *122*, e1149–e1158. https://doi.org/10.1542/peds.208-1057

Lavelle, T. A., Weinstein, M. C., Newhouse, J. P., Munir, K., Kuhlthau, K. A., & Prosser, L. A. (2014). Economic burden of childhood autism spectrum disorders. *Pediatrics*, *133*, e520–e529. https://doi.org/10.1542/peds.2013-0763

Leiter, V., Krauss, M. W., Anderson, B., & Wells, N. (2004). The consequences of caring: Effects of mothering a child with special needs. *Journal of Family Issues*, *25*, 379–403.

Liptak, G. S., Stuart, T., & Auinger, P. (2006). Health care utilization and expenditure for children with autism: Data from U.S. national samples. *Journal of Autism and Developmental Disorders*, *36*, 871–879. https://doi.org/10.1007/s10803-006-0119-9

Loprest, P., & Davidoff, A. (2004). How children with special health care needs affect the employment decisions of low-income parents. *Maternal and Child Health Journal*, *8*(3), 171–182. https://doi.org/10.1023/b:maci.0000037650.83572.81

Lynch, F., & Dickerson, J. (2012). Validity and reliability of the family economic impact interview: A new instrument to measure costs

related to child mental health conditions (abstract). *Clinical Medicine & Research*, *10*(3), 181.

Lynch, F. L., Vuckovic, N., Schneider, J., & Firemark, A. J. (2007, July 8–11). Development of and preliminary results of a survey instrument to measure the family costs associated with child mental health conditions. *6th International Health Economics Association (IHEA) World Congress*, Copenhagen, Denmark.

Maenner, M. J., Shaw, K. A., Bakian, A. V., Bilder, D. A., Durkin, M. S., Esler, A., Furnier, S. M., Hallas, L., Hall-Lande, J., Hudson, A., Hughes, M. M., Patrick, M., Pierce, K., Poynter, J. N., Salinas, A., Shenouda, J., Vehorn, A., Warren, Z., Constantino, J. N., … Cogswell, M. E. (2021). Prevalence and characteristics of autism Spectrum disorder among children aged 8 years – autism and developmental disabilities monitoring network, 11 sites, United States, 2018. *Morbidity and Mortality Weekly Report*, *70*(11), 1–16. https://doi.org/10.15585/mmwr.ss7011a1

McCall, B. P., & Starr, E. M. (2016). Effects of autism spectrum disorder on parental employment in the United States: Evidence from the National Health Interview Survey. *Community, Work & Family*, *21*, 367–392. https://doi.org/10.1080/13668803.2016.1241217

Montes, G., & Halterman, J. (2008). Association of childhood autism spectrum disorders and loss of family income. *Pediatrics*, *121*, e821–e826. https://doi.org/10.1542/peds.2007-1594

Pastor-Cerezuela, G., Fernández-Andrés, M. I., Tárraga-Mínguez, R., & Navarro-Peña, J. M. (2016). Parental stress and ASD: Relationship with autism symptom severity, IQ, and resilience. *Focus on Autism and Other Developmental Disabilities*, *31*(4), 300–311.

Picardi, A., Gigantesco, A., Tarolla, E., Stoppioni, V., Cerbo, R., Cremonte, M., Alessandri, G., Lega, I., & Nardocci, F. (2018). Parental burden, and its correlates in families of children with autism Spectrum disorder: A multicentre study with two comparison groups. *Clinical Practice and Epidemiology in Mental Health*, *31*(14), 143–176.

Porterfield, S. L. (2002). Work choices of mothers in families with children with disabilities. *Journal of Marriage and Family*, *64*, 972–981.

Powers, E. T. (2003). Children's health and maternal work activity: Estimates under alternative disability definitions. *The Journal of Human Resources*, *38*, 522–556. https://doi.org/10.2307/1558767

Richard, P., Gaskin, D. J., Alexandre, P. K., Burke, L. S., & Younis, M. (2014). Children's emotional and behavioral problems and their mothers' labor supply. *Inquiry*, *51*, 0046958014557946. https://doi.org/10.1177/0046958014557946

Rosenzweig, J. M., Brennan, E. M., Huffstutter, K., & Bradley, J. R. (2008). Child care and employed parents of children with emotional or behavioral disorders. *Journal of Emotional and Behavioral Disorders.*, *16*(2), 78–89. https://doi.org/10.1177/1063426607312538

Schomer, S. (2021, November 4). Early mornings, late nights: How this working mom juggles business and parenthood. Benefit News. https://www.benefitnews.com/news/how-flexible-workplace-policies-can-support-working-parents

Silva, N., Crespo, C., Carona, C., & Canavarro, M. C. (2015). Mapping the caregiving process in paediatric asthma: Parental burden, acceptance and denial coping strategies and quality of life. *Psychology & Health*, *30*(8), 949–968. https://doi.org/10.1080/08870446.2015.1007981

Snow Jones, A., Miller, D. J., & Salkever, D. S. (1999). Parental use of alcohol and children's behavioural health: A household production analysis. *Health Economics*, *8*(8), 661–683. https://doi.org/10.1002/(sici)1099-1050(199912)8:8<661::aid-hec481>3.0.co;2-o

Stewart, L., Stutz, H., & Lile, W. (2018). The continuum of dependent family care: A theoretical explanation and model. *Community, Work & Family*, *21*(5), 599–619. https://doi.org/10.1080/13668803.2018.1530637

Varni, J. W., Seid, M., & Kurtin, P. S. (2001). PedsQL 4.0: Reliability and validity of the pediatric quality of life inventory version 4.0 generic core scales in healthy and patient populations. *Medical Care*, *39*(8), 800–812. https://doi.org/10.1097/00005650-200108000-00006

Winston, P. (2014, March). Work-Family Supports for Low-Income Families: Key Research Findings and Policy Trends. Office of Human Services Policy, US Department of Health and Human Services, Office of the Assistant Secretary for Planning and Evaluation. https://aspe.hhs.gov/reports/work-family-supports-low-income-families-key-research-findings-policy-trends-0

Yeatts, K. B., Stucky, B., Thissen, D., Irwin, D., Varni, J. W., DeWitt, E. M., Lai, J.-S., & DeWalt, D. A. (2010). Construction of the Pediatric Asthma Impact Scale (PAIS) for the patient-reported outcomes measurement information system (PROMIS). *The Journal of Asthma*, *47*(3), 295–302.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Lynch, F. L., Bulkley, J. E., Varga, A., Crawford, P., Croen, L. A., Daida, Y. G., Fombonne, E., Hatch, B., Massolo, M., & Dickerson, J. F. (2023). The impact of autism spectrum disorder on parent employment: Results from the r-Kids study. *Autism Research*, *16*(3), 642–652. https://doi.org/10.1002/aur.2882

ARTICLE

The Lifetime Distribution of the Incremental Societal Costs of Autism

Michael L. Ganz, MS, PhD

Objective: To describe the age-specific and lifetime incremental societal costs of autism in the United States.

Design: Estimates of use and costs of direct medical and nonmedical care were obtained from a literature review and database analysis. A human capital approach was used to estimate lost productivity. These costs were projected across the life span, and discounted incremental age-specific costs were computed.

Setting: United States.

Participants: Hypothetical incident autism cohort born in 2000 and diagnosed in 2003.

Main Outcome Measures: Discounted per capita incremental societal costs.

Results: The lifetime per capita incremental societal cost of autism is $3.2 million. Lost productivity and adult care are the largest components of costs. The distribution of costs over the life span varies by cost category.

Conclusions: Although autism is typically thought of as a disorder of childhood, its costs can be felt well into adulthood. The substantial costs resulting from adult care and lost productivity of both individuals with autism and their parents have important implications for those aging members of the baby boom generation approaching retirement, including large financial burdens affecting not only those families but also potentially society in general. These results may imply that physicians and other care professionals should consider recommending that parents of children with autism seek financial counseling to help plan for the transition into adulthood.

Arch Pediatr Adolesc Med. 2007;161:343-349

AUTISM IS A VERY EXPENSIVE disorder costing our society upwards of $35 billion in direct (both medical and nonmedical) and indirect costs to care for all individuals diagnosed each year over their lifetimes.[1] Given the financial and nonfinancial costs we face and given increasingly more options for treatment and possibly for prevention, information on the distribution of costs is needed to help us decide on how to best allocate scarce resources to support individuals with autism and their families. Because the complementary (or competing) treatment and prevention strategies currently available, or yet to be developed, vary in effectiveness or implementation costs, understanding how total costs due to autism are distributed across the life cycle is important to make better decisions.

Relatively little is known about the societal costs of autism, in total and at different points across the life cycle. In earlier work, the per capita and total societal costs for individuals with autism were described.[1] Although the per capita and societal costs were described overall and across 17 components of direct medical, direct nonmedical, and indirect costs, age-specific costs were not. Because certain categories are more relevant and more costly and because these costs are borne by different parties at different ages, presenting the age distribution of the costs of autism can provide policy makers information that is helpful for cost-utility analyses and for current and future resource planning activities. The focus of this study is to present estimates of the costs of autism along with some detail on how the estimates were constructed. Although no clinical data are presented, these data should be useful to health care professionals, families, and agencies in planning for future care, especially with respect to nonmedical costs.

Author Affiliations: Abt Associates Inc, Lexington, and Harvard School of Public Health, Boston, Mass.

METHODS

A detailed description of the sources of data and computational methods used to compile the costs of autism has been presented elsewhere.[1] Briefly, cross-sectional cost data from different age groups were used to create prevalence-based cost estimates that approximate incidence-based estimates (ie, those constructed by longitudinally tracking an incident cohort over time). A prevalence-based cohort, also known as a synthetic, or hypothetical, cohort,[2] allows us to approximate the lifetime experiences of a single incident cohort by using the prevalence-based cost patterns as if

they were observed longitudinally from an incident cohort. Although an incidence-based cost-of-illness approach is more appropriate because it captures the full experience of autism, including any comorbid conditions, formidable data requirements preclude it.[3]

The total costs of autism equal the sum of its direct and indirect costs. Direct costs measure the value of goods and services used and indirect costs measure the value of lost productivity due to autism. These direct and indirect costs represent the value of other activities that these resources could have purchased (ie, opportunity costs).[4,5] Physician and other professional services, hospital and emergency department services, drugs, equipment and other supplies, and medically related travel and time costs are typical components of direct medical costs. Direct medical costs were obtained either from the literature or from an analysis of the Medical Expenditure Panel Survey (MEPS)[6] and the National Health Interview Survey (NHIS).[7] Special education, transportation, child care and babysitting, respite care, out-of-home placement, home and vehicle modifications, and supported employment services are typical components of direct nonmedical costs. Nonmedical costs were obtained from the literature. Multiple cost estimates within categories were averaged to obtain a single cost estimate for each category. Indirect costs are the value of lost or impaired work time (income), benefits, and household services of individuals with autism and their caregivers because of missed time at work, reduced work hours, switching to a lower-paying but more flexible job, or leaving the workforce. Indirect costs were computed using a human capital approach[3,8] that combines average earnings, benefits, and household services with information on average work-life expectancies and labor force participation rates for men and women at different ages.

In the analyses that follow, the incremental costs of autism are presented, which are defined as those additional costs that are due exclusively to autism. For example, costs due to use of medical services for periodic well-child preventive care or care related to the common cold are not considered herein because those costs are common to children with and without autism; however, costs specifically due to autism are considered herein. When incremental costs were not available or otherwise specifically presented in the source materials, they were computed by subtracting national average costs calculated from the MEPS from the costs reported in the source documents. For example, if a source document presented an average cost of $\$X$ for all children with autism and the national average for all children for that same category was $\$Y$, then the incremental cost was computed as $\$(Y–X)$. Because of the broad impact of autism on families, insurers, taxpayers, and society and because of the considerable public autism funding, a societal perspective was used, as recommended by the Panel on Cost-effectiveness in Health and Medicine.[8]

The Harvard School of Public Health Human Subjects Committee had previously exempted this study from institutional approval.

DIRECT COSTS

Literature Review

An in-depth targeted literature review concentrating on US-based studies was conducted to obtain data on use and costs. British and Canadian studies were also used when data were otherwise unavailable. Data on physician, outpatient, clinic services, dental care,[9] prescription medications,[9-11] complementary and alternative therapies,[12-18] behavioral therapies,[19-22] hospital and emergency services,[9,23] allied health, equipment and supplies, home health,[9] and medically related travel[9] were classified as direct medical. Data on child care,[9,19] adult care,[19,20] respite and family care,[9,19,20] home and care modifications,[9,24] special education,[19,20,25-27] supported employment,[20,28-34] and other costs[9,24] were classified as direct nonmedical. Although some dimensions of care may be misclassified between direct medical and direct nonmedical (for example, many special education programs provide behavioral therapies), because the degree of misclassification is not known, no corrections were made. Costs, as reported in the source materials, were inflated to 2003 US dollars using the all-item consumer price index.[35] State-specific costs were transformed to national averages[36] and foreign costs were converted to US costs using the latest available Federal Reserve exchange rates.[37] Use measures were translated to costs by multiplying the use measures by age group–specific survey-adjusted average costs from the MEPS.[6] More in-depth information on how the cost estimates were constructed from these sources is available elsewhere[1] and in a technical appendix available on request.

Survey Analysis

Data from the NHIS[7] and the MEPS[6] were also used to supplement data on costs of autism and to also compute average costs for use in deriving the incremental costs of autism. Because confidentiality concerns constrain the MEPS to only report the first 3 digits of diagnosis codes, individuals with an *International Classification of Diseases, Ninth Revision* (*ICD-9*) diagnosis code of 299, which includes autism diagnoses (299.0*x*) as well as disintegrative psychoses (299.1*x*) and early childhood psychoses (299.8*x*/299.9*x*), were used as proxies for individuals with autism. Specific autism questions were available in the NHIS during 1997-2000. Information from those questions was combined with an *ICD-9* diagnosis code of 299 in the NHIS and was linked to the MEPS to increase the number of usable cases. Survey-adjusted means for expenditures were then computed as described earlier. Further information is available elsewhere[1] and from the technical appendix.

INDIRECT COSTS

Productivity losses for people with autism were estimated by combining standard average work-life expectancies for all men and women taken from the economics literature (ages 23-57 years for men and 23-53 years for women),[34] with average income and benefits (from Tables 696 and 628 of the *Statistical Abstract of the United States*[36]) and estimates of age- and sex-specific labor force participation rates.[38] Average incomes are projected for future years based on estimated productivity growth rates[35] to estimate average total earnings and benefits at each age. These estimates are adjusted for the fact that while some adults with autism are unable to work, others are (35% of adults with lower levels of disability and 10% of adults with higher levels of disability work in supported work environments). Finally, the lost value of sex-specific household services is added.[3,39] These estimates do not account for the effects of taxes or lost leisure time. Similar methods were used to estimate productivity for parents. Fathers of children with lower levels of disability were assumed to be unemployed 10% of the time (and working full-time during the remaining 90%) and mothers were assumed to be unemployed 55% of time (and were working half-time 25% of the time and full-time, 20%).[40,41] Fathers of children with higher levels of disability were assumed to be unemployed 20% of the time and mothers were assumed to be unemployed 60% of time (and were working half-time 30% of the time and full-time, 10%). These assumptions were combined with the same average earnings, benefits, productivity growth, labor force participation rates as used for individuals with autism, and the appropriate work-life expectances. These estimates assumed households in which both a mother and a father care for 1 child with autism. These estimates will differ based on different family configurations.

Table 1. Age-Specific and Lifetime per Capita Incremental Societal Costs of Autism*

Age Group, y	Average Per Capita Cost per Age Group			Total Per Capita Cost
	Direct Medical	Direct Nonmedical	Indirect	
3-7	35 370	10 805	43 066	446 203
8-12	6013	15 708	41 138	314 297
13-17	5014	13 550	38 453	285 082
18-22	2879	10 720	36 090	248 446
23-27	1574	27 539	51 740	404 260
28-32	1454	23 755	35 757	304 828
33-37	1389	20 492	30 852	263 662
38-42	1283	17 676	29 132	240 457
43-47	1440	15 248	26 600	216 439
48-52	1447	13 152	24 531	195 650
53-57	1290	11 292	17 776	151 790
58-62	1218	9489	0	53 535
63-66	1027	7908	0	35 738
Total lifetime costs	305 956	978 761	1 875 667	3 160 384

*Costs presented in 2003 dollars. Costs for age 4 years and older are discounted to 2003 dollars using a discount rate of 3%. Life expectancy for men is age 66 years and for women, age 65 years.

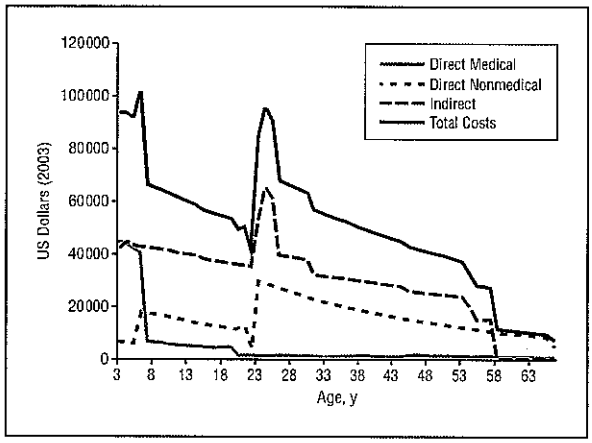


Figure 1. Age distribution of incremental societal costs of autism (present value).

CALCULATING COSTS

To the extent possible, cost estimates were derived for higher- and lower-functioning individuals as they were presented in the literature. Semidependent, independent, or those individuals described as having high-functioning autism were classified in the higher-functioning category. Dependent individuals or those not described as having high-functioning autism were classified in the lower-functioning category. Based on data presented in Fombonne,[42] the prevalence of higher-functioning autism is assumed to be 54%. The male-female ratio is assumed to be 4:1. Weighted average per capita costs were computed based on the assumed distribution of lower- and higher-functioning status and the male-female ratio. Age 3 years was considered to be the baseline age (age at diagnosis) and 2003 was the baseline year. Because there is some evidence that people with autism have reduced life expectancies,[43-46] costs were tabulated through age 66 years for males and through age 65 years for females. Costs were discounted to present value (to age 3 years) using a discount rate of 3% as recommended by the Panel on Cost-effectiveness in Health and Medicine.[8] Costs in future years were discounted, or deflated, to reflect the time value of money: a dollar today is worth more than a dollar in the future. In doing so, all costs were adjusted for the different periods in which they were incurred. In other words, dollars at different ages become comparable. Because health care resource investments, such as in the case of autism research and treatment budgets, incur costs in the present and potentially realize the benefits in the future, it is common to discount future flows of costs (and benefits) to present value. Although 3% is the currently used standard for a discount rate, this rate is varied in the sensitivity analyses described in the next subsection.

SENSITIVITY ANALYSES

In previous work, the robustness of the overall cost estimates was assessed using 1-way sensitivity analyses and conclusions were mostly robust to changes in many key parameters.[1] However, the total costs were found to be most sensitive to changes in the discount rate and to changes in the assumed level of indirect costs. Because variations in indirect costs will not substantially change the pattern of costs over the life cycle, herein focus is placed on the discount rate.[8] The discount rate is varied between 2% and 5% as suggested by Gold et al.[8]

DEFINITION OF AUTISM

Many of the sources of data simply used the term *autism* and did not differentiate between the different autism spectrum disorders. Reflecting the literature, the term *autism* herein is used in an inclusive manner to mean all disorders in the spectrum. Given the nature of many of the nonmedical and indirect costs, it is likely that those costs are more representative of more disabled individuals. Older sources[9] may have only included lower-functioning children and individuals in their definitions of autism. However, varying the proportions of lower- and higher-functioning individuals does not substantially change conclusions about overall lifetime costs.[1]

RESULTS

In the Tables that follow, the average per capita costs by category are presented in 5-year intervals (the full Tables

Table 2. Age-Specific and Lifetime per Capita Incremental Societal Direct Costs of Autism*

Age Group, y	Average per Capita Cost per Age Group						
	Physician and Dental	Drugs	CAM Therapies	Behavioral Therapies	Emergency and Hospital	Home Health	Travel
3-7	1147	147	198	32 501	828	467	81
8-12	577	153	109	4033	768	303	70
13-17	435	131	50	3479	591	267	60
18-22	426	129	33	1254	852	132	52
23-27	496	124	28	0	774	106	45
28-32	507	114	25	0	682	87	39
33-37	547	98	21	0	598	93	33
38-42	540	84	18	0	522	90	29
43-47	765	72	16	0	426	137	25
48-52	845	61	14	0	352	154	21
53-57	851	52	12	0	292	65	18
58-62	810	44	10	0	323	14	16
63-66	632	34	9	0	301	39	14
Total lifetime costs	42 259	6180	2704	206 337	36 235	9738	2503

Abbreviation: CAM, complementary and alternative medicine.
*Costs presented in 2003 dollars. Costs for age 4 years and older are discounted to 2003 dollars using a discount rate of 3%. Life expectancy for men is age 66 years and for women, age 65 years.

Table 3. Age-Specific and Lifetime per Capita Incremental Societal Direct Nonmedical Costs of Autism*

Age Group, y	Average per Capita Cost per Age Group						
	Child Care	Adult Care	Respite Care	Home Improvements	Special Education	Supported Work	Other
3-7	4636	0	1100	161	4585	0	323
8-12	3999	0	948	139	10 343	0	278
13-17	3450	0	818	120	8922	0	240
18-22	2907	0	706	10	6247	0	851
23-27	0	25 064	0	9	0	836	1630
28-32	0	21 620	0	8	0	721	1406
33-37	0	18 650	0	7	0	622	1213
38-42	0	16 087	0	6	0	537	1046
43-47	0	13 877	0	5	0	463	903
48-52	0	11 970	0	4	0	399	778
53-57	0	10 326	0	4	0	291	672
58-62	0	8907	0	3	0	0	579
63-66	0	7423	0	3	0	0	483
Total lifetime costs	74 963	662 192	17 858	2388	150 483	19 349	51 528

*Costs presented in 2003 dollars. Costs for age 4 years and older are discounted to 2003 dollars using a discount rate of 3%. Life expectancy for men is age 66 years and for women, age 65 years.

are available as eTables 1-4 at http://archpediatrics.com). **Table 1** and **Figure 1** display the incremental societal direct medical, direct nonmedical, and indirect costs. Direct medical costs are quite high for the first 5 years of life (average of around $35 000), start to decline substantially by age 8 years (around $6000), and continue to decline through the end of life to around $1000. Direct nonmedical costs vary around $10 000 to approximately $16 000 during the first 20 years of life, peak in the 23- to 27-year age range (around $27 500), and then steadily decline to the end of life to around $8000 in the last age group. Indirect costs also display a similar pattern, decreasing from around $43 000 in early life, peaking at ages 23 to 27 years (around $52 000), and declining through the end of life to $0.

Table 2 displays the individual components of the incremental societal direct medical costs. Considered over the entire life span, direct medical costs make up 9.7% of total discounted lifetime costs. Behavioral therapies, which are the largest component of direct medical costs, make up 6.5% of total discounted lifetime costs.[1] However, behavioral therapies, as presented herein, are only relevant for children 19 years or younger. The large direct medical costs early in life are driven primarily by behavioral therapies that cost around $32 000 during the first 5-year age group and decline from about $4000 in the 8- to 12-year age group to around $1250 for the 18- to 22-year age group. Physician and dental costs are initially high, then decrease, but increase again in later life. Prescription drugs, complementary and alternative therapies, and hospital and emergency services are also relatively

Table 4. Age-Specific and Lifetime per Capita Incremental Societal Indirect Costs of Autism*

Age Group, y	Average per Capita Cost per Age Group	
	Own Indirect	Not Own Indirect
3-7	0	43 066
8-12	0	41 138
13-17	0	38 453
18-22	0	36 090
23-27	32 703	19 036
28-32	32 620	3136
33-37	30 852	0
38-42	29 132	0
43-47	26 600	0
48-52	24 531	0
53-57	17 776	0
58-62	0	0
63-66	0	0
Total lifetime costs	971 072	904 595

*Costs presented in 2003 dollars. Costs for age 4 years and older are discounted to 2003 dollars using a discount rate of 3%. Life expectancy for men is age 66 years and for women, age 65 years.

high initially but steadily decline. Some costs decline less smoothly than others because of different availability of cost-by-age estimates in the literature.

Table 3 displays the individual components of the incremental societal direct nonmedical costs. Nonmedical costs, except during ages 3 to 7 years, are more expensive than direct medical costs and make up 31% of total discounted lifetime costs.[1] Different costs become relevant at different ages, which contributes to the dips and spikes in the direct nonmedical line in Figure 1. Child care and respite costs, which average about $5700 in early ages to around $3600 at ages 18 to 22 years, contribute far less (3% of total discounted lifetime costs) than adult care costs (21% of total discounted lifetime costs), which range from around $25 000 at ages 23 to 27 years to around $7400 at ages 63 to 66 years. Special education costs, which make up 4.8% of total discounted lifetime costs, range from around $12 000 at age 6 years (costs for ages 3-5 years are assumed to be zero) to around $6200 at ages 18 to 22 years, and supported employment costs range from around $800 at ages 23 to 37 years to around $300 at ages 53 to 57 years (age 57 years is the assumed end of working life).

Table 4 displays the components of the incremental societal indirect costs. Indirect costs are by far the largest component of the total incremental societal costs of autism (59.3% of total discounted lifetime costs).[1] Own indirect costs, which make up 30.7% of total discounted lifetime costs, range from around $33 000 at ages 23 to 27 years to around $18 000 at ages 53 to 57 years. Not own (assumed herein to be parents') indirect costs, which make up 28.6% of total discounted lifetime costs, range from around $43 000 at ages 3 to 7 years, when parents are assumed to be about 33 to 37 years of age, to around $19 000 at ages 23 to 27 years, when parents are assumed to be 53 to 57 years of age, to around $3000 per year for the next 5 years until the end of the average work life. Although total indirect costs spike at ages 23

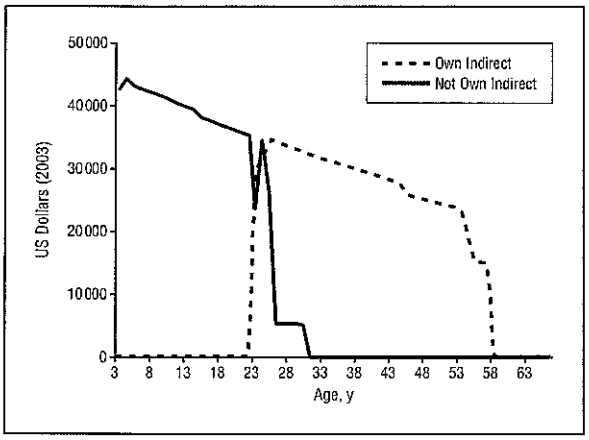


Figure 2. Age distribution of own and not own indirect incremental costs (present value).

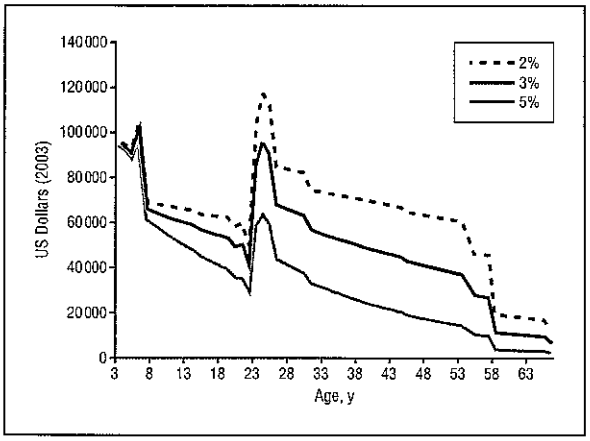


Figure 3. Age distribution of total incremental societal costs of autism computed at different discount rates.

to 27 years, because of the overlapping own and not own indirect costs, as **Figure 2** indicates, at any given time from age 3 years through age 57 years, there is a substantial and smoothly declining level of indirect costs. Figure 2 also dramatically illustrates, at least for this model, the transition from exclusive parental lost productivity almost immediately to lost own productivity.

SENSITIVITY ANALYSES

Sensitivity analyses using 2% and 5%, which are common upper and lower bounds, reveal that the patterns of age-specific expenditures are similarly shaped. **Figure 3** displays total costs using 2%, 3%, and 5% as the discount rates. There is an inverse relationship between the discount rate and the weight placed on future costs: lower discount rates place greater weight on future costs and higher rates place less weight on future costs. As a result, total present value costs will be larger the smaller the discount rate. The maximum difference in total costs between the 5% scenario and the 2% scenario (about $53 000) occurs at age 24 years and the average difference in costs between the 5% and 2% scenarios is about $31 000.

COMMENT

This article presents the first description, to my knowledge, of the societal costs of autism in the United States across all ages of the life span and contributes not only to the literature on the costs of autism but also to the literature on age-specific health care costs in general. As was previously reported, the total annual societal per capita cost of caring for and treating a person with autism in the United States was estimated to be $3.2 million and about $35 billion for an entire birth cohort of people with autism.[1] Sensitivity analyses revealed that these lifetime costs could range from $13 billion to $76 billion depending on the underlying assumptions of the model. Although those estimates are highly conservative because they exclude a number of important elements (such as legal costs that families incur to secure services[47,48]; lost productivity of those other than parents; the costs of genetic testing; the full costs of alternative therapies, including diets; the costs of adverse outcomes of potentially dangerous treatment modalities; and costs associated with immunization-avoidance behaviors[48]), they are valuable because they add information to a relatively underdeveloped literature. As treatment and, perhaps prevention, strategies are developed, knowledge of when costs are incurred relative to when benefits are expected is important for clinical decision-making and cost-effectiveness analysis efforts.

Knowledge about age-specific per capita incremental societal costs is particularly important because, as opposed to the summary lifetime data presented previously,[1,25,47] age-specific data illuminate the relative magnitudes of different types of costs at different ages. Given that at different ages different segments of society are responsible for absorbing these costs, this detailed disaggregation of costs can provide even more valuable information to planners, policy makers, and even to families making decisions that can affect current and future financial health, especially as they consider the fact that at various points in the life cycle different costs are more germane than others.

Although autism is typically thought of as a disorder of childhood, its costs can be felt well into adulthood. Adult care, which has the largest lifetime cost of all direct costs, is typically more than 5 times larger than the next 3 largest costs, which include care incurred during childhood (behavioral therapies, child/respite care, and special education). Alemayehu and Warner[49] reported that the typical American spends about $317 000 over his or her lifetime in direct medical costs, incurring 60% of those costs after age 65 years. In contrast, people with autism incur about $306 000 in incremental direct medical costs, implying that people with autism spend twice as much as the typical American over their lifetimes and spend 60% of those incremental direct medical costs after age 21 years.

These results, especially on the substantial costs resulting from lost productivity of both individuals with autism and their parents and from rather large adult care costs, have important implications for those aging members of the baby boom generation approaching retirement. As those individuals retire, many of their adult children with autism will be transitioning into adult care settings. Those costs, combined with very limited to nonexistent income for their adult children with autism combined with potentially lower levels of savings because of decreased income and benefits while employed, may create a large financial burden affecting not only those families but potentially society in general. Perhaps physicians and other care professionals should consider recommending that parents of children with autism seek financial counseling to help plan for the transition into adulthood.

Although this study is limited by a number of factors, it is the first of its kind, to my knowledge, and can shed insight into the lifetime distribution of autism costs and also motivate future, more rigorous studies. The cost model presented herein is based on a number of simplifying assumptions and relies on sometimes incomplete and old information. These caveats should be kept in mind when using these estimates for policy or practice decision making. The results presented herein for direct medical costs are consistent with recently published data on health care use and costs for children with autism. Gurney et al[50] reported that, relative to children without autism, children with autism, as reported by their parents, experience a significantly higher number of preventive visits and emergency and nonemergency hospital visits. Croen et al[51] reported, based on administrative data from the Northern California Kaiser Permanente Medical Care program, that children with autism incurred 2.5 times as much outpatient costs, 2.9 times as much inpatient costs, and 7.6 times as much medication costs as randomly selected children without autism. Pursuing a research agenda of both carefully and systematically documenting the costs of autism in the United States can be helpful in improving these estimates. Prospectively tracking the life experiences of individuals with autism and their families and obtaining a wide variety of data on the different sources of services for people with autism can provide this more complete picture. Prospectively collected clinical and quality-of-life data combined with cost data will be even more useful for understanding the societal costs, both financial and nonfinancial, of caring for those members of our society with autism at every age of the life course.

Accepted for Publication: November 16, 2006.
Correspondence: Michael L. Ganz, MS, PhD, Abt Associates Inc, 181 Spring St, Lexington, MA 02421 (mganz@hsph.harvard.edu).
Financial Disclosure: None reported.
Additional Information: eTables 1-4 are available at http://archpediatrics.com.

REFERENCES

1. Ganz ML. The costs of autism. In: Moldin SO, Rubenstein JLR, eds. *Understanding Autism: From Basic Neuroscience to Treatment.* Boca Raton, Fla: Taylor and Francis Group; 2006.
2. National Center for Health Statistics. NCHS definitions: synthetic cohort. December 16, 2004. http://www.cdc.gov/nchs/datawh/nchsdefs/syntheticcohort.htm. Accessed January 5, 2005.

3. Waitzman NJ, Scheffler RM, Romano PS. *The Costs of Birth Defects: Estimates of the Value of Prevention*. Lanham, Md: University Press of America, Inc; 1996.
4. Pindyck RS, Rubinfeld DL. *Microeconomics*. 5th ed. Upper Saddle River, NJ: Prentice Hall; 2000.
5. Rice DP, Hodgson TA, Kopstein AN. The economic costs of illness: a replication and update. *Health Care Financ Rev*. 1985;7:61-80.
6. Agency for Healthcare Research and Quality. The Medical Expenditure Panel Survey. http://www.ahrq.gov/data/mepsix.htm. Accessed January 3, 2005.
7. Centers for Disease Control and Prevention. The National Health Interview Survey. December 16, 2004. http://www.cdc.gov/nchs/nhis.htm. Accessed January 3, 2005.
8. Gold MR, Siegel JE, Russell LB, Weinstein MC, eds. *Cost-Effectiveness in Health and Medicine*. New York, NY: Oxford University Press; 1996.
9. Birenbaum A, Guyot D, Cohen HJ. *Health Care Financing for Severe Developmental Disabilities*. Washington, DC: American Association on Mental Retardation; 1990.
10. Aman MG, Van Bourgondien ME, Wolford PL, Sarphare G. Psychotropic and anticonvulsant drugs in subjects with autism: prevalence and patterns of use. *J Am Acad Child Adolesc Psychiatry*. 1995;34:1672-1681.
11. Martin A, Scahill L, Klin A, Volkmar FR. Higher-functioning pervasive developmental disorders: rates and patterns of psychotropic drug use. *J Am Acad Child Adolesc Psychiatry*. 1999;38:923-931.
12. Aman MG, Lam KS, Collier-Crespin A. Prevalence and patterns of use of psychoactive medicines among individuals with autism in the Autism Society of Ohio. *J Autism Dev Disord*. 2003;33:527-534.
13. Eisenberg DM, Kessler RC, Foster C, Norlock FE, Calkins DR, Delbanco TL. Unconventional medicine in the United States: prevalence, costs, and patterns of use. *N Engl J Med*. 1993;328:246-252.
14. Green VA, Pituch KA, Itchon J, Choi A, O'Reilly M, Sigafoos J. Internet survey of treatments used by parents of children with autism. *Res Dev Disabil*. 2006; 27:70-84.
15. Langworthy-Lam KS, Aman MG, Van Bourgondien ME. Prevalence and patterns of use of psychoactive medicines in individuals with autism in the Autism Society of North Carolina. *J Child Adolesc Psychopharmacol*. 2002;12: 311-321.
16. Levy SE, Mandell DS, Merhar S, Ittenbach RF, Pinto-Martin JA. Use of complementary and alternative medicine among children recently diagnosed with autistic spectrum disorder. *J Dev Behav Pediatr*. 2003;24:418-423.
17. Nickel RE. Controversial therapies for young children with developmental disabilities. *Infants Young Child*. 1996;8:29-40.
18. Yussman SM, Ryan SA, Auinger P, Weitzman M. Visits to complementary and alternative medicine providers by children and adolescents in the United States. *Ambul Pediatr*. 2004;4:429-435.
19. Hildebrand DG. *Cost-Benefit Analysis of Lovaas Treatment for Autism and Autism Spectrum Disorder (ASD)*. Vancouver, British Columbia: Columbia Pacific Consulting; 1999.
20. Jacobson JW, Mulick JA, Green G. Cost-benefit estimates for early intensive behavioral intervention for young children with autism—general model and single state case. *Behav Intervent*. 1998;13:201-226.
21. Lovaas OI. Behavioral treatment and normal educational and intellectual functioning in young autistic children. *J Consult Clin Psychol*. 1987;55:3-9.
22. McEachin JJ, Smith T, Lovaas OI. Long-term outcome for children with autism who received early intensive behavioral treatment. *Am J Ment Retard*. 1993; 97:359-372.
23. Walsh KK, Kastner T, Criscione T. Characteristics of hospitalizations for people with developmental disabilities: utilization, costs, and impact of care coordination. *Am J Ment Retard*. 1997;101:505-520.
24. Fujiura GT, Roccoforte JA, Braddock D. Costs of family care for adults with mental retardation and related developmental disabilities. *Am J Ment Retard*. 1994; 99:250-261.
25. Järbrink K, Knapp M. The economic impact of autism in Britain. *Autism*. 2001;5: 7-22.
26. Parrish T, Harr J, Wolman J, Anthony J, Merickel A, Esra P. *State Special Education Finance Systems, 1999–2000. Part II: Special Education Revenues and Expenditures*. Palo Alto, Calif: Center for Special Education Finance; 2004.
27. Yeargin-Allsopp M, Rice C, Karapurkar T, Doernberg N, Boyle C, Murphy C. Prevalence of autism in a US metropolitan area. *JAMA*. 2003;289:49-55.
28. Bureau of Labor Statistics. *Occupational Outlook Handbook, 2004-05 Edition*. Washington, DC: US Dept of Labor; 2004.
29. Capo LC. Autism, employment, and the role of occupational therapy. *Work*. 2001; 16:201-207.
30. Heal LW, McCaughrin WB, Tines JJ. Methodological nuances and pitfalls of benefit-cost analysis: a critique. *Res Dev Disabil*. 1989;10:201-212.
31. Keel JH, Mesibov GB, Woods AV. TEACCH-supported employment program. *J Autism Dev Disord*. 1997;27:3-9.
32. Mawhood L, Howlin P. The outcome of a supported employment scheme for high functioning adults with autism or Asperger syndrome. *Autism*. 1999;3:229-254.
33. Rusch FR, Conley RW, McCaughrin B. Benefit-cost analysis of supported employment in Illinois: a statewide evaluation. *Am J Ment Retard*. 1990;95: 44-54.
34. Skoog GR, Ciecka JE. The Markov (increment-decrement) model of labor force activity: extended tables of central tendency, variation, and probability intervals. *J Legal Econ*. 2001;11:23-87.
35. Congressional Budget Office. The budget and economic outlook: an update. August 2003. http://www.cbo.gov/showdoc.cfm?index=4493&sequence=3. Accessed January 4, 2005.
36. US Department of Commerce. *Statistical Abstract of the United States*. Washington, DC: Bureau of the Census; 2004.
37. Board of Governors of the Federal Reserve System. Foreign Exchange Rates Historical Data Series H.10. http://www.federalreserve.gov/releases/H10/hist/. Accessed January 4, 2005.
38. Congressional Budget Office. CBO's projections of the labor force. September 2004. http://www.cbo.gov/showdoc.cfm?index=5803&sequence=0. Accessed January 4, 2005.
39. American Academy of Pediatrics. The pediatrician's role in the diagnosis and management of autistic spectrum disorder in children. *Pediatrics*. 2001;107:1221-1226.
40. Butter EM, Wynn J, Mulick JA. Early intervention critical to autism treatment. *Pediatr Ann*. 2003;32:677-684.
41. Population Division. *Annual Estimates of the Population by Sex and Five-Year Age Groups for the United States: April 1, 2000 to July 1, 2003*. Washington, DC: US Census Bureau; 2004. www.census.gov/popest/national/asrh/NCEST2003/NC-EST2003-01.pdf. Accessed January 4, 2005.
42. Fombonne E. Epidemiological surveys of autism and other pervasive developmental disorders: an update. *J Autism Dev Disord*. 2003;33:365-382.
43. Fombonne E. The life expectancy of children diagnosed with a pervasive developmental disorder. *J Autism Dev Disord*. 2003;33:361.
44. Gillberg C. Outcome in autism and autistic-like conditions. *J Am Acad Child Adolesc Psychiatry*. 1991;30:375-382.
45. Shavelle RM, Strauss D. Comparative mortality of persons with autism in California, 1980-1996. *J Insur Med*. 1998;30:220-225.
46. Shavelle RM, Strauss DJ, Pickett J. Causes of death in autism. *J Autism Dev Disord*. 2001;31:569-576.
47. Maltby J. The costs of autism: more than meets the eye. *Advocate*. 2000;33(6): 12-16. http://www.autisminfo.com/Advocate.pdf. Accessed December 24, 2004.
48. Folstein SE, Rosen-Sheidley B. Genetics of autism: complex aetiology for a heterogeneous disorder. *Nat Rev Genet*. 2001;2:943-955.
49. Alemayehu B, Warner KE. The lifetime distribution of health care costs. *Health Serv Res*. 2004;39:627-642.
50. Gurney JG, McPheeters ML, Davis MM. Parental report of health conditions and health care use among children with and without autism: National Survey of Children's Health. *Arch Pediatr Adolesc Med*. 2006;160:825-830.
51. Croen LA, Najjar DV, Ray GT, Lotspeich L, Bernal P. A comparison of health care utilization and costs of children with and without autism spectrum disorders in a large group-model health plan. *Pediatrics*. 2006;118:e1203-e1211 http://pediatrics.aappublications.org/cgi/content/full/118/4/e1203.

Research in Autism Spectrum Disorders 72 (2020) 101502

Contents lists available at ScienceDirect

Research in Autism Spectrum Disorders

journal homepage: www.elsevier.com/locate/rasd

The lifetime social cost of autism: 1990–2029

Janet Cakir[a,*,1], Richard E. Frye[b], Stephen J. Walker[c]

[a] North Carolina State University, Raleigh, NC, United States
[b] University of Phoenix, Phoenix, AZ, United States
[c] Wake Forest School of Medicine, Winston Salem, NC, United States

ARTICLE INFO

Number of reviews completed is 2

Keywords:
Autism
Autism cost
Autism rates
Autism policy
US autism
ASD

ABSTRACT

This cost of illness analysis computes a baseline and future estimate of lifetime social costs associated with autism spectrum disorder (ASD) for the 50 states in the United States (US). The number of cases of ASD are estimated, then multiplied by annual direct and indirect medical and non-medical costs identified in the peer-reviewed literature. This amount is then extrapolated across the number of years each cost type is expected to be incurred to calculate a total lifetime cost for each state in the US from 1990–2019, and to project future cost for 2020–2029. From 1990–2019, there have been an estimated 2 million new cases of (ASD), with social costs of more than $7 trillion. If the future prevalence of ASD remains unchanged over the next decade, there will be an estimated additional 1 million new cases, resulting in an additional $4 trillion to the United States in social costs, however if the rate of increase in prevalence continues, costs could reach nearly $15 trillion by 2029. The financial burden of ASD is significant and identifying the modifiable causes of ASD has the potential to provide tangible benefits.

1. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that is behaviorally defined and includes impairments in social communication as well as stereotypical and/or repetitive and/or restrictive behaviors. Standard of care therapy for ASD commonly involves intensive (full-time) behavioral and educational therapy, with many children requiring life-long care, resulting in what others have characterized as substantial costs (e.g. Buescher, Cidav, & Knapp, 2014; Ganz, 2007; Horlin, Falkmer, Parsons, Albrecht, & Falkmer, 2014; Lavelle et al., 2014; Leigh & Du, 2015). There have been some attempts to estimate the social cost of ASD, and typically a standard method of '*average per capita cost*' is the focus (Leigh & Du, 2015). Others like Buescher et al. (2014); Ganz (2007), and Leigh and Du (2015) have calculated the national social costs of ASD as a function of (a) the estimated number of affected individuals, (b) severity, (c) the age at diagnosis, and (d) associated expenses for treatment and care.

The present study, by carefully reviewing the prior published methods used to estimate the social cost of ASD, presents an applied national cost analysis at the individual state level utilizing methods found in earlier analyses. This new model, designed to be geographically specific (i.e., accounting for cost differences from state-to-state), provides a baseline estimate for incurred past, and a projected future estimate of the lifetime social costs of ASD for each state in the United States, and for the nation, beginning in 1990 and projecting out to 2029.

* Corresponding author.
E-mail addresses: jyfergus@ncsu.edu (J. Cakir), rfrye@phoenixchildrens.com (R.E. Frye), swalker@wakehealth.edu (S.J. Walker).
[1] Dr. Cakir holds an adjunct appointment at North Carolina State University.

https://doi.org/10.1016/j.rasd.2019.101502
Received 13 June 2019; Received in revised form 23 December 2019; Accepted 26 December 2019
Available online 28 January 2020
1750-9467/ Published by Elsevier Ltd.

1.1. Per capita annual and lifetime costs associated with autism

Having a diagnosis of ASD carries with it a lifetime of direct and indirect costs (de la Cuesta, 2009; Ganz, 2007; Horlin et al., 2014; Knapp, Romeo, & Beecham, 2009). Recently, a review by Rogge and Janssen (2019) distinguished between six types of ASD-related costs: medical and healthcare related service costs, therapeutic costs, (special) education costs, costs of production loss for adults with ASD, costs of informal care and lost productivity for family/caregivers, and costs of accommodation, respite care, and out-of-pocket expenses. Most models used to estimate the cost of ASD attempt to either (1) characterize the lifetime social costs (Buescher et al., 2014; Ganz, 2006, 2007; Ganz, 2008; Knapp et al., 2009; Leigh & Du, 2015) or (2) illustrate the cost savings or benefits of a particular intervention associated with avoided expenses from reduced needs and improved outcomes (Chasson, Harris, & Neely, 2007; Horlin et al., 2014; Jacobson, Mulick, & Green, 1998).

It is difficult to assess if there is general agreement regarding the social cost of ASD because the earlier published studies do not have a uniform method (unit of measure) for reporting costs and, most importantly, each study has its own inherent assumptions that influence the final cost estimate. We explored methods used to calculate social costs of ASD in a variety of international studies; however, estimated costs for other nations have less relevance to the United States due to worldwide social, cultural, and regional differences in expenses that can influence the per capita lifetime social cost associated with ASD. For example, in contrast to studies of western societies that identify the largest source of cost as special education for children (Järbrink, 2007; Leigh & Du, 2015), and lost productivity (Horlin et al., 2014) or care for adults (Buescher et al., 2014; Ganz, 2007; Knapp et al., 2009; Nydén, Myrén, & Gillberg, 2008); a study conducted in China found the largest cost associated with an ASD is early intervention and behavioral therapy, which can exceed the per capita income of as many as 20 % of urban and 38 % of rural Chinese families (Wang et al., 2012).

Despite the heterogeneity, previously conducted studies have several key methodological strengths, such as the identification of costs dependent on the age of the individual (Buescher et al., 2014; Ganz, 2007; Jacobson et al., 1998; Leigh and Du, 2015), an average annual per capita cost (Horlin et al., 2014; Järbrink, 2007; Nydén et al., 2008) or lifetime per capita cost (Ganz, 2007; Järbrink & Knapp, 2001; Knapp et al., 2009), extrapolation to a population based on prevalence of the disorder (Buescher et al., 2014; Ganz, 2007; Jacobson et al., 1998; Järbrink & Knapp, 2001; Knapp et al., 2009; Leigh & Du, 2015), apportionment of costs based on severity or ID (Buescher et al., 2014; Ganz, 2007; Horlin et al., 2014; Knapp et al., 2009; Leigh & Du, 2015), and analysis of alternate future scenarios (Leigh & Du, 2015). This study borrows these methodological strengths and applies them to United States population-based estimates of individuals with ASD.

1.2. Number of autism cases

Because national per capita cost is multiplied by the number of cases in a society to derive total social cost, the estimated prevalence of existing, and incidence of new cases of affected individuals is a key determining factor in aggregated social cost for a specific nation or state. Most studies of the social cost of ASD estimate the total number of cases of ASD by calculating the number of individuals expressed as the percentage of population affected, or prevalence (Buescher et al., 2014; Knapp et al., 2009; Leigh & Du, 2015). The most recent studies by Buescher et al. (2014) and Leigh and Du (2015) both applied the prevalence or rate of ASD reported by the Centers for Disease Control (CDC), which Buescher et al. (2014) considered the most reliable and accepted estimates. Others have constructed cohorts, allowing for the approximation of lifetime costs for age groups (Buescher et al., 2014; Ganz, 2007; Knapp et al., 2009; Leigh & Du, 2015), which is important when there is changing rate over time, as has been reported by Van Naarden Braun et al. (2015).

There has been some disagreement concerning whether the rate of ASD is static or changing over time. Fombonne (2005) examined studies from 14 countries and found that the best estimate for the rate of ASD is 0.6 %, and Baxter et al. (2015) explored studies with samples up to 27 years of age and identified a global rate of 7.6 per 1000 (0.8 %); neither of these global studies was able to definitively reveal a change in prevalence of ASD over time. In the United States in 2007, surveys of parents reporting a diagnosis indicated the prevalence of ASD was 110 / 10,000 (1.1 %) (Kogan et al., 2009). This is within the range of a national surveillance program for 8-year-old children in metropolitan Atlanta that showed a rate of 4.2 per 1000 (0.4 %) in 1996, and 15.5 per 1000 (1.6 %) in 2010 (Van Naarden Braun et al., 2015). The ADDM network reported a prevalence of 14.7 per 1000 (1.47 %) of children aged 8 in reporting year 2010 (ADDM Network Principal Investigators, 2014) and 16.8 per 1000 (1.68 %) for reporting year 2014 among 8-year-old children (Baio, Wiggins, & Christensen, 2018). The most recent estimates of the prevalence of ASD from the National Health Interview Survey (NHIS), which was conducted by the National Center for Health Statistics and designed to yield a nationally representative sample, were reported as 2.76 % (27.6 in 1000) in 2016 (Zablotsky, Black, & Blumberg, 2017). Recently, Nevison, Blaxill, and Zahorodny, (2018) analyzed temporal trends of ASD prevalence using constant age tracking and age-resolved snapshots and found a strong, statistically significant upward trend over time in the State of California, which realized an increase of 1,000 % between 1931 and 2012. This study also reported statistically significant increases in ASD prevalence over time in one-half of the states in the ADDM network.

In the United States, each state reports to the United States Department of Education, which reports to Congress the number of students served under the Individuals with Disabilities Education Act of 2004 (IDEA), including students served under the classification of an ASD. Mandell and Palmer (2005) found that these state-reported cases of ASD were associated with education related spending and the unique education system characteristics of each state. These state- reported numbers do not capture those in private and home schools, but to the extent the education system in each state remains the same, the change in ASD reported over time may be able to shed light on changes in prevalence.

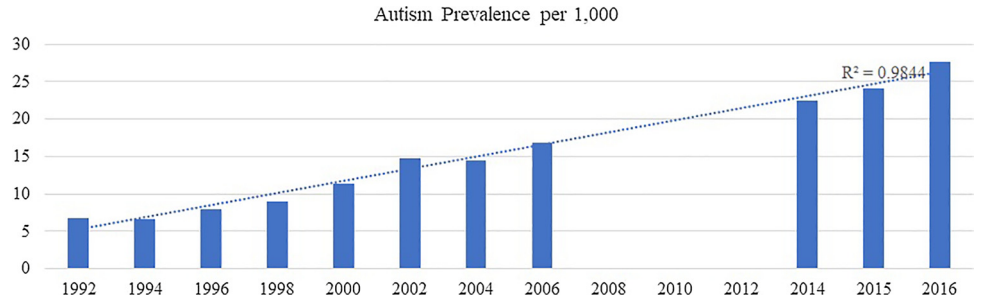


Fig. 1. Prevalence of ASD Over Time.

2. Methods

This study has two essential components: 1. Estimation of the number of cases of ASD for each state for each decade 1990–2019, and 2020–2029. 2. Estimation of the lifetime cost of ASD per person. To estimate the number of cases for each state, we computed an average national prevalence of ASD for each decade 1990–2019 using prevalence reported in CDC (2018) and Zablotsky et al. (2017) (also shown in Fig. 1), and applied it as a percentage to the total state-by-state, decade by decade population of children to estimate a number of individuals with ASD for each decade for each state. State decade estimates of the number of individuals with ASD were then multiplied by a lifetime per capita cost of the disorder based on reported annual average costs for additional medical and education needs, lowered productivity, and lower rates of independent living associated with an ASD. Like Leigh and Du (2015), we used scenario analysis to explore a base case of future percentage incidence identical to the prior decade, and an alternate scenario of increase in percentage incidence equal to the increase of past prevalence over the prior two decades using two separate data sources.

2.1. Total estimated cases of ASD

To calculate an estimated number of American children carrying an ASD diagnosis, we averaged the prevalence of ASD from federal monitoring programs, expressed it as a percentage, and applied it to the federal 2010 Census (United States Census Bureau, 2019a), and CDC WONDER population projections for the decades of 1990–2019 and 2020–2029 (CDC WONDER Online Database, 2005). CDC ADDM reported ASD prevalence per 1,000 for birth years between 1990 and 1999 are as follows: 6.7 (1992), 6.6 (1994), 8.0 (1996), and 9.0 (1998) for an average of 7.6 in 1,000 for United States children born in years 1990 – 1999. For the 2000s, reported prevalence by birth year was 11.3 (2000), 14.7 (2002), 14.6 (2004), and 16.8 (2006) (CDC, 2018) for an average prevalence of 14.4 in 1000 for birth years 2000 – 2009. The ADDM Network does not cover birth years 2010–2019 yet, so instead we applied the National Center for Health Statistics survey of ASD in children aged 3–17, which documented a prevalence of 22.4 (2014), 24.1 (2015), and 27.6 (2016) (Zablotsky et al., 2017), averaging to 24.7 for birth years 2010 – 2019. Fig. 1 graphs the United States estimates based on the ADDM (CDC, 2018) and NHIS (Zablotsky et al., 2017).

For our base case future scenario 2020–2029, we assumed the future incidence of ASD will remain unchanged from the average prevalence of the prior decade 2010–2019, and as an alternate scenario, we projected a future incidence of ASD based on the average rate of prevalence increase for each study decade, 1990–2019, (i.e. 180.77 % increase per decade). As a third scenario, we apply the

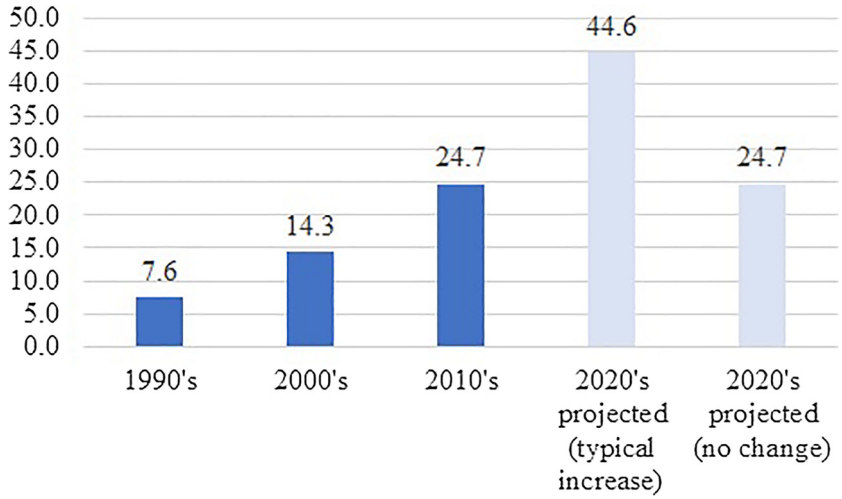


Fig. 2. Average prevalence of ASD per 1000 calculated from data from CDC (2018) and (Zablotsky et al., 2017).

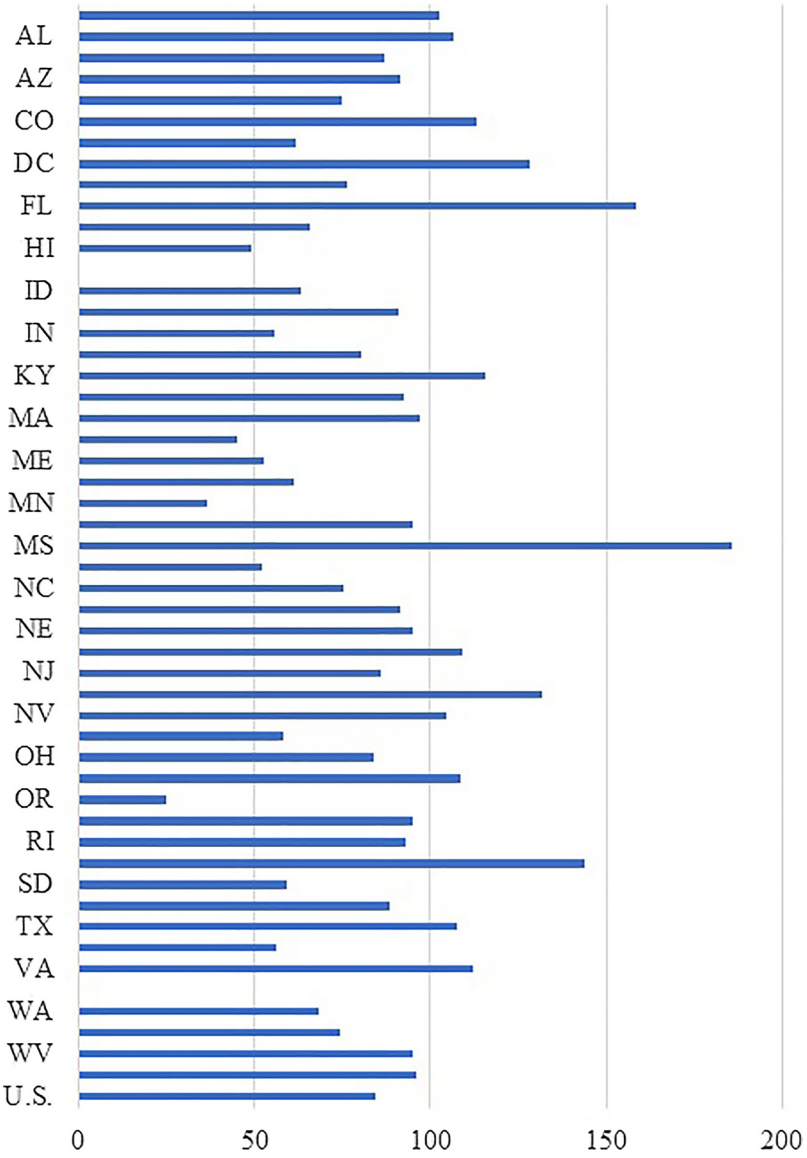


Fig. 3. Percent change in ASD 2008–2015 (United States Department of Education, 2017).

state-by-state rate of change between 2008 and 2015 from the United States Department of Education's 39th annual report to Congress on the implementation of IDEA, 2017 (United States Department of Education, 2017). Fig. 2 displays the decadal average prevalence of ASD and the projected future scenarios of incidence for the 2020′s (24.7 per 1000 for a baseline of no change, and 44.6 per 1000, assuming the historical rate of increase continues). Fig. 3 shows our state-by-state change in ASD scenario based on 2008–2015 change as reported by states for IDEA (United States Department of Education, 2017). The change for Iowa was less than .05 % and not reported, and Vermont did not provide data, so a 0 % change from 2010 to 2019 is applied for these states for this scenario so that a conservative and reasonable national aggregate can be computed. Table 1 lists the total population of children from the Census and CDC population projections and provides our estimated number of children with ASD for each state for each study decade 1990–2019. Table 2 provides the three alternate future scenarios for 2020–2029, reported as whole numbers.

2.2. Application of estimates for lifetime per capita cost

Estimating the social cost of ASD is a complex undertaking because the treatment and care of affected individuals is fragmented, taking place in medical, educational, and residential settings (Leslie & Martin, 2007). Others like Järbrink (2007) and Lavelle et al. (2014) have focused on the cost for children, or the cost of a specific need for either an adult or child (e.g. Liptak, Stuart, & Auinger, 2006; Shimabukuro, Grosse, & Rice, 2008; Vohra, Madhavan, & Sambamoorthi, 2017). Some of the cost for children or adults with an ASD can be found in government and non-profit organization reports related to a specific cost parameter (e.g. Chambers, Parrish, &

Table 1
Estimated Number of ASD Cases Based on Population and Average Prevalence for Each Decade 1990–2019.

State	Children Born				Estimated Autism Diagnoses		
	1990-1999	2000-2009	2010–2019	2020–2029	1990-1999	2000-2009	2010–2019
AL	663,126	613,186	594,849	618,790	5,040	8,830	14,693
AK	102,957	104,883	132,659	145,156	782	1510	3,277
AZ	910,246	909,395	1,229,605	1,487,522	6,918	13,095	30,371
AR	401,364	394,566	405,430	439,732	3,050	5,682	10,014
CA	5,414,870	5,037,172	6,096,218	6,206,327	41,153	72,535	150,577
CO	672,129	692,563	761,831	839,160	5,108	9,973	18,817
CT	491,099	424,677	466,796	455,493	3,732	6,115	11,530
DE	121,431	112,372	121,750	121,957	923	1,618	3,007
DC	64,960	58,760	65,276	58,145	494	846	1,612
FL	2,359,229	2,153,761	2,751,410	3,230,947	17,930	31,014	67,960
GA	1,399,683	1,381,946	1,603,712	1,801,993	10,638	19,900	39,612
HI	167,533	170,768	193,434	181,751	1,273	2,459	4,778
ID	232,314	242,967	250,213	269,901	1,766	3,499	6,180
IL	1,801,540	1,694,982	1,858,071	1,841,184	13,692	24,408	45,894
IN	927,686	878,896	907,911	949,875	7,050	12,656	22,425
IA	417,741	402,769	379,413	359,033	3,175	5,800	9,372
KS	402,705	407,939	399,047	392,373	3,061	5,874	9,856
KY	580,949	565,255	549,206	576,143	4,415	8,140	13,565
LA	633,615	620,622	641,217	645,405	4,815	8,937	15,838
ME	167,323	143,636	149,703	136,146	1,272	2,068	3,698
MD	785,270	731,356	924,920	968,761	5,968	10,532	22,846
MA	868,369	752,774	827,840	856,265	6,600	10,840	20,448
MI	1,414,815	1,234,070	1,390,491	1,332,435	10,753	17,771	34,345
MN	720,171	711,040	815,408	836,858	5,473	10,239	20,141
MS	432,867	416,628	395,721	393,531	3,290	5,999	9,774
MO	820,711	780,700	809,635	831,172	6,237	11,242	19,998
MT	127,848	123,188	120,147	113,272	972	1,774	2,968
NE	251,636	260,836	254,164	253,436	1,912	3,756	6,278
NV	365,773	370,555	503,795	618,599	2,780	5,336	12,444
NH	178,240	147,562	187,070	193,913	1,355	2,125	4,621
NJ	1,185,434	1,105,770	1,222,533	1,233,084	9,009	15,923	30,197
NM	291,552	288,289	279,372	252,816	2,216	4,151	6,900
NY	2,577,734	2,319,777	2,487,470	2,409,578	19,591	33,405	61,441
NC	1,290,695	1,267,985	1,476,349	1,750,081	9,809	18,259	36,466
ND	87,264	84,671	74,167	68,122	663	1,219	1,832
OH	1,598,381	1,468,745	1,504,189	1,462,851	12,148	21,150	37,153
OK	518,148	523,462	511,177	545,425	3,938	7,538	12,626
OR	497,413	474,770	564,057	628,944	3,780	6,837	13,932
PA	1,696,217	1,483,173	1,542,709	1,485,967	12,891	21,358	38,105
RI	143,870	117,888	140,482	133,381	1,093	1,698	3,470
SC	626,275	598,150	594,547	633,251	4,760	8,613	14,685
SD	111,588	115,152	108,756	108,589	848	1,658	2,686
TN	856,127	819,994	895,989	1,013,628	6,507	11,808	22,131
TX	3,765,007	3,856,707	4,547,455	5,130,534	28,614	55,537	112,322
UT	449,041	513,496	512,219	606,742	3,413	7,394	12,652
VT	83,649	66,606	75,378	71,773	636	959	1,862
VA	1,062,211	1,021,474	1,180,363	1,299,862	8,073	14,709	29,155
WA	900,361	869,534	977,872	1,099,440	6,843	12,521	24,153
WV	229,137	210,076	186,577	176,570	1,741	3,025	4,608
WI	775,136	727,060	767,196	742,678	5,891	10,470	18,950
WY	74,097	77,416	59,983	53,266	563	1,115	1,482
United States	42,717,537	40,550,019	45,495,782	48,061,857	324,653	583,920	1,123,746

Note. Population data from the 2010 United States Census (United States Census Bureau, 2019a) and CDC WONDER Online Database (CDC WONDER Online Database, 2005).

Harr, 2002). Recently, Rogge and Janssen (2019) developed a literature review of the economic costs of ASD, however no previous studies have developed a state-by-state estimate of the lifetime per capita cost, which is the goal of this study. Similar to others, we identified previously-published annual average per-capita incremental needs and costs above that of typical individuals conducted from 2000 to present, then multiplied each type of cost (medical, education, care, and productivity) by the number of appropriate years in childhood or adulthood similar to Buescher et al. (2014) and Knapp et al. (2009), to develop a per capita lifetime total cost on a state-by-state level.

To develop a state level estimate, we reviewed the literature for prior studies that estimated the cost of ASD associated needs, then inflated reported costs to 2019 dollars using the United States Department of Labor, Bureau of Labor Statistics Consumer Price Index Inflation Calculator, January to January adjustment. The calculator can be found at https://www.bls.gov/data/inflation_calculator.

Table 2
Estimated Number of ASD Cases for Each of Three Future Scenarios for 2020–2029.

State	Scenarios of Future Incidence		
	2020–2029 no increase in rate	2020–2029 consistent rate of increase	IDEA percent increase reported to congress compared to 2010–2019 estimates
AL	15,284	27,598	30,385
AK	3,585	6,474	6,645
AZ	36,742	66,343	58,131
AR	10,861	19,612	18,736
CA	153,296	276,802	263,660
CO	20,727	37,427	40,137
CT	11,251	20,315	18,655
DE	3,012	5,439	5,305
DC	1,436	2,593	3,683
FL	79,804	144,100	175,608
GA	44,509	80,369	65,637
HI	4,489	8,106	7,133
ID	6,667	12,038	10,099
IL	45,477	82,117	87,612
IN	23,462	42,364	34,894
IA	8,868	16,013	9,372
KS	9,692	17,500	17,781
KY	14,231	25,696	29,288
LA	15,942	28,785	30,472
ME	3,363	6,072	5,650
MD	23,928	43,207	33,126
MA	21,150	38,189	40,302
MI	32,911	59,427	55,433
MN	20,670	37,324	27,472
MS	9,720	17,551	27,915
MO	20,530	37,070	39,036
MT	2,798	5,052	4,514
NE	6,260	11,303	12,254
NV	15,279	27,590	25,460
NH	4,790	8,649	9,671
NJ	30,457	54,996	56,105
NM	6,245	11,276	15,982
NY	59,517	107,467	97,383
NC	43,227	78,054	63,888
ND	1,683	3,038	3,510
OH	36,132	65,243	68,437
OK	13,472	24,326	26,325
OR	15,535	28,051	17,443
PA	36,703	66,274	74,305
RI	3,295	5,949	6,707
SC	15,641	28,243	35,847
SD	2,682	4,843	4,282
TN	25,037	45,208	41,761
TX	126,724	228,822	233,518
UT	14,987	27,061	19,800
VT	1,773	3,201	1,862
VA	32,107	57,974	61,838
WA	27,156	49,035	40,674
WV	4,361	7,875	8,991
WI	18,344	33,123	33,086
WY	1,316	2,376	2,904
United States	1,187,128	2,143,559	$2,108,713

htm. We discuss the literature and data sources available to make transparent informed decisions on the average per-capita annual cost each of medical care, special education, adult care, and productivity loss, then multiply across number of years of need for a lifetime per-capita cost per affected individual. This is then multiplied by the number of estimated cases of ASD in each state and aggregated for a national estimate. Where possible, we applied the most up to date state-by-state cost data aggregated and reported by federal programs or public-private partnerships, non-profits, and foundations to produce an estimate of costs from needs that are being met.

2.2.1. Direct medical costs

The additional cost of medical care for individuals with an ASD has been reported in numerous studies that have documented the incremental cost reflective of a core set of medical needs. Vohra et al. (2017); Lavelle et al. (2014); Shimabukuro et al. (2008); Ganz

Table 3
Direct Medical Costs.

Incremental annual average per capita cost (2019 dollars)	Calculated expenses	Study dollars: Annual average per capita cost	Study dollar year	Adult / child	Author Reference
$8,077	In and Outpatient Hospital Pharmacy Physician Non-physician Emergency care Other health care Home healthcare	$6,132 (ASD) $860 (typical) $5,272 difference	1999	child	Liptak et al. (2006)
$21,425 (excluded)	Physician Dental Pharmacy CAM Therapy Hospital/Emergency Home Health and Supplies Early Intensive Behavioral Intervention	$15,466	2003	child	Ganz (2007)
$6,497	In and Outpatient Hospitals Pharmacy Physician Non-physician Medical equipment Facility fees Diagnostic services Laboratory services Speech and Occupational Therapy when covered by insurance	$4,110 low $6,200 high $4,690 (average amount above typical)	2003	Child (to age 21)	Shimabukuro et al. (2008)
$3,852	In and Outpatient hospital services Physician Nonphysician Home health care Pharmacy Vision Equipment and supplies Therapy	$3,020 (health care above typical) $350 (therapies) $3,370 total	2011	child	Lavelle et al. (2014)
$2,092	Physician Dental Pharmacy CAM Therapy Hospital/Emergency Home Health and Supplies	$1,510 (average of years 18+) (average amount above typical)	2003	adult	Ganz (2007)
$6,129	In and Outpatient hospitalizations Physician Emergency Pharmacy Other associated healthcare expenses	$13,700 (ASD) $8,560 (typical) $5,140 difference	2008	adult	Vohra et al. (2017)

(2007), and Liptak et al. (2006) all reported costs for inpatient and outpatient care, physician visits, hospitalizations, emergency medicine, and pharmacy costs. Leslie and Martin (2007) examined in and out-patient visits and pharmacy usage, however their reported costs are in total, not incremental to typically developing individuals. Other medical needs like medical supplies, therapy, laboratory and diagnostic services, facility fees, dental services, and other unspecified needs were included inconsistently across studies (detailed in Table 3).

Most studies reporting on the incremental cost of medical care for individuals with an ASD rely on surveys about private health insurance usage like the Medical Expenditure Panel Survey (MEPS), or surveys of health care providers, which include the National Ambulatory Medical Care Survey (NAMCS) and National Hospital Ambulatory Medical Care Survey (NHAMCS). Liptak et al. (2006) studied the MEPS, NAMCS and NHAMCS. Lavelle et al. (2014) and Ganz (2007) used the MEPS and National Health Interview Survey (NHIS), a survey of households. Shimabukuro et al. (2008) analyzed the MarketScan database of health insurance claims. Vohra et al. (2017) studied Medicaid claims.

Ganz (2007) provides a unique cost for each year of life, and when those are averaged by childhood (under 18) and adulthood (18 and older) the average is the highest reported. Ganz's estimate is also unique in reflecting the cost of medical need, as opposed to the cost of medical spending, or needs that have been met. Additionally, Ganz includes Early Intensive Behavioral Intervention (EIBI) in early childhood, while others do not.

There is some confusion between therapy and EIBI. Services that help you keep, learn, or improve skills and functioning for daily living, including speech-language pathology, physical, and occupational therapy, are called "habilitative services" (Healthcare.gov.,

2019) and are covered as an essential benefit under the Affordable Care Act (Centers for Medicare & Medicaid Services, 2019), however health insurance usually caps the number of therapy visits. Liptak et al. (2006), may have captured habilitative services under the category of "other" or non-physician services, Shimabukuro et al. (2008) included speech and occupational therapy in their estimate, Vohra et al. (2017) captured "all-cause" usage of insurance, and would have included habilitative services where covered, and Lavelle et al. (2014) provides an estimate for therapy as separate from health care, which we included in our analysis of direct medical costs for consistency. The childhood average calculated from Ganz's (2007) is an outlier, highlighting an important issue: the possibility of a treatment gap between costs that are being met, and the needs of individuals with ASD; and the difference between habilitative services which can include therapy, and EIBI, which is sometimes thought of as a therapy, and sometimes thought of as an educational approach. For this study, we exclude the Ganz (2007) childhood average estimate, but provide it as a reference in Table 3, maintaining the focus of this study on direct medical costs for the portion of needs that are being met, as opposed to unmet needs.

Future studies using similar approaches to those listed in Table 3 may reflect the higher EIBI costs due to changes that are now slowly unfolding in the insurance industry regarding coverage for ASD and EIBI. For example, the Blue Cross Blue Shield's standard benefit plan for Federal Employees covers, with pre-approval, Applied Behavioral Analysis (ABA), a form of EIBI, but denies coverage for ABA when offered as part of a school or educational program (Blue Cross Blue Shield, 2019). Another large health insurance provider, Cigna, covers ABA for autism, but denies coverage for other EIBIs, like Lovaas therapy (Cigna, 2019).

Early intensive behavioral interventions such as Lovaas Therapy, Discreet Trial Training, the Early Start Denver Model, and ABA, range in annual per-capita cost from $20,000 when there is parent involvement ($24,871 in 2019 dollars) (Chasson et al., 2007; Rogge & Janssen, 2019) to $60,000 in 2003 dollars ($83,119 in 2019 dollars) (Butter, Wynn, & Mulick, 2003; Rogge & Janssen, 2019). Though the cost of EIBI is high, studies show the costs are later offset with benefits in lower special education costs (Chasson et al., 2007; Rogge & Janssen, 2019). Further comprehensive studies similar to Jacobson et al. (1998), which measure how other later health, productivity, and care costs are impacted by EIBI could work toward incentivizing its coverage under health plans or the development of publicly available opportunities to make the treatment more accessible to children with an ASD.

To develop an incremental annual average per capita medical cost for this study, we took the adjusted 2019-dollar average of studies of children and multiplied by 15, assuming a diagnosis age of 3, and the average of adult studies and multiplied by 49, assuming a life expectancy of 67 years. Like Buescher et al. (2014), we apply life expectancy based on Shavelle and Strauss (1998) who projected that at the age of 5, ASD males would live, on average, another 62.0 years, and females another 62.5 years for an average life expectancy of 67 years. This means an adult will require another 49 years of medical care beyond the age of 18. Our review results indicate that for children with an ASD, an average of about $6,142 more is spent on medical care each year, and for adults the annual incremental average is $4,110.5. Across the individual's lifetime, this adds up to about $293,545 added cost for medical care associated with ASD.

2.2.2. Direct non-medical costs (education)

In the United States, the current average per pupil spending to educate a K-12 student is $12,201 for fiscal year 2017 ($12,647 in 2019 dollars) (United States Census Bureau, 2019b). Under IDEA, children with disabilities are legally entitled to a free and appropriate public education in the least restrictive setting. This means that a child with an ASD qualifies for special education instruction, which can be provided in a typical classroom with typically developing peers, or other setting as needed to meet minimal education needs (National Council on Disability, 2018). These costs are in addition to the typical cost of education.

The cost of special education would naturally be higher if a student has greater need, for example in children with multiple disabilities, as can be the case with individuals with an ASD. Buescher et al. (2014) reported a figure for education costs for children with an ASD both with, and without, an intellectual disability (ID), splitting the population in a 40/60 ratio. The rate of 40 % of individuals with a co-occurring intellectual disability is further supported by Van Naarden Braun et al.'s (2015) study of data from Atlanta Georgia. Others, however, report a rate as low as 31 % (Baio et al., 2018). For this study, we include a 40/60 (ID / No ID) weighted average to replicate Buescher et al. (2014) and apply Baio et al.'s 31/69 (ID / No ID) as a sensitivity analysis. Table 4 summarizes the literature concerning the cost of special education for children with an ASD.

The cost of special education for children with an ASD is less well studied than the cost of medical care, with greater variability

Table 4
Direct Non-Medical Costs (Education).

Incremental annual average per capita cost (2019 dollars)	Calculated Expenses	Study dollars: Annual average per capita cost	Study dollar year	Adult / child	Author Reference
$17,213	Special education cost in general	$11,543	2000	child	Chambers et al. (2002)
$11,031	Special Education cost	$7,963 (child)	2003	child	Ganz (2007)
$31,959 (child with ID) $15,980 (child without ID) $22,372 (weighted average 40/60) $20,932.80 (weighted 31/69)	Special education cost	$27,961 (child with ID 6–17) $13,980 (child without ID 6–17)	2011	child	Buescher et al. (2014)
$9,841	Additional total school cost	$8,610	2011	child	Lavelle et al. (2014)

across the estimates of cost. For our estimate, we averaged across the available studies. We apply a 40/60 split between ID/No ID, and a 31/69 split as a sensitivity analysis for the estimate calculated from Buescher et al. (2014). In our final average across estimates, we use the more generally accepted 40/60 split producing an annual per-capita special education cost of $15,114.25 per year. Across an assumed 13 years of special education need (K-12), the total estimated incremental per-capita education cost is $196,485. Applying a 31/69 ID/No ID ratio for Buescher et al. (2014) decreases the overall lifetime figure slightly by about 2 %.

2.2.3. Indirect costs (loss of productivity of individual and family members)

Indirect costs can include the loss of productivity of individuals with an ASD from unemployment, and loss of productivity of family members through decreased hours worked, lower wages, and shifted household duties. Ganz (2007) provided an annual average per capita estimate of $27,745 ($38,436 in 2019 dollars) for the lost work productivity across the population of affected individuals, assuming 45 % would be employed. Buescher et al. (2014) provides an estimate of $10,718 productivity loss of the individual with an ASD, however it is not reflective of only United States studies. More recent studies on rates of employment for the ASD population are 61.42 % (Ohl et al., 2017), 53.4 % (Roux et al., 2013), 55.1 % (Shattuck, Nerendorf, Cooper, Sterzing, & Wagner, 2012).

For lost productivity of caregivers due to lower household income, the lowest reported estimate of loss was based on a survey of children enrolled in afterschool programs collected as part of the National Household Education Survey of After School Programs and Activities in 2005 (Montes & Halterman, 2008). It is possible that the population of children with an ASD able to attend afterschool programs could be less severely affected overall, thus producing a lower estimate.

The highest estimate of lost productivity of family members is by Ganz (2007). The Ganz estimate is more complete and was developed based on the average income and benefits of the United States labor force, adjusted by age and sex, then applying assumptions regarding reduced employment dependent on level of disability, resulting in a metric that declines over time as the individual with an ASD ages. The estimate eventually turns to zero as the average work life expectancy of the parents is assumed to end when the individual with ASD reaches about 30 years old.

Lavelle et al. (2014) estimated the value of shifted household activities for parents of children with an ASD, however they did not estimate loss of family income, therefore we focused on other studies for a more complete estimate of indirect costs due to lost productivity. To assess the impact this may have on our final estimate, we performed a sensitivity analysis by adding Lavelle's value of shifted household activity to the two lost family income figures by Montes and Halterman (2008), and Cidav, Marcus, and Mandell (2012), then re-average the indirect costs for childhood years to estimate the impact the cost of shifted household activity has on the overall estimate of lifetime indirect costs. Table 5 summarizes the literature concerning the loss of productivity associated with an ASD.

For this study, we averaged estimates 2019 inflated estimates from Ganz (2007); Montes and Halterman (2008), and Cidav et al. (2012) ($28,668.67), and multiplied by 15 childhood years assuming diagnosis at age 3 for a childhood years family productivity loss of $430,030. Like Ganz (2007), we assumed retirement by caregivers of adults after an additional 12 more work years during the affected individual's adulthood, each year bringing losses valued at $31,042 for a total productivity loss for families of $372,504 during the affected individuals adulthood. For the loss of productivity of affected individuals with ASD, we adopt Ganz's estimate of 34 years of lost employment by each valued, on average, at $27,745 ($38,436 in 2019 dollars) for a total of $1,306,824 in 2019 dollars. Together, all these forms of lost productivity lead to an estimated total lifetime per capita indirect cost of $2,109,358.

In our sensitivity analysis, if we add the cost of shift in household activity as reported by Lavelle et al. (2014), which is $5089 ($5,817 in 2019 dollars) to Montes and Halterman (2008) and Cidav et al. (2012) which focused on lost income only, then re-calculate average annual per capita figures across the studies for childhood years, the lifetime total increases by 3 % to $2,167,528. The minimal effect on the overall figure indicates that shifts in household services are a minor factor in an overall large monetary loss due to lower wages, lost work hours, and lost employment for individuals with an ASD and their families. This does not mean, however, that this shift in household services has a minimal impact on the families for other, non-monetary reasons.

2.2.4. Direct non-medical costs (residential living supports: general care and accommodation)

In addition to the cost of medical care, individuals with an ASD and/or their families are faced with the cost of general care and accommodation for the individual with an ASD. In the United States, the Medicaid home and community-based services (HCBS) waiver program provides reimbursement for services to enable home-based care and accommodation when the individual would otherwise require institutional placement. HCBS waivers can fund supports such as home health, personal care, day care, habilitation, and respite care to enable family members to continue to provide in-home care for qualifying children and adults if the cost is less than institutional care (Medicaid & CHIP Payment & Access Commission, 2019).

In comparison to adults with other types of disabilities, a higher percentage of individuals with an ASD continue to live with a family member (Anderson, Shattuck, Cooper, Rou, & Wagner, 2014; Hewitt et al., 2017; Newman et al., 2011), and the percentage that live independently as young adults is as low as 17 % (Newman et al., 2011). In studies on the cost of ASD, Buescher et al. (2014) estimated that 1 % age five and below, 5 % under the age of 18, and 19 % of adults with an ASD are cared for in an intermediate care facility. Ganz (2007) and Buescher et al. (2014) based estimates for the cost of care on earlier studies that included Medicaid data, however more recent studies of the HCBS Waiver program enable an analysis of actual expenditures.

In the United States, as of 2009, there were 562,067 persons with a developmental and intellectual disability receiving HCBS waiver services (Lakin, Larson, Salmi, & Webster, 2010), and by 2017, HCBS 1915 (c) waiver spending for this population reached $34 billion. For this study, we assume that all adults with ASD and combined ID will need and qualify for an HCBS waiver through Medicaid, and we adopt the most recently documented per capita cost for HCBS waivers for the developmentally and intellectually

Table 5
Loss of Productivity of individual and family members.

Incremental annual average per capita cost (2019 dollars)		Study dollars: Annual average per capita cost	Study dollar year	Adult / child	Author Reference
$38,436 (adults with ASD) $56,640 (caregivers of children with ASD) $31,042 (caregivers of adults with ASD)	Lost work productivity of the individual. Lost contribution to household services. Lost employment by care giver.	$27,745 (adults with ASD) $40,886 (caregivers of children with ASD) $22,408 (caregivers of adults with ASD)	2003	Childhood and adult years	Ganz (2007)
$8,184	Loss of household income from families with children in an afterschool program	$6,200	2005	Childhood years	Montes and Halterman (2008)
$21,182	Lost employment and decreased wages.	$17,763 (families of a single child with an ASD)	2011	Childhood years	Cidav et al. (2012)

Table 6
HCBS Waiver Cost Per Enrollee With a Developmental and Intellectual Disability (Lakin et al., 2010; Musumeci et al., 2019).

State	HCBS spending per enrollee in 2009 dollars (Lakin et al. (2010)	HCBS spending per enrollee adjusted to 2019 dollars (Lakin et al. (2010)	HCBS spending per enrollee (2017 dollars) Musumeci et al. (2019)	HCBS spending per enrollee adjusted to 2019 dollars Musumeci et al. (2019)	Lifetime Per Capita Cost (2019 dollars)
AL	$49,859	$59,439	$63,600	$65,924	$3,230,273
AK	$64,017	$76,318	$78,700	$81,576	$3,997,209
AZ	$26,805	$31,956	no data	no data	no data
AR	$34,469	$41,092	$48,300	$50,065	$2,453,179
CA	$26,794	$31,943	$26,200	$27,157	$1,330,710
CO	$41,472	$49,441	$36,600	$37,937	$1,858,931
CT	$63,394	$75,574	$87,400	$90,594	$4,439,086
DE	$107,453	$128,099	$110,800	$114,849	$5,627,583
DC	$92,190	$109,903	$69,000	$71,521	$3,504,542
FL	$29,215	$34,828	$28,400	$29,438	$1,442,449
GA	$28,901	$34,454	$43,700	$45,297	$2,219,543
HI	$41,441	$49,403	$40,600	$42,084	$2,062,093
ID	$30,196	$35,997	$29,300	$30,371	$1,488,160
IL	$32,264	$38,463	$3,100	$3,213	$157,450
IN	$45,389	$54,110	$32,600	$33,791	$1,655,769
IA	$23,147	$27,595	$35,300	$36,590	$1,792,903
KS	$36,224	$43,184	$45,100	$46,748	$2,290,650
KY	$48,831	$58,214	$43,700	$45,297	$2,219,543
LA	$50,665	$60,399	$39,200	$40,632	$1,990,986
ME	$72,821	$86,813	$68,900	$71,418	$3,499,463
MD	$48,305	$57,586	$58,800	$60,949	$2,986,479
MA	$56,241	$67,048	$82,300	$85,307	$4,180,055
MI	$44,865	$53,486	$66,500	$68,930	$3,377,566
MN	$66,158	$78,869	$69,600	$72,143	$3,535,016
MS	$21,789	$25,975	$35,800	$37,108	$1,818,298
MO	$48,765	$58,135	$60,600	$62,814	$3,077,902
MT	$36,022	$42,944	$40,300	$41,773	$2,046,856
NE	$44,304	$52,817	$35,400	$36,694	$1,797,982
NV	$45,941	$54,769	$47,700	$49,443	$2,422,705
NH	$40,370	$48,126	$44,400	$46,022	$2,255,096
NJ	$54,142	$64,545	$56,500	$58,565	$2,869,661
NM	$71,517	$85,258	$70,300	$72,869	$3,570,569
NY	$69,752	$83,155	$70,300	$72,869	$3,570,569
NC	$45,697	$54,477	$52,300	$54,211	$2,656,341
ND	$22,467	$26,784	$38,200	$39,596	$1,940,196
OH	$44,208	$52,702	$45,300	$46,955	$2,300,808
OK	$52,099	$62,109	$55,200	$57,217	$2,803,633
OR	$40,295	$48,037	$4,400	$4,561	$223,478
PA	$44,062	$52,528	$44,900	$46,541	$2,280,492
RI	$74,206	$88,463	no data	no data	no data
SC	$38,228	$45,573	$30,400	$31,511	$1,544,030
SD	$31,297	$37,311	$31,800	$32,962	$1,615,137
TN	$75,411	$89,900	$82,400	$85,411	$4,185,134
TX	$39,125	$46,643	$37,700	$39,078	$1,914,800
UT	$33,329	$39,733	$47,500	$49,236	$2,412,547
VT	$54,151	$64,556	no data	no data	no data
VA	$57,570	$68,632	$67,000	$69,448	$3,402,961
WA	$35,822	$42,705	$35,400	$36,694	$1,797,982
WV	$60,839	$72,529	$66,400	$68,826	$3,372,487
WI	$39,989	$47,672	$21,800	$22,597	$1,107,232
WY	$46,002	$54,840	$47,600	$49,339	$2,417,626
National Average (2019)		$56,767		$51,337	$2,515,503

disabled to reflect current spending associated with the current administration of the HCBS waiver program policies. We assume those with a combined ASD and ID will need care across their adult life, and apply the same 40/60 ID/No ID ratio, with a 31/69 ID/No ID ratio as a sensitivity analysis. Life expectancy is assumed to be an average of 67 years, meaning 49 total per-capita years of needed adult care for those with both an ASD and ID. Table 6 shows each state's average per person HCBS waiver cost for accommodation associated care in 2009 (Lakin et al., 2010) and 2017 (Musumeci, Chidambaram, & Watts, 2019). We calculated the per capita lifetime cost of care for individuals with both an ASD and intellectual disability by inflating the most recent data on the cost of HCBS waivers to 2019 dollars.

HCBS waiver cost varies from state to state due to differences in services provided and cost of living. In addition to inter-state variability in cost and services covered, HCBS waiver spending has changed over time. A detailed description of the variability in Medicaid coverage is beyond the scope of this study, however we provide two separate estimates of state-by-state HCBS waiver cost

for developmentally and intellectually disabled individuals (Lakin et al., 2010; Musumeci et al., 2019) to show that the HCBS waiver program changes over time. For more information about exactly what is offered in each state, a point-in-time description of each state's HCBS Medicaid waiver program can be accessed at https://www.medicaid.gov/medicaid/ltss/downloads/asd-state-of-the-states-report.pdf.

To reflect the current state of HCBS service, our study applied the most recent data available on a state-by-state basis, and our final state-level calculations reflect the Lifetime Per Capita Cost in 2019 dollars shown in Table 6. On average nationally, a year of service under a Medicaid HCBS waiver for the developmentally and intellectually disabled costs $51,337 in 2017, down by 11 % from 2009 when adjusted to 2019 dollars. Applying the 2019 adjusted national annual average from fiscal year 2017 to 49 years of adult care results in a lifetime per capita average cost for an HCBS waiver (general care and accommodation) of $2,515,503. For the three states with no data in Musumeci et al. (2019), we applied Lakin et al. (2010) adjusted to 2019 dollars, which decreased the national average to $2,418,732. Not all adults with an ASD living at home will qualify for an HCBS waiver, therefore we assume that 40 % will qualify based on a combined developmental (ASD) and intellectual disability (ID), decreasing the average lifetime per capita cost of care across the entire ASD population by 60 % to $967,493. Under a scenario of only 31 % with a co-occurring ID requiring an HCBS waiver, the average national lifetime per capita cost of care could be as low as $749,807. For state-level analysis, we weight by 40% and round to the nearest whole, or integer number.

3. Results

Using the previously described methods, the average per capita lifetime cost of ASD in the United States is $3,566,881 in 2019 dollars. The total population level lifetime cost varies on a state-by-state basis, largely based on the size of the population and cost of Medicaid HCBS waivers, which vary in cost due to the amount and cost of services eligible for reimbursement under the State-defined programs. Applying what is known about the prevalence of ASD to the United States population of children since 1990, we estimate that there are approximately 2 million diagnoses 1990–2019, and the associated lifetime social costs of the disorder have reached more than $7 trillion in 2019 dollars. Even if the future incidence of ASD remains the same as the current prevalence, that total is projected to increase to over 3 million affected, with associated lifetime social costs of $11.5 trillion by 2029. If, however, the incidence of ASD increases at the same average rate per decade as the past increase in prevalence, or continues to increase at the United States Department of Education reported rate, we project that the number affected will increase to over 4 million individuals with an ASD, and a lifetime social cost of $14.5 to $14.9 trillion for cases of ASD 1990–2029. Table 7 provides the state-by-state results of our analysis for 1990–2019 and Table 8 provides the results of our three scenario analyses.

4. Discussion & implications

Our estimate of approximately $3.6 million in lifetime social cost for an individual with an ASD is within the range of existing studies. Buescher et al. (2014) estimated the cost of ASD at $1.4 million for individuals without, and $2.4 million for individuals with a co-occurring ID ($1.6 and $2.7 million in 2019 dollars), and Ganz (2007) estimated $3.2 million ($4.4 million in 2019 dollars). To the best of our ability, we based our cost estimate on studies of actual expenditures, or productivity losses. Ganz's higher estimate includes EIBI, a need that is currently not met by any existing public programs and may be beyond the ability of most families to afford, naturally increasing his estimate substantially. Our estimate is higher than Buescher et al. (2014), however, their estimate of productivity loss for individuals with an ASD for adults is only $10,718 ($12,251 in 2019 dollars). We base our productivity loss estimate on Ganz (2007) which is $27,745 ($38,436 in 2019 dollars), who's estimate is more complete and includes the average earnings in the United States and earnings of those in supported employment. Fig. 4 provides a comparison of estimates of the lifetime per capita cost of ASD. Like Ganz (2007), we found the largest cost to society is from lost productivity and adult care for the affected individuals (Fig. 5).

On a state-by-state basis, the lifetime per capita cost of ASD varies considerably depending largely on the cost of an HCBS waiver, which depends on the cost of living and covered services. Fig. 6 shows the geographic distribution of the per capita lifetime cost, illustrating that there is no clear geographic pattern to the cost of ASD on a per-capita basis.

The analyses presented here represent an estimate of the lifetime social cost of ASD to states and to the country. To our knowledge, this is the first time the cost of ASD has been estimated at the level of the individual state. While we readily acknowledge that these are imperfect calculations and that there is uncertainty inherent in the estimates of population, existing prevalence, and future incidence of autism, and even incremental cost, we point out that the analyses have been conducted using well-accepted data from the most current studies on ASD rates and cost to produce a reasonable, baseline and projected future estimate of the cost of ASD to society.

Most importantly, our findings show that the largest proportion of cost rests with lost productivity for families and the individual, and the cost of care for affected adults. The lack of accessibility of EIBI points to an area where cost savings could be potentially gained with the development of publicly funded EIBI school programs. A maximum cost of $83,119 in 2019 dollars for about four years (Butter et al., 2003; Rogge & Janssen, 2019), may result in cost savings that could span the remainder of the individual's life in the areas of special education, medical care, less productivity loss, and lower adult care costs, but more studies on the benefits of EIBI are needed to draw a definitive conclusion. EIBI typically takes place during the preschool years, which makes early diagnosis imperative. Even though the cost of ASD can decrease with early intervention (Jacobson et al., 1998), it can increase with delays in diagnosis (Horlin et al., 2014) and any lifetime social cost estimates of ASD would be affected if a portion of the population recovered from the disorder. For example, a survey of 673,000 children revealed that 40 % of parents with children age 3–17 stated that their

Table 7
State-by-State Results 1990–2019.

State	Estimated Population Level Lifetime Cost of Autism Per Decade in 2019 Dollars		
	1990-1999	2000-2009	2010–2019
AL	$19,613,144,880	$34,361,918,510	$57,177,765,421
AK	$3,283,048,704	$6,339,390,720	$13,757,737,344
AZ	$18,070,992,060	$34,206,366,150	$79,334,215,070
AR	$10,921,013,000	$20,345,310,120	$35,856,729,240
CA	$128,877,697,816	$227,155,828,520	$471,557,774,744
CO	$17,075,839,680	$33,339,340,080	$62,904,478,320
CT	$16,327,582,104	$26,753,259,530	$50,444,003,660
DE	$4,476,938,583	$7,847,981,178	$14,585,215,947
DC	$1,976,595,270	$3,385,019,430	$6,449,942,460
FL	$56,952,278,240	$98,511,877,152	$215,865,969,280
GA	$37,096,886,790	$69,395,379,500	$138,135,164,460
HI	$4,359,038,425	$8,420,169,275	$16,360,947,050
ID	$5,641,755,432	$11,178,087,348	$19,742,949,360
IL	$36,453,142,656	$64,983,078,144	$122,186,716,992
IN	$22,994,956,800	$41,280,024,576	$73,143,532,800
IA	$10,530,043,075	$19,235,984,200	$31,082,697,228
KS	$10,761,398,528	$20,650,916,352	$34,650,226,688
KY	$15,396,010,075	$28,385,848,700	$47,303,935,825
LA	$16,350,690,330	$30,348,103,734	$53,782,395,316
ME	$5,086,948,056	$8,270,289,764	$14,788,941,754
MD	$22,642,472,640	$39,958,197,360	$86,677,267,080
MA	$28,191,306,000	$46,302,084,400	$87,341,791,680
MI	$42,478,801,742	$70,202,807,194	$135,676,968,830
MN	$21,965,305,362	$41,093,141,166	$80,833,768,554
MS	$10,944,866,030	$19,956,915,293	$32,515,234,218
MO	$23,891,134,113	$43,063,031,858	$76,603,318,902
MT	$3,322,422,360	$6,063,762,620	$10,145,009,840
NE	$6,345,126,872	$12,464,590,236	$20,834,051,518
NV	$9,920,346,600	$19,041,355,920	$44,406,040,680
NH	$4,744,433,585	$7,440,532,375	$16,180,094,167
NJ	$33,758,993,268	$59,667,493,596	$113,155,768,644
NM	$8,925,197,056	$16,718,634,016	$27,790,550,400
NY	$78,905,025,056	$134,542,512,480	$247,460,754,656
NC	$35,919,812,516	$66,863,070,316	$133,535,720,584
ND	$2,237,933,958	$4,114,693,054	$6,183,853,712
OH	$42,757,449,228	$74,441,887,650	$130,767,822,783
OK	$14,652,671,858	$28,047,699,458	$46,979,338,466
OR	$10,163,584,620	$18,383,182,023	$37,460,069,028
PA	$45,267,842,235	$75,000,432,430	$133,808,946,425
RI	$3,940,536,064	$6,121,711,104	$12,510,210,560
SC	$15,312,920,000	$27,708,021,000	$47,241,645,000
SD	$2,752,135,664	$5,380,944,494	$8,717,259,898
TN	$27,807,287,094	$50,460,803,136	$94,575,544,902
TX	$96,294,923,112	$186,899,110,396	$377,998,125,176
UT	$12,165,321,091	$26,355,225,358	$45,096,877,364
VT	$1,669,633,560	$2,517,576,390	$4,888,141,020
VA	$31,973,697,756	$58,256,053,548	$115,470,476,660
WA	$22,709,049,783	$41,551,952,701	$80,153,686,893
WV	$6,874,134,803	$11,943,858,575	$18,194,148,864
WI	$17,922,077,371	$31,852,682,070	$57,651,224,950
WY	$2,007,904,594	$3,976,578,370	$5,285,461,116
United States Total	$1,157,998,515,441	$2,082,772,970,329	$4,008,267,903,678

child was once diagnosed with an ASD and no longer had the disorder at the time of the survey (Kogan et al., 2009). This contrasts with others that indicate for social cost analysis, the disorder results in lifelong impairment and social costs (Buescher et al., 2014; Ganz, 2007; Järbrink & Knapp, 2001; Knapp et al., 2009; Newschaffer & Curran, 2003).

This is a lifetime social cost analysis that does not incorporate the personal cost of ASD, and it in no way represents what families pay to treat the disorder. Individual family costs can be quite high and will vary depending on health insurance, symptom severity, and the presence of a variety of co-occurring disorders. Examples of personal costs not included in the social cost estimates are (1) uncovered medications; (2) supplements; (3) privately funded EIBI, speech, occupational, physical, vision, and applied behavior analysis therapy; (4) dietary interventions; (5) transportation to and from medical appointments.

These calculations reflect the lifelong costs associated with ASD based on estimated cases of ASD. The social cost of a lifetime will be spread over 72 years, however due to the lack of a known cure for ASD, it is assumed that each new case will incur lifetime costs.

Table 8
Projected Cost of ASD Based on Three Scenarios of Future Incidence.

State	Projected Population Level Lifetime Cost of Autism Per Decade in 2019 Dollars		
	2020–2029 no increase in rate	2020–2029 consistent rate of increase	IDEA percent increase reported to congress compared to 2010–2019 estimates
AL	$59,477,640,148	$107,397,534,206	$118,243,136,345
AK	$15,050,805,120	$27,179,612,928	$27,897,517,440
AZ	$95,976,350,140	$173,299,194,310	$151,848,054,270
AR	$38,889,548,260	$70,223,903,920	$67,087,245,760
CA	$480,072,790,912	$866,853,072,944	$825,696,639,520
CO	$69,289,531,920	$125,116,963,920	$134,176,385,520
CT	$49,223,372,522	$88,878,571,930	$81,616,035,410
DE	$14,609,468,052	$26,381,439,819	$25,731,483,405
DC	$5,745,730,380	$10,375,124,565	$14,736,438,015
FL	$253,486,871,872	$457,714,628,800	$557,795,631,744
GA	$155,212,007,345	$280,263,178,645	$228,889,674,585
HI	$15,371,346,025	$27,756,767,850	$24,424,996,925
ID	$21,298,744,884	$38,457,220,776	$32,262,790,548
IL	$121,076,509,536	$218,625,673,056	$233,255,385,216
IN	$76,525,911,552	$138,178,489,344	$113,813,620,224
IA	$29,411,156,532	$53,107,899,137	$31,082,697,228
KS	$34,073,660,416	$61,523,840,000	$62,511,737,088
KY	$49,626,414,355	$89,607,219,680	$102,133,260,040
LA	$54,135,556,644	$97,747,584,870	$103,476,269,104
ME	$13,449,218,799	$24,282,978,456	$22,595,327,450
MD	$90,782,353,440	$163,926,493,860	$125,679,381,480
MA	$90,340,321,500	$163,120,876,490	$172,146,365,820
MI	$130,012,075,154	$234,761,252,778	$218,983,299,262
MN	$82,956,853,980	$149,795,917,656	$110,255,959,968
MS	$32,335,592,040	$58,387,034,557	$92,865,025,905
MO	$78,641,170,970	$141,998,451,430	$149,529,310,764
MT	$9,563,927,740	$17,268,392,760	$15,429,438,820
NE	$20,774,317,060	$37,509,921,043	$40,665,891,574
NV	$54,522,653,130	$98,454,087,300	$90,853,246,200
NH	$16,771,835,330	$30,283,842,123	$33,862,300,517
NJ	$114,130,054,164	$206,083,870,992	$210,239,573,460
NM	$25,152,461,920	$45,415,398,016	$64,369,358,912
NY	$239,711,621,472	$432,835,808,672	$392,221,328,928
NC	$158,293,988,748	$285,827,815,896	$233,953,000,512
ND	$5,680,909,278	$10,254,665,708	$11,847,885,660
OH	$127,174,197,852	$229,636,504,773	$240,878,461,707
OK	$50,127,169,952	$90,513,178,166	$97,951,139,325
OR	$41,770,181,765	$75,422,939,729	$46,900,372,097
PA	$128,885,704,255	$232,726,784,290	$260,928,323,425
RI	$11,879,292,160	$21,447,620,352	$24,180,398,336
SC	$50,317,097,000	$90,857,731,000	$115,319,799,000
SD	$8,704,278,126	$15,717,680,449	$13,896,986,926
TN	$106,994,167,354	$193,193,765,936	$178,463,211,362
TX	$426,465,290,992	$770,056,507,176	$785,859,993,544
UT	$53,419,767,709	$96,456,417,827	$70,575,258,600
VT	$4,654,497,330	$8,403,297,210	$4,888,141,020
VA	$127,162,085,204	$229,610,201,128	$244,913,851,336
WA	$90,119,385,636	$162,726,619,335	$134,979,963,594
WV	$17,218,898,263	$31,093,516,125	$35,499,911,553
WI	$55,807,602,664	$100,769,473,563	$100,656,909,166
WY	$4,693,432,408	$8,473,856,688	$10,356,935,952
United States Total	$4,234,343,935,336	$7,645,819,196,990	$7,288,425,350,562

The lifespan reported for ASD is shorter than the typical life span due to premature mortality from increased suicidality (Cassidy et al., 2014; Croen et al., 2015; Demirkaya, Tutkunkardaş, & Mukaddes, 2016; Paquette-Smith, Weiss, & Lunsky, 2014), which is particularly true for high-functioning individuals (Hirvikoski et al., 2016), as well as shortened life expectancy due to epilepsy in low-functioning individuals (Hirvikoski et al., 2016; Pickett, Xiu, Tuchman, Dawson, & Lajonchere, 2011).

To keep the calculations simple and understandable, the costs applied in this analysis are based on studies conducted in the current decade. In some other studies, sometimes costs incurred over decades in the future are subjected to future-year discounting based on the idea that current generations value less the costs that must be paid by future generations. There is no evidence that current generations value less the cost of ASD to future generations, thus we did not apply a future year cost discount.

Such large numbers are not typically dealt with by most people. For perspective, the average household income in the United States is $61,937 (United States Census Bureau, 2018). United States wage earners earned a total of $10.4 trillion in 2017 (United

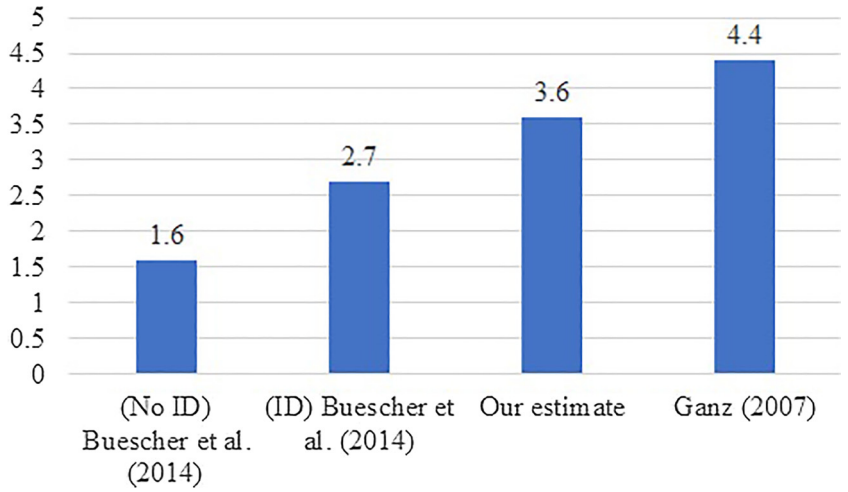


Fig. 4. Comparison of estimates of Lifetime per capita social costs associated with ASD in millions of 2019 dollars.

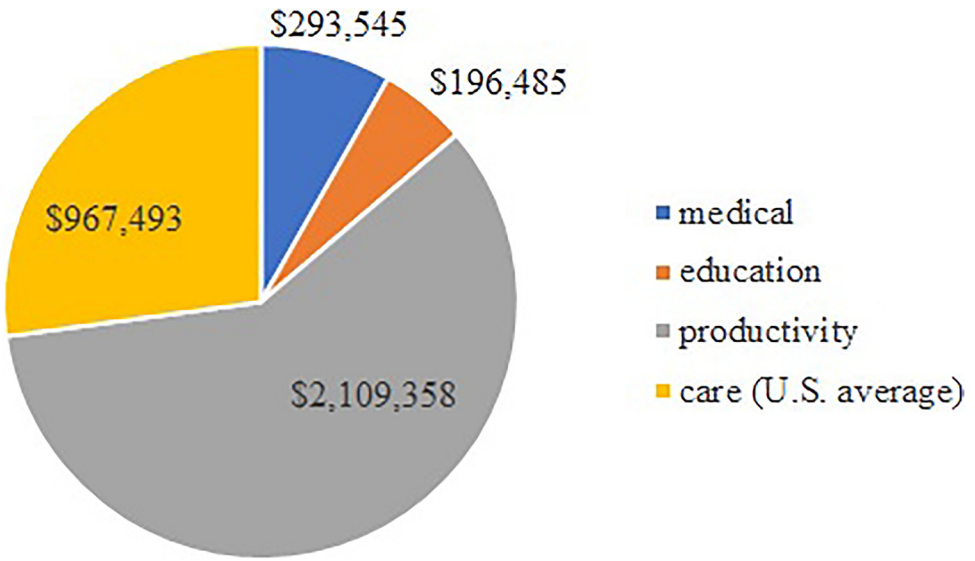


Fig. 5. Percentage distribution of the types of cost associated with an ASD in 2019 dollars.

States Bureau of Economic Analysis, 2019). The total annual revenue of the United States government is $3.4 trillion (United States Department of the Treasury, 2018). Federal minimum wage is $7.25 per hour (United States Department of Labor, 2019). If we use these data to translate the social cost of autism into something meaningful to everyday people, the total lifetime cost of ASD in the United States for the estimated cases to date (1990–2019) is the equivalent of 8 months of wages for all workers in the United States, or the total United States revenue from taxes and other sources for more than two years, or 1 trillion hours of minimum wage work.

Most of the funding for research in ASD comes from federal sources through agencies like the National Institute of Health (NIH), and in 2016, the United States spent about $272 million (Interagency Autism Coordinating Committee, 2016), or one one-hundredth of a percent (0.01 %) of total annual federal revenue on research to understand the biology of individuals affected by ASD, what causes the disorder, and ways to prevent it.

5. Conclusion

For *all* of the individuals with ASD identified in the 3 decades from 1990–2019, the lifetime social cost for the US is estimated to be more than $7 trillion in 2019 dollars. Even if one assumes that the rate of increase in prevalence of ASD is static for the next decade (2020–2029), the projected cost estimate for ASD in the US will increase to $11.5 trillion in 2019 dollars. If, however, the prevalence of ASD continues at the current average rate of increase per decade, those costs are projected to reach nearly $15 trillion by 2029 (accounting for an estimated 4 million affected individuals). These numbers serve to reinforce the conclusions of Leigh and Du (2015), who stated that the burden is significant and alarming, and the national policy response should be to focus the bulk of funding

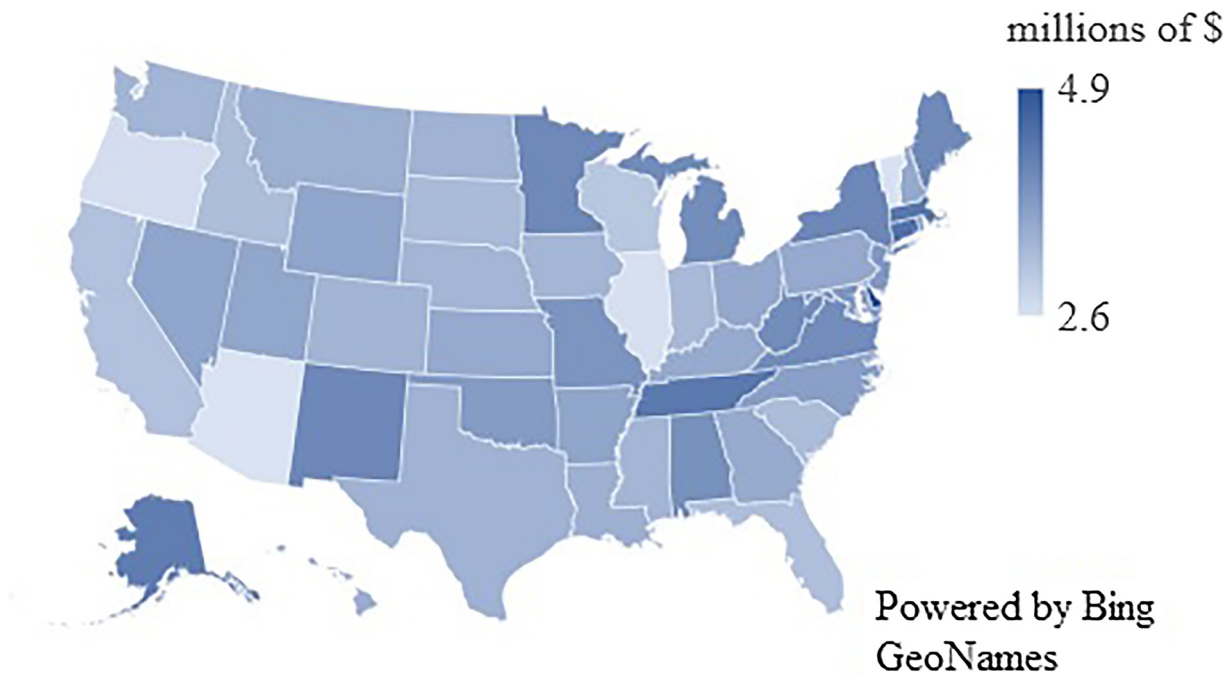


Fig. 6. Per Capita Lifetime Cost of ASD.

and resources on understanding the modifiable causes of ASD and then avoid those risk factors because prevention is far less expensive than treatment and management. A variety of approaches in the field of vulnerability assessment and risk management could be used to identify known modifiable risk factors that could potentially reduce rates, thereby reducing costs. Due to the high cost of care for affected adults, focusing on understanding risks associated with the severe forms of ASD, where there is co-occurring ID, holds the greatest potential benefit to society.

Funding

This work was not funded.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Author contributions statement

Author A – Population and cost analysis.
Author B – Topic conceptualization, interpretation and discussion.
Author C – Topic conceptualization, interpretation and discussion.

Declaration of Competing Interest

Author A has a child with encephalopathy and features of autism.
Author B has no declared conflicts of interest.
Author C has no declared conflicts of interest.

References

ADDM Network Principal Investigators (2014). *Prevalence of autism Spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States, 2010. Morbidity and mortality weekly report: Surveillance summaries*Surveillance Year 20101–21. https://www.jstor.org/stable/24806108.

Anderson, K. A., Shattuck, P. T., Cooper, B. P., Rou, A. M., & Wagner, M. (2014). Prevalence and correlates of postsecondary residential status among young adults with an autism spectrum disorder. *Autism, 18*(5), 562–570. https://doi.org/10.1177/1362361313481860.

Baio, J., Wiggins, L., Christensen, D. L., et al. (2018). Prevalence of autism spectrum disorder among children aged 8 years — autism and developmental disabilities monitoring network, 11 sites, United States, 2014. *Morbidity and Mortality Weekly Report Surveillance Summaries, 67*(SS-6), 1–23. https://doi.org/10.15585/mmwr.ss6706a1.

Baxter, A. J., Brugha, T. S., Erskine, H. E., Scheurer, R. W., Vos, T., & Scott, J. G. (2015). The epidemiology and global burden of autism spectrum disorders. *Psychological Medicine, 45*, 601–613. https://doi.org/10.1017/S003329171400172X.

Blue Cross Blue Shield (2019). *2019 Blue cross and blue shield service benefit plan brochure – standard and basic option.* Retrieved fromhttps://www.fepblue.org/benefit-plans/benefit-plans-brochures-and-forms.

Buescher, A. V. S., Cidav, Z., & Knapp, M. (2014). Costs of autism Spectrum disorders in the United Kingdom and the United States. *JAMA Pediatrics, 168*(8), 721–728. https://doi.org/10.1001/jamapediatrics.2014.210.

Butter, E. M., Wynn, J., & Mulick, J. (2003). Early intervention critical to autism treatment. *Pediatric Annals, 32*(10), 677–684.

Cassidy, S., Bradley, P., Robinson, J., Allison, C., McHugh, M., & Baron-Cohen, S. (2014). Suicidal ideation and suicide plans or attempts in adults with Asperger's syndrome attending a specialist diagnostic clinic: A clinical cohort study. *The Lancet Psychiatry, 2*, 142–147. https://doi.org/10.1016/S2215-0366(14)70248-2.

Centers for Medicare and Medicaid Services (2019). *The center for consumer information and oversight.* Retrieved fromhttps://www.cms.gov/CCIIO/Resources/Data-Resources/ehb.

Chasson, G. S., Harris, G. E., & Neely, W. J. (2007). Cost comparison of early intensive behavioral intervention and special education for children with autism. *Journal of Child and Family Studies, 16*, 401–413. https://doi.org/10.1007/s10826-006-9094-1.

Chambers, J. G., Parrish, T. B., & Harr, J. J. (2002). *What are we spending on special education services in the United States, 1999–2000? Report. Special education expenditure project (SEEP)*Washington, DC: American Institutes for Research in the Behavioral Sciences, Palo Alto, CA. Center for Special Education Finance. Special Education Programs (ED/OSERS). https://files.eric.ed.gov/fulltext/ED471888.pdf.

Cigna (2019). *Medical coverage policy*. Retrieved fromhttps://cignaforhcp.cigna.com/public/content/pdf/coveragePolicies/medical/mm_0499_coveragepositioncriteria_intensive_behavioral_interventions.pdf.

CDC (2018). *Autism Spectrum disorder (ASD): Data and statistics, prevalence.* Retrieved fromhttps://www.cdc.gov/ncbddd/autism/data.html.

CDC WONDER Online Database (2005). *Population Projections, United States, 2004–2030, by state, age, and sex.* Retrieved from athttp://wonder.cdc.gov/population-projections.html.

Cidav, Z., Marcus, S. C., & Mandell, D. S. (2012). Implications of childhood autism for parental employment and earnings. *Pediatrics, 129*(4), https://doi.org/10.1542/peds.2011-2700.

Croen, L. A., Zerbo, O., Qian, Y., Massolo, M. L., Rich, S., Sidney, S., et al. (2015). The health status of adults on the autism spectrum. *Autism, 7*, 814–823. https://doi.org/10.1177/1362361315577517.

de la Cuesta, G. G. (2009). Trends in the economic costs of autism in the UK. *Tizard Learning Disability Review; Brighton, 14*(3), 41.

Demirkaya, K.ç, Tutkunkardaş, M. D., & Mukaddes, N. M. (2016). Assessment of suicidality in children and adolescents with diagnosis of high functioning autism spectrum disorder in a Turkish clinical sample. *Neuropsychiatric Disease and Treatment, 12*, 2921–2926.

Fombonne, E. (2005). The changing epidemiology of autism. *Journal of Applied Research in Intellectual Disabilities, 18*(4), 281–294. https://doi.org/10.1111/j.1468-3148.2005.00266.x.

Ganz, M. (2008). *The costs of autism, technical appendix.* Retrieved fromHarvard University School of Public Health)https://www.researchgate.net/profile/Michael_Ganz/publication/248380724_The_Costs_of_Autism/links/541e2b1a0cf203f155c046c5/The-Costs-of-Autism.pdf.

Ganz, M. L. (2007). The lifetime distribution of the incremental societal costs of autism. *Archives of Pediatrics and Adolescent Medicine, 161*, 343–349. https://doi.org/10.1001/archpedi.161.4.343.

Ganz, M. L. (2006). The costs of autism. In S. O. Rubenstein, & J. L. R. Moldin (Eds.). *Understanding autism: From basic neuroscience to treatment*. Boca Raton, FL: Taylor and Francis Group.

Healthcare.gov (2019). *Habilitative/habilitation services.* Retrieved fromhttps://www.healthcare.gov/glossary/habilitative-habilitation-services/.

Hewitt, A. S., Stancliffe, R. J., Hall-Lande, J., Nord, D., Pettingell, S. L., Hamre, K., et al. (2017). *Research in autism spectrum disorders, Vol. 34*, 1–9.

Hirvikoski, T., Mittendorfer-Rutz, E., Bowman, M., Larsson, H., Lichtenstein, P., & Bolte, S. (2016). Premature mortality in autism spectrum disorder. *The British Journal of Psychiatry, 208*, 232–238. https://doi.org/10.1192/djp.bp.114.160192.

Horlin, C., Falkmer, M., Parsons, R., Albrecht, M. A., & Falkmer, T. (2014). The cost of autism spectrum disorders. *PloS One, 9*(9), https://doi.org/10.1371/journal.pone.0106552.

Interagency Autism Coordinating Committee (2016). *2016 IACC autism spectrum disorder research portfolio analysis report*https://iacc.hhs.gov/publications/portfolio-analysis/2016/portfolio_analysis_2016.pdf.

Jacobson, J. W., Mulick, J. A., & Green, G. (1998). Cost-benefit estimates for early intensive behavioral intervention for young children with autism - general model and single state case. *Behavioral Interventions, 13*, 201–226. https://doi.org/10.1002/(SICI)1099-078X(199811)13:4<201::AID-BIN17>3.0.CO;2-R.

Järbrink, K. (2007). The economic consequences of autistic spectrum disorder among children in a Swedish municipality. *Autism, 11*(5), 453–463. https://doi.org/10.1177/1362361307079602.

Järbrink, K., & Knapp, M. A. (2001). The economic impact of autism in Britain. *Autism, 5*(1), 7–22. https://doi.org/10.1177/1362361301005001002.

Knapp, M., Romeo, R., & Beecham, J. (2009). Economic cost of autism in the UK. *Autism, 13*(3), 317–336. https://doi.org/10.1177/1362361309104246.

Kogan, M. D., Blumberg, S. J., Schieve, L. A., Boyle, C. A., Perrin, J. M., Ghandour, R. M., et al. (2009). Prevalence of parent-reported diagnosis of autism spectrum disorder among children in the US, 2007. *Pediatrics, 124*(5).

Lakin, K. C., Larson, S., Salmi, P., & Webster, A. (2010). *Residential services for persons with developmental disabilities: Status and trends through 2009. Residential services for persons with developmental disabilities: Status and Trends through 2009*. College of Education and Human Development, University of Minnesota. Retrieved from http://rtc.umn.edu/docs/risp2009.pdf.

Leslie, D. L., & Martin, A. (2007). Health care expenditures associated with autism spectrum disorders. *Archives of Pediatrics & Adolescent Medicine, 161*(4), 350–355. https://doi.org/10.1001/archpedi.161.4.350.

Lavelle, T. A., Weinstein, M. C., Newhouse, J. P., Munir, K., Kuhlthau, K. A., & Prosser, L. A. (2014). Economic burden of childhood autism Spectrum disorders. *Pediatrics, 133*(3), e520–e529.

Leigh, J. P., & Du, J. (2015). Brief report: Forecasting the economic burden of autism in 2015 and 2025 in the United States. *Journal of Autism and Developmental Disorders, 45*(12), 4135–4139. https://doi.org/10.1007/s10803-015-2521-7.

Liptak, G. S., Stuart, T., & Auinger, P. (2006). Health care utilization and expenditures for children with autism: Data from United States National Samples. *Journal of Autism and Developmental Discord, 36*, 871–879. https://doi.org/10.1007/s10803-006-0119-9.

Mandell, D. S., & Palmer, R. (2005). Differences among states in the identification of autistic spectrum disorders. *Archives of Pediatrics & Adolescent Medicine, 159*(3), 266–269. https://doi.org/10.1001/archpedi.159.3.266.

Medicaid and CHIP Payment and Access Commission (2019). *Medicaid and CHIP payment and access commission.* Retrieved fromhttps://www.macpac.gov/medicaid-101/waivers/.

Musumeci, M. B., Chidambaram, P., & Watts, M. (2019). *Medicaid home and community-based services enrollment and spending. Kaiser family foundation and watts health policy consulting. April 2019 issue brief*. Retrieved from https://www.kff.org/medicaid/issue-brief/medicaid-home-and-community-based-services-enrollment-and-spending/.

Montes, G., & Halterman, J. S. (2008). Association of childhood autism spectrum disorders and loss of family income. *Pediatrics, 121*(4), 821–826.

Nevison, C., Blaxill, M., & Zahorodny, W. (2018). California autism prevalence trends from 1931 to 2014 and comparison to national ASD data from IDEA and ADDM. *Journal of Autism and Developmental Disabilities,* 1–15. https://doi.org/10.1007/s10803-018-3670-2.

Newman, L., Wagner, M., Konkey, A. M., Marder, C., Nagle, K., Shaver, D., et al. (2011). *The post-high school outcomes of young adults with disabilities up to 8 years after high school. A report from the National Longitudinal Transition Study – 2 (NLTS2) (NCSER; 2011-3005*. Menlo Park, CA: SRI International. Retrieved from http://www.nlts2.org/reports/.

Newschaffer, C., & Curran, L. K. (2003). *Autism: An emerging public health problem. Public health reports*118. https://doi.org/10.1093/phr/118.5.393.

Nydén, A., Myrén, K.-J., & Gillberg, C. (2008). Long-term psychosocial and health economy consequences of ADHD, autism, and reading-writing disorder: A prospective service education project. *Journal of Attention Disorders, 12*(2), 141–148. https://doi.org/10.1177/1087054707306116.

National Council on Disability (2018). *Idea Series: Broken promises: The underfunding of IDEA.* Retrieved fromNational Council on Disability (NCD) 1331 F Street NW, Suite 850 Washington, DC 20004https://ncd.gov/sites/default/files/NCD_BrokenPromises_508.pdf.

Ohl, A., Sheff, M. G., Small, S., Nguyen, J., Paskor, K., & Zanjirian, A. (2017). Predictors of employment status among adults with autism spectrum disorder. *Work, 56*, 345–355.

Paquette-Smith, M. J., Weiss, J., & Lunsky, Y. (2014). History of suicide attempts in adults with Asperger syndrome. *Crisis, 35*(4), 273–277. https://doi.org/10.1027/0227-5910/a000263.

Pickett, J., Xiu, E., Tuchman, R., Dawson, G., & Lajonchere, adn C. (2011). Mortality in individuals with autism, with and without epilepsy. *Journal of Child Neurology, 26*(8), 932–939. https://doi.org/10.1177/0883073811402203.

Rogge, N., & Janssen, J. (2019). The economic costs of autism spectrum disorder: A literature review. *Journal of Autism and Developmental Disorders, 49*(7), 2873–2900. https://doi.org/10.1007/s10803-019-04014-z.

Roux, A. M., Shattuck, P. T., Cooper, B. P., Anderson, K. A., Wagner, M., & Narendorf, S. C. (2013). Postsecondary employment experiences among young adults with an autism spectrum disorder. *Journal of the American Academy of Child and Adolescent Psychiatry, 52*(9), 931–939.

Shattuck, P. T., Nerendorf, S. C., Cooper, B., Sterzing, P. R., & Wagner, M. (2012). Postsecondary education and employment aming youth with an autism spectrum disorder. *Pediatrics, 129*(6), 1042–1049. https://doi.org/10.1542/peds.2011-2864.

Shavelle, R., & Strauss, D. (1998). Comparative mortality of persons with autism in California, 1980–1996. *Journal of Insurance Medicine, 30*, 67–72.

Shimabukuro, T. T., Grosse, S. D., & Rice, C. (2008). Medical expenditures for children with an autism spectrum disorder in a privately insured population. *Journal of Autism and Developmental Disorders, 38*(3), 546–552.

United States Bureau of Economic Analysis (2019). *Regional data: GDP and personal income. SAGDP4N compensation fo employees 1/. GeoFips 00000.* Retrieved fromhttps://www.bea.gov/data/gdp/gdp-state.

United States Census Bureau (2019a). *2010 Decennial census of population and housing.* Retrieved fromhttps://www.census.gov/programs-surveys/decennial-census/decade.2010.html.

United States Census Bureau (2019b). *2017 public elementary-secondary education finance data. Summary table 20.* Retrieved fromhttps://www.census.gov/data/tables/2017/econ/school-finances/secondary-education-finance.html.

United States Census Bureau (2018). *Household Income: 2018.* Retrieved fromhttps://www.census.gov/content/dam/Census/library/publications/2019/acs/acsbr18-01.pdf.

United States Department of Education (2017). *39th annual report to congress on the implementation of the Individuals with Disabilities Education Act, 2017*Retrieved fromhttps://www2.ed.gov/about/reports/annual/osep/2017/parts-b-c/39th-arc-for-idea.pdf.

United States Department of Labor (2019). *Minimum wage.* Retrieved fromhttps://www.dol.gov/general/topic/wages/minimumwage.

United States Department of the Treasury (2018). *Financial report of the United States government: FY2018*Retrieved fromhttps://fiscal.treasury.gov/files/reports-statements/financial-report/2018/03282019-FR(Final).pdf.

Van Naarden Braun, K., Christensen, D., Doernberg, N., Schieve, L., Rice, C., Wiggins, L., et al. (2015). Trends in the prevalence of autism Spectrum disorder, cerebral palsy, hearing loss, intellectual disability, and vision impairment, Metropolitan Atlanta, 1991–2010. *PloS One,* 1–21. https://doi.org/10.1371/journal.pone.0124120.

Vohra, R., Madhavan, S., & Sambamoorthi, U. (2017). Comorbidity prevalence, healthcare utilization, and expenditures of Medicaid enrolled adults with autism spectrum disorders. *Autism, 21*(8), 995–1009. https://doi.org/10.1177/1362361316665222.

Wang, J., Zhou, X., Xia, W., Sun, C.-H., Wu, L.-J., Wang, J.-L., et al. (2012). Parent-reported health care expenditures associated with autism spectrum disorders in Heilongjiang province, China. *BMC Health Services Research, 12*(7), https://doi.org/10.1186/1472-6963-12-7.

Zablotsky, B., Black, L. I., & Blumberg, S. J. (2017). *Estimated prevalence of children with diagnosed developmental disabilities in the United States 2014–2016*Retrieved from doi:. National Center for Health Statistics Report (NCHS Data Brief No. 291)https://www.cdc.gov/nchs/data/databriefs/db291.pdf.

Journal of Family Psychology
2010, Vol. 24, No. 4, 449–457

0893-3200/10/$12.00 DOI: 10.1037/a0019847

The Relative Risk and Timing of Divorce in Families of Children With an Autism Spectrum Disorder

Sigan L. Hartley, Erin T. Barker, and Marsha Mailick Seltzer
University of Wisconsin–Madison

Frank Floyd
Georgia State University

Jan Greenberg
University of Wisconsin–Madison

Gael Orsmond
Boston University

Daniel Bolt
University of Wisconsin–Madison

We compared the occurrence and timing of divorce in 391 parents of children with an autism spectrum disorder (ASD) and a matched representative sample of parents of children without disabilities using a survival analysis. Parents of children with an ASD had a higher rate of divorce than the comparison group (23.5% vs. 13.8%). The rate of divorce remained high throughout the son's or daughter's childhood, adolescence, and early adulthood for parents of children with an ASD, whereas it decreased following the son's or daughter's childhood (after about age 8 years) in the comparison group. Younger maternal age when the child with ASD was born and having the child born later in the birth order were positively predictive of divorce for parents of children with an ASD. Findings have implications for interventions focused on ameliorating ongoing and long-term marital strains for parents of children with an ASD.

Keywords: autism spectrum disorders, divorce, marital relationship, parent

Autism spectrum disorders (ASDs) are lifelong neurodevelopmental disorders involving a triad of impairments in communication and social reciprocity and increases in repetitive/restricted interests and behaviors (American Psychiatric Association, 2000). Parenting a son or daughter with an ASD poses several unique challenges (e.g., Seltzer, Krauss, Orsmond, & Vestal, 2000), which may take a toll on marriages. The extent of this toll in terms of divorce has been the topic of wide speculation in the media, with divorce rates of 80% and higher mentioned (Doherty, 2008; Solomon & Thierry, 2006), but the issue has not yet been addressed by empirical research. In this study, we compared the occurrence and timing of divorce among parents with an adolescent or adult with an ASD with a closely matched sample of parents of adolescents and adults without a disability drawn from a nationally representative sample. Family characteristics predictive of divorce are also identified.

Several studies have examined parental divorce in heterogeneous samples of children with a variety of disabilities or specific populations of children with disabilities other than ASD. Some of these studies indicate that parents of children with a disability have an increased risk of divorce as compared with parents of children without a disability (Breslau & Davis, 1986; Witt, Riley, & Coiro, 2003; Wymbs et al., 2008). Other studies, however, have not shown an adverse impact of having a child with a disability on divorce (Joesch & Smith, 1997; Urbano & Hodapp, 2007), possibly suggesting that some disabilities exact a heavy toll on marriages, but others have little impact (Joesch & Smith, 1997; Risdal & Singer, 2004).

Few disabilities appear to be more taxing on parents than ASDs (Seltzer et al., 2001). Parents of children with an ASD fare worse on a variety of measures of well-being than parents of children without disabilities as well as parents of children with other types of disabilities (e.g., Abbeduto et al., 2004; Eisenhower, Baker, & Blacher, 2005). Several factors have been proposed to account for the poorer well-being of parents of children with an ASD, including the uncertainty surrounding ASD diagnosis and the long-term prognosis of individuals with an ASD, the stressful nature of autistic symptoms and associated behavior problems, and

Sigan L. Hartley, Erin T. Barker, Marsha Mailick Seltzer, Jan Greenberg, and Daniel Bolt, Waisman Center, University of Wisconsin–Madison; Frank Floyd, Psychology Department, Georgia State University; Gael Orsmond, Department of Occupational Therapy, Boston University.

This research was supported by the National Institutes of Health Grants R01 AG08768 (to M. Seltzer), P01 AG20166, P30 HD03352 (to M. Seltzer), and T32 HD07489.

Correspondence concerning this article should be addressed to Sigan L. Hartley, Waisman Center, University of Wisconsin–Madison, 1500 Highland Avenue, Madison, WI 53705. E-mail: hartley@waisman.wisc.edu

the lack of public understanding of and tolerance for the behaviors of children with an ASD (Gray & Holden, 1992). Moreover, because of familial linkages, these parents may be caring for multiple children with special needs (Orsmond, Lin, & Seltzer, 2007), and are themselves at risk for evidencing a broader autism phenotype, which includes subtle impairments in social reciprocity and communication and increases in restricted/repetitive interests and behaviors (e.g., Piven, 2001) and psychiatric symptoms (e.g., Szatmari et al., 1995). Thus, in addition to the challenging nature of ASD, some parents may have more limited resources to cope with these demands, which may elevate their risk of family disruption, including marital dissolution.

In addition to examining the rate of divorce, it is important to understand the timing of divorce in families of children with an ASD to detect when couples may be most vulnerable to marital dissolution. Parents in the general population have the greatest risk of divorce prior to the child's teenage years (Bramlett & Mosher, 2002; Cherlin, 1992; Shiono & Quinn, 1994). This vulnerability to divorce is believed to be related to the high level of parenting demands and stress of having children and the subsequent reduction in responsiveness to the needs of one's spouse during these years (e.g., Shapiro, Gottman, & Carrere, 2000; Shiono & Quinn, 1994). If couples can survive these early years, however, their risk of divorce decreases, although there may be another smaller increase in risk of divorce during midlife (Furstenberg & Kiernan, 2001; Cherlin, 1992). In contrast to this typical pattern, parents of sons and daughters with an ASD continue to experience a high level of parenting demands and report elevated levels of stress into their child's adolescence and adulthood (Abbeduto et al., 2004; Smith et al., 2010). Thus, these parents may experience a prolonged period of vulnerability to divorce that starts in their son's or daughter's childhood and persists into their adolescence and adulthood.

It is also important to explore the family characteristics that place parents of children with an ASD at risk for divorce. In families of children without a disability, maternal ethnicity/race has been shown to be related to divorce, with some minority groups (e.g., African Americans) evidencing an increased risk of divorce (Bramlett & Mosher, 2002; Tzeng & Mare, 1995). Risk of divorce is also higher if the parents are less educated, marry younger, and have children early in the marriage (Bramlett & Mosher, 2002; Karney & Bradbury, 1995; Ono, 2009). Younger maternal age and lower maternal education were also risk factors for divorce in parents of children with Down syndrome and other types of congenital disabilities (Urbano & Hodapp, 2007), and thus may have similar effects in families of children with an ASD.

Characteristics related to the child with an ASD may also be important predictors of parental divorce. Studies of children with disabilities indicate that parental stress and marital satisfaction are more strongly associated with the child's behavior problems than his or her intellectual delay (e.g., Baker, Blacher, Crnic, & Edelbrock, 2002). Thus, whereas the presence of intellectual disability (ID) in addition to an ASD may not increase the risk of divorce, severity of aberrant behaviors may be related to marital dissolution. Having multiple children with an ASD in the family may also increase divorce, as parenting resources may be particularly taxed (Orsmond et al., 2007). Birth order of the child with an ASD may also play a role in parental divorce. Urbano and Hodapp (2007) found that divorce was less likely in families of children with Down syndrome when the child was born later (i.e., second vs. first born) in the birth order. It is unknown whether birth order is similarly predictive of divorce in families of children with an ASD.

In this study, we examined the occurrence and timing of divorce in 391 parents who have an adolescent or adult with an ASD, collected as part of an ongoing longitudinal study, as compared with a closely matched sample of 391 parents of adolescents and adults without a disability, drawn from a nationally representative sample, using a survival analysis. We also examined whether correlates of divorce reported in the literature for parents in the general population and with children with other types of disabilities also predicted divorce in parents of children with an ASD. We hypothesized that parents of children with an ASD would evidence more divorce than parents of children without a disability, and that the risk of divorce would remain high as their son or daughter moved through childhood, adolescence, and into adulthood. Mothers who were younger when they had their child and less educated were predicted to have a higher rate of divorce. The severity of the child's aberrant behaviors and having multiple children with ASD in the family were also expected to be associated with an increased risk of divorce.

Method

Adolescents and Adults With Autism Study (AAA)

AAA is an ongoing longitudinal study of families of 406 adolescents and adults with an ASD in Massachusetts and Wisconsin (Lounds, Seltzer, Greenberg, & Shattuck, 2007; Seltzer et al., 2003). The present study used data from the first (collected in 1998–1999) through fourth (collected in 2003–2004) waves of data collection because data from the normative comparison group were also available for this same time period. Criteria for inclusion in the AAA study were that the son or daughter was age 10 or older at the beginning of the study, had received an ASD diagnosis (autistic disorder, Asperger disorder, or pervasive development disorders–not otherwise specified [PDD-NOS]) from an educational or health professional, and had a research-administered Autism Diagnostic Interview—Revised (ADI–R; Lord, Rutter, & Le Couteur, 1994) profile consistent with an ASD diagnosis. Almost all (94.6%) of the sons and daughters met the ADI–R lifetime criteria for a diagnosis of autistic disorder, and the remainder met criteria for Asperger disorder or PDD-NOS. Approximately half of the participants lived in Wisconsin ($n = 202$) and half in Massachusetts ($n = 204$), and were recruited through service agencies, schools, and clinics.

The 406 adolescents and adults with an ASD included 11 children from families with more than one child with an ASD. In such families, one son or daughter with an

ASD was selected to be the target child for the present analysis according to the following criteria: (a) the child who lived in the family home, (b) the older child if both children were living at home, and (c) a child was randomly selected in the case of triplets, all of whom lived at home. In an additional four families, mothers had never married the biological father of the son or daughter with an ASD, and these families were excluded from the present analyses, resulting in a sample for the present analysis of 391 families.

National Survey of Midlife in the United States (MIDUS)

The normative comparison group came from the MIDUS national survey of 7,108 English-speaking adults 25 to 74 years of age, which was first completed in 1994–1996 (MIDUS I; Brim, Ryff, & Kessler, 2004). A national random digit dialing sampling procedure was used. For each household contacted, a household member between 25 and 74 years of age was randomly selected and invited to participate in the study. In addition, subsamples of siblings of these individuals and twin pairs and oversamples from metropolitan areas were included (additional information can be found at http://www.midus.wisc.edu/). Follow-up data were collected in 2004–2006 (MIDUS II) from 4,963 (70%) of the MIDUS I participants. MIDUS II data were used to construct the comparison group for the present study because they coincided with the timing of the fourth wave of data collection in the AAA sample. Compared with the U.S. Census Bureau, the MIDUS sample was similar in demographics, with the exception of being slightly underrepresentative of adults with a high school education or less and African Americans (Brim et al., 2004).

Of the MIDUS II participants, 4,316 (87.0%) were parents, but 433 (10.03%) reported that they had at least one child with a disability or mental health condition and were excluded, leaving 3,883 MIDUS participants as potential members of the comparison group for the present analysis. The following procedure was used to create a matched comparison group with the AAA sample. First, because we were interested in examining divorce related to a particular child (i.e., the child with an ASD), we randomly selected a target child within each MIDUS II family. Then, the MIDUS II sample was stratified on the basis of the mother's ethnicity (Caucasian vs. non-Caucasian) and education level (less than high school, high school, some college, and more than college). Within each stratum, we created a random number associated with each case and ordered the cases according to their random number. Using this new random order of cases, we selected the first case that matched an AAA family on the basis of mother's ethnicity, education level, mother's age (within 4 years) and index child's sex, child's age (within 3 years), and birth order (first vs. later born) until all of the AAA families were matched. Table 1 displays the participant characteristics of the 391 families in the MIDUS II comparison group.

Table 1
Characteristics of Families

Characteristic	ASD (n = 391)	No disability (n = 391)
Mother		
Mean (*SD*) age (years)	56.1 (10.5)	57.4 (10.0)
Range	37.6–86.2	38.0–84.9
White, non-Hispanic, *n* (%)	364 (93.1)	364 (93.1)
High school education, *n* (%)	11 (2.8)	11 (2.8)
High school graduate, *n* (%)	95 (24.3)	95 (24.3)
Some college/bachelor's degree, *n* (%)	110 (28.1)	110 (28.1)
Postbachelor's/graduate degree, *n* (%)	175 (44.8)	175 (44.8)
Child		
Biological, *n* (%)	382 (97.7)	382 (97.7)
Adoptive, *n* (%)	9 (2.3)	9 (2.3)
Male, *n* (%)	287 (73.4)	287 (73.4)
Female, *n* (%)	104 (26.6)	104 (26.6)
Mean (*SD*) age (years)	26.9 (9.5)	27.8 (9.7)
Range	14.6–56.9	13.88–58.12
First born (n, %)	184 (47.1)	184 (47.1)
Mean (*SD*) total children	2.8 (1.3)	2.8 (1.5)
Range	1–8	1–11

Note. ASD = autism spectrum disorder. Race/ethnicity breakdown for ASD: African American (8), Hispanic (7), American Indian (2), Asian or Pacific Islander (6), and other (4). Race/ethnicity breakdown for no disability: African American (10), Hispanic (7), American Indian (2), Asian or Pacific Islander (2), and other (6).

There was not a significant difference on any of the matched variables between the AAA and MIDUS II comparison groups. Although not used in matching, there also was not a significant difference in total number of children between the AAA and MIDUS II comparison groups. There was not a significant difference in the average length of marriages (in years) of the biological or adoptive parents of the target child for couples who did not divorce between the AAA (M = 26.56, SD = 9.50) and MIDUS II (M = 27.54, SD = 9.47) groups.

Procedure and Measures

Mothers in the AAA sample participated in 2- to 3-hr, in-home interviews and completed self-administered measures. Parents in the MIDUS II sample participated in an hour-long telephone interview and completed self-administered measures.

Divorce. Information on divorce was collected through self-reported questionnaires in the AAA sample and telephone interviews in the MIDUS II sample. Only divorce involving the biological or adoptive parents of the target child was examined. The month and year the divorce was finalized were used in analyses. Of the 391 AAA families, 84 dropped out of the study prior to the fourth wave of data collection. Dropouts occurred because the family decided to no longer participate in the study (n = 64) or the family could not be located (n = 20). In an additional six families,

either the mother or child with an ASD died. Information on divorce in these 90 cases was obtained through searching the publicly accessible divorce records using the online Wisconsin Circuit Court Access system and the Massachusetts county court records. Records were searched using mothers' first and last names and then verified using mothers' date of birth, address, and husbands' first and last names.

There were 10 cases in the AAA sample for which mothers reported being divorced, but the date of divorce could not be obtained by searching the Massachusetts or Wisconsin divorce records. It is likely that these families divorced in another state. Similarly, there were six cases in the MIDUS II comparison group for which mothers reported being divorced but did not report the date of divorce. These cases were not included in analyses regarding the timing of divorce.

Characteristics of families. We examined characteristics of the family as predictors of divorce, including mother's age, race/ethnicity (White, non-Hispanic vs. other), education (less than a high school degree, high school degree or some college, college graduate, and more than college degree), and age when the target child was born. We also examined the target child's age, sex, and birth order (first vs. later born) and family size (single child vs. multiple children).

For families of children with an ASD, the presence of ID was determined by assessing cognitive functioning on the Wide Range Intelligence Test (Glutting, Adams, & Sheslow, 2000) and adaptive behavior on the Vineland Screener (Sparrow, Carter, & Cicchetti, 1993). These assessments were administered in 2004. Children with scores above 75 on both measures were classified as not having ID and those with scores below 75 on both measures were classified as having ID. For children with scores below 75 on only one of the two measures or for whom there were missing data, a review of available records (historical assessments, parent report of prior diagnoses, clinical and school records) combined with a clinical consensus procedure were used to determine ID status.

Aberrant behaviors were assessed through two measures of the child's autism symptoms: report of their child's age when problems were first noticed and the ADI–R (Lord et al., 1994) lifetime rating or rating of autism symptoms at their most severe manifestation (mainly rated at age 4–5 years). These measures were chosen because they capture the child's aberrant behavior preceding the divorce in most cases. The ADI–R has good test–retest reliability and diagnostic and convergent validity (Hill, Bolte, Petrova, Beltcheva, Tacheva, & Poustka, 2001; Lord et al., 1994). The average interrater agreement between interviewers and two supervising psychologists in the present study was 88%. Items on the ADI–R are coded as 0 (*symptom not present*), 1 (*symptom present but not severe/frequent enough to meet criterion*), and 2 (*symptom present and meets criterion*). A score of 3 is recoded as a 2 in the algorithm. To include nonverbal children with ASD as well as verbal children, we excluded verbal items from this summary score.

Data Analysis Plan

The prevalence of divorce in the 391 parents of children with an ASD was statistically compared against the matched sample of 391 parents of children without a disability using Pearson's chi-square test. A Kaplan–Meier survival analysis was conducted to evaluate differences in the marriage survival distributions of parents of children with an ASD and parents of children without a disability. This is a method for modeling time to event data; in this context, divorce is the event and years of marriage following the birth of the target child (i.e., latency to divorce following the birth of the child) is the time variable. The Kaplan–Meier survival analysis has the advantage of accounting for "censored" data (i.e., likelihood that divorce occurs subsequent to the end of data collection). The 381 families of children with an ASD and 385 families of children without a disability for whom years married following the birth of the target child could be determined were included in the survival analysis. The Breslow (1979) statistic was used to test for differences in latency to divorce between parents of children with an ASD and parents of children without a disability.

Binary logistic regression analyses were conducted to examine the impact of family characteristics on divorce in parents of children with an ASD and parents of children without a disability. This analysis provides the relative risk ratios (i.e., likelihood of divorce) given the presence of a specific risk factor, controlling other risk factors. Mother's education (0 = less than college degree, 1 = college degree of more), race/ethnicity (0 = White, non-Hispanic, 1 = other), maternal age when the target child was born (in years), target child sex (0 = female, 1 = male), family size (0 = single child, 1 = multiple children), and birth order (0 = first born, 1 = later born) were entered into the regression model for both parents of children with and without an ASD. The following ASD-specific variables were also entered into the model for parents of children with an ASD: age when problems were first noticed (in months), ADI–R lifetime score, and presence of other children with an ASD in the family (0 = no, 1 = yes).

Results

Prevalence and Timing of Divorce

The prevalence of divorce was significantly higher among the parents of children with an ASD ($n = 92$, 23.53%) than the parents of children without a disability ($n = 54$, 13.81%), Pearson's $\chi^2(1, N = 781) = 12.16$, $p < .001$. As shown in Figure 1, there was a significant difference in the survival distributions of parents of children with an ASD and parents of children without a disability, Breslow $\chi^2(1) = 8.55$, $p = .003$. The rate of decline in the remaining marriages for parents of children without a disability tapers off in the target child's late childhood (i.e., about age 8 years), and continues to flatten out until the

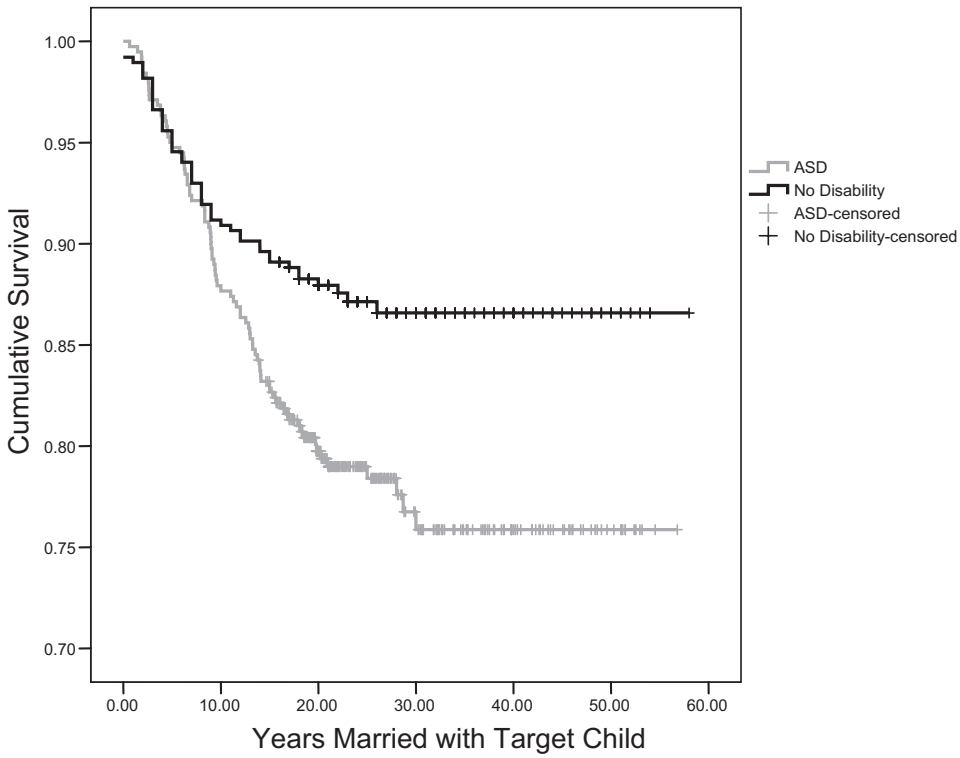


Figure 1. Survival plot for divorce in parents of children with an autism spectrum disorder (ASD) and parents of children without a disability.

target child has reached age 26 years, when the rate of divorce is virtually nonexistent. In other words, the risk of divorce begins to decrease in the child's late childhood for parents of children without a disability, and is extremely low by the time the son or daughter is a young adult. In contrast, the risk of divorce for parents of children with an ASD remains steep throughout the child's adolescence and early adulthood and does not decrease until the target child has reached age 30 years. Thus, parents of children with an ASD continue to have a high risk of divorce through the son's or daughter's childhood, adolescence, and early adulthood.

Follow-up Analyses

Follow-up analyses were conducted to ensure that results were robust against possible selection differences between the two samples. First, we examined the impact of parent death on the prevalence of divorce. Of the couples who did not experience a divorce, a parent death occurred in 33 (8.43%) AAA families and 37 (9.46%) MIDUS II families, which is not a significant difference. Because divorce is no longer an option for these families, we reran the survival analysis excluding these cases. Parents of children with an ASD continued to have a shorter latency to divorce than parents of children without a disability, Breslow $\chi^2(1) = 9.24$, $p = .002$. The pattern of decline in marriages did not change.

Second, we performed a series of calculations to estimate the impact of differences in the way that dropouts were handled within our two samples. To obtain complete divorce information through 2004 for both groups, we selected our comparison group of families of children without a disability from the second wave of MIDUS data collection (MIDUS II); thus, families who dropped out of the study prior to 2004 were not included. In contrast, all families of children with an ASD originally enrolled in the AAA study were included in this sample by searching divorce records for the 84 families who dropped out of the study prior to 2004. We recalculated the Pearson chi-square statistic for the overall likelihood of divorce using the smaller sample of 307 AAA families who remained in the study through 2004 and 307 matched MIDUS II families. The prevalence of divorce continued to be significantly higher among the parents of children with an ASD ($n = 72$, 23.92%) than the parents of children without a disability ($n = 47$, 15.60%), Pearson's $\chi^2(1, N = 601) = 6.03$, $p = .01$. Results from the Kaplan–Meier survival analysis for this smaller sample continued to indicate that parents of children with an ASD had a shorter latency to divorce than parents of children without a disability, Breslow $\chi^2(1) = 4.90$, $p = .03$. The pattern of decline in marriages was also similar.

Third, we examined whether the prevalence of divorce differed within the states from which the two samples were drawn. The MIDUS II comparison group included families

living in 45 states, whereas the AAA families resided in Wisconsin or Massachusetts. The averaged divorce rate (per 1,000 total population residing in the state) reported by the Division of Vital Statistics, National Center for Health Statistics, Centers for Disease Control and Prevention (2007) for Wisconsin and Massachusetts, is *lower* than the national average divorce rate by a magnitude of 0.38. Thus, the heightened prevalence of divorce in parents of children with an ASD as compared with parents of children without a disability may be *more* pronounced than what was captured in our study. However, by closely matching our samples on maternal characteristics, regional differences in divorce rates may have been accounted for in our analyses.

Family Characteristic Predictors of Divorce

Table 2 presents the binary logistic regression results for the impact of family characteristics on divorce in parents of children with an ASD and without a disability. For parents of children with an ASD, maternal age at which she had the target child and birth order significantly predicted divorce. The rate of divorce was greater when mothers were younger when they had the child with an ASD and when the child with an ASD was born later in the birth order. The effect size is reported with respect to the odds ratio (i.e., exp(B), indicating how the odds of divorce change for each unit change in the predictor). For example, the odds ratio effect size of 0.90 for maternal age at childbirth implies that the odds ratio of divorce decreases by multiples of 0.90 for each year increase in maternal age, assuming all other variables are equal. There were no significant family characteristic predictors of divorce in the comparison group.

Discussion

This study is the first systematic examination of the relative risk and timing of divorce in a large sample of parents of children with an ASD. The present study also constitutes the first step toward identifying family characteristic predictors of divorce within families of children with an ASD. Fully three fourths of the marriages of parents of children with an ASD in our sample remained intact, at least through the time of the present analysis, indicating that most marriages survive despite having a child with an ASD. Nevertheless, the rate of divorce was nearly twice the rate of the comparison group, which was closely matched on key characteristics and drawn from a nationally representative sample. The heightened risk of divorce in parents of children with an ASD is consistent with findings that these families experience an extraordinary level of stress (e.g.,

Table 2
Statistics for Binary Logistic Regression Assessing Prospective Prediction of Divorce

Variable	*B*	*SE*	Wald	OR Exp(*B*)	95% CI
		ASD			
Constant	2.69	1.71	2.49	14.76	
Mother					
Education	−0.06	0.27	0.05	1.06	[0.63, 1.80]
Race/ethnicity	−0.07	0.52	0.02	0.93	[0.33, 2.56]
Age had child	−0.11	0.03	12.94**	0.90	[0.85, 0.95]
Child					
Sex	0.58	0.31	3.41	1.78	[0.97, 3.27]
Birth order	0.82	0.31	7.19**	0.44	[0.24, 0.80]
Family size	−0.49	0.45	1.15	1.63	[0.67, 3.95]
ID	0.33	0.30	1.19	0.72	[0.40, 1.30]
Age first noticed problems	−0.01	0.01	0.90	0.99	[0.97, 1.01]
ADI–R lifetime	0.02	0.02	0.80	0.98	[0.94, 1.02]
Siblings with an ASD	0.17	0.85	0.04	1.18	[0.23, 6.19]
		No disability			
Constant	−1.50	1.20	1.57	0.22	
Mother					
Education	−0.56	0.34	2.68	1.74	[0.84, 3.08]
Race/ethnicity	−0.93	0.49	3.63	2.53	[0.97, 6.55]
Age had child	−0.04	0.03	1.64	0.96	[0.89, 1.01]
Child					
Sex	0.71	0.41	2.99	2.03	[0.91, 4.52]
Birth order	−0.06	0.36	0.02	0.95	[0.47, 1.90]
Family size	0.31	0.60	0.27	0.73	[0.23, 2.38]

Note. OR = odds ratio; CI = confidence interval; ASD = autism spectrum disorder; ID = intellectual disability; ADI–R = Autism Diagnostic Interview—Revised. ASD: overall $\chi^2(8, N = 382) = 23.90$, $p = .01$, Nagelkerke $R^2 = .09$, $p < .01$. No ASD: $\chi^2(5, N = 385) = 15.46$, $p = .02$, Nagelkerke $R^2 = .07$. Education: less than a college degree = 0, college degree or more = 1. Race/ethnicity: White, non-Hispanic = 0, other = 1. Child sex: girl = 0, boy = 1. Birth order: first born = 0, later born = 1. Family size: single child = 0, multiple children = 1. ID: no ID = 0, ID = 1. Siblings with ASD: no = 0, yes = 1.
* $p \leq .05$. ** $p \leq .01$.

Seltzer et al., 2000; Smith et al., 2010). This finding is similar to the heightened rate of divorce found in parents of children with attention-deficit/hyperactivity disorder, who were also twice as likely to divorce as parents of typically developing children (Wymbs et al., 2008).

As expected, parents of children with an ASD had a prolonged period of vulnerability to divorce. Specifically, there was a relatively high, and equivalent, risk of divorce for both the comparison group and families of children with an ASD during the son's or daughter's early childhood (until age 8 years). However, the risk of divorce markedly decreased in the son's or daughter's late childhood for our comparison group. A similar pattern has been found in the general population; the risk of divorce has been shown to be highest during the first several years of marriage, when children are young (e.g., Bramlett & Mosher, 2002; Shiono & Quinn, 1994), and in part, is attributed to the high level of parenting demands and stress and subsequent lack of attention devoted to one's spouse during these years (Shapiro et al., 2000). As children without a disability age, they launch into their own independent lives and parenting demands and stress often decrease, affording a renewed focus on the marital relationship. In contrast to this normative pattern, the risk of divorce remained high into the son's or daughter's early adulthood (age 30 years) for parents of children with an ASD. Parents of children with an ASD often continue to have a "full nest" (i.e., children living at home; Seltzer et al., 2000) and high levels of parenting demands and stress (e.g., Smith et al., 2010); subsequently, they may continue to experience marital strain into their son's or daughter's early adulthood. In our sample, nearly all (94.6%) of the parents who divorced coresided with their child with ASD at the time of their divorce. Moreover, parents are faced with a unique set of challenges as their child with an ASD ages into adolescence and adulthood, including assisting their son or daughter in transitioning out of school and into job and community settings and planning for long-term care, which may add new strains on parents' marriages. It is interesting that there was not a significant difference in the prevalence of divorce between parents of children with an ASD and our comparison group during the son's or daughter's early childhood (prior to age 8 years). Although different in nature, the challenges of having a young child with an ASD may not place more strain on marriages than the challenges of having a young child without a disability, given that parenting demands and stress are high in both cases.

There were significant family characteristic predictors of divorce. As expected, mothers of children with an ASD who were younger when they had their child were at greater risk of divorce. This finding has also been shown in studies of parents of children without a disability (e.g., Bramlett & Mosher, 2002) as well as parents of children with Down syndrome (Urbano & Hodapp, 2007), although it was not significantly associated with divorce in our comparison group. An elevated rate of divorce was also related to having a child with an ASD later in the birth order (i.e., second or later born). This finding is in contrast to findings of an increased risk of divorce being related to earlier birth order in families of children with Down syndrome (Urbano & Hodapp, 2007). The mechanisms driving this syndrome difference are not clear and should be examined in future studies. Birth order of the target child was not significantly related to divorce in families of children without a disability. Unexpectedly, there was not a significant relation between having multiple children with an ASD and divorce, which may be due to the small number of families ($n = 10$) with more than one child with an ASD in our sample.

In contrast to our prediction, onset and severity of early autism symptoms were also not significantly predictive of divorce. This finding may be related to several factors. First, dimensions of autism symptoms (e.g., predictability and course over time) other than onset and severity of early autism symptoms may take a greater toll on marriages. Moreover, among parents of children with an ASD, parenting stress is often more related to co-occurring behavior problems (e.g., aggression and hyperactivity) than autism symptoms (e.g., Lecavalier, Leone, & Wiltz, 2006). Thus, it may be that these co-occurring behavior problems are also more predictive of divorce within families of children with an ASD than autism symptoms. Second, parents' coping strategies or their own presence of a broader autism phenotype (i.e., milder autism features and impairments) and psychiatric problems may greatly moderate the degree to which their child's autism symptoms affect marriages. The mechanisms leading to divorce likely involve cascading effects of the interplay of these and other family characteristics with spousal behaviors and life experiences over time and should be the focus of future studies with larger samples and a longer follow-up period.

There are several other limitations to this study. Our comparison group, which was selected from a large study of adults in their midlife (MIDUS), has the advantage of offering a normative sample of families of children without a disability. A disadvantage of this comparison group is that the sampling procedures differed from those used in the AAA study. However, several follow-up analyses were conducted, and these sampling differences were estimated to have little impact on the overall pattern of findings in the survival analysis. The prevalence of divorce may have been slightly underestimated in our sample of parents of children with an ASD. Our sample of families of children with an ASD resided in Wisconsin and Massachusetts, states with relatively low rates of divorce, whereas our comparison group was located throughout the United States. The heightened risk of divorce in parents of children with an ASD may be more pronounced when controlling for this regional difference. However, by closely matching on several maternal characteristics, we likely accounted for some of the regional differences between our groups. It is also possible that divorced parents were less likely to volunteer for the study. Also, parents who dropped out of the study may have gotten a divorce in a state other than Wisconsin and Massachusetts and were thus falsely counted as not divorced. Moreover, the prevalence of divorces occurring at later stages (i.e., during the child's middle to late adulthood) may not have been fully captured in this

study. Generalizations of findings from this study should be made cautiously. The rate, timing, and correlates of divorce in families who do not participate in research studies may differ from those of families who do participate. Families in this study were also predominately White, non-Hispanic and college-educated, and thus findings may not generalize to less educated and minority families of children with an ASD. Finally, family characteristics, including the onset and severity of the child's autism symptoms, were reported by mothers and may be open to bias and not representative of fathers' perspectives. However, the likelihood that maternal perceptions biased ratings of autism symptoms is low given that the ADI–R has a standardized interview procedure and trained coding system.

Even given these limitations, this study provides the first large-scale examination of divorce in parents of children with an ASD using a closely matched comparison group. Findings have important implications for enhancing services for families of children with an ASD. Service providers should be educated about the heightened risk and timing of divorce in families of children an ASD. Parents should be guided in identifying strategies to enhance their marital relationship in an ongoing way, such as learning how to best communicate with and support their spouse and carving out "couple time." Given their prolonged period of vulnerability to divorce, couples may need to remain vigilant to recurring and compounding marital strains throughout the course of their child's development, including into adulthood. Finally, it may be reassuring for parents to know that most marriages survive and thus their marriage is not destined for divorce, as is often incorrectly presented in the media.

References

Abbeduto, L., Seltzer, M. M., Shattuck, P., Krauss, M. W., Orsmond, G., & Murphey, M. (2004). Stress and coping in mothers of youths with Down syndrome, autism, and fragile X syndrome. *American Journal on Mental Retardation, 109,* 237–254.

American Psychiatric Association. (2000). *Diagnostic and statistical manual of mental disorders* (4th ed., text rev.). Washington, DC: Author.

Baker, B. L., Blacher, J., Crnic, K. A., & Edelbrock, C. (2002). Behavior problems and parenting stress in families of three-year-old children with and without developmental delays. *American Journal of Mental Retardation, 107,* 433–444.

Bramlett, M. D., & Mosher, W. D. (2002). *Cohabitation, marriage, divorce, and remarriage in the United States* (Vol. 23). Washington, DC: National Center for Health Statistics.

Breslau, N. N., & Davis, G. C. (1986). Chronic stress and major depression. *Archives of General Psychiatry, 43,* 309–314.

Breslow, N. (1979). Statistical methods for censored survival data. *Environmental Health Perspectives, 32,* 181–192.

Brim, O. G., Ryff, C. D., & Kessler, R. C. (2004). The MIDUS national survey: An overview. In O. G. Brim, C. D. Ryff, & R. C. Kessler (Eds.), *How healthy are we? A national study of well-being at midlife* (pp. 1–36). Chicago: University of Chicago Press.

Centers for Disease Control and Prevention. (2007). National Center for Health Statistics, Division of Vital Statistics. Retrieved from http://www.cdc.gov/nchs/mardiv.htm#state_tables

Cherlin, A. (1992). *Marriage, divorce, and remarriage.* Cambridge, MA: Harvard University Press.

Doherty, S. (2008, July 2). Arrested development: Day-to-day struggles of autistic children affect entire family. *The Capital Times,* p. 25.

Eisenhower, A. S., Baker, B. L., & Blacher, J. (2005). Preschool children with intellectual disability: Syndrome specificity, behaviour problems, and maternal well-being. *Journal of Intellectual Disability Research, 49,* 657–671.

Furstenberg, F. F., & Kiernan, K. E. (2001). Delayed parental divorce: How much do children benefit? *Journal of Marriage and Family, 63,* 446–457.

Glutting, J., Adams, W., & Sheslow, D. (2000). *Wide Range Intelligence Test.* Wilmington, DE: Wide Range.

Gray, D. E., & Holden, W. J. (1992). Psycho-social well-being among parents of children with autism. *Australia and New Zealand Journal of Developmental Disabilities, 18,* 83–93.

Hill, A., Bolte, S., Petrova, G., Beltcheva, D., Tacheva, S., & Poustka, F. (2001). Stability and interpersonal agreement of the interview-based diagnosis of autism. *Psychopathology, 34*(4), 187–191.

Joesch, J. M., & Smith, K. R. (1997). Children's health and their mothers' risk of divorce or separation. *Social Biology, 44,* 159–169.

Karney, B. R., & Bradbury, T. N. (1995). The longitudinal course of marital quality and stability: A review of theory, method, and research. *Psychological Bulletin, 118,* 3–34.

Lecavalier, L., Leone, S., & Wiltz, J. (2006). The impact of behaviour problems on caregiver stress in young people with autism spectrum disorders. *Journal of Intellectual Disability Research, 50,* 172–183.

Lord, C., Rutter, M., & Le Couteur, A. (1994). Autism Diagnostic Interview—Revised: A revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *Journal of Autism and Developmental Disorders, 24,* 659–685.

Lounds, J., Seltzer, M. M., Greenberg, J. S., & Shattuck, P. (2007). Transition and change in adolescent and young adults with autism: Longitudinal effects on maternal well-being. *American Journal on Mental Retardation, 112,* 401–417.

Ono, H. (200). Husbands' and wives' education and divorce in the United States and Japan, 1946–2000. *Journal of Family History, 34,* 292–322.

Orsmond, G. I., Lin, L.-Y., & Seltzer, M. M. (2007). Mothers of adolescents and adults with autism: Parenting multiple children with disabilities. *Intellectual and Developmental Disabilities, 45,* 257–260.

Piven, J. (2001). The broad autism phenotype: A complementary strategy for molecular genetic studies of autism. *American Journal of Medical Genetics, 105,* 34–35.

Risdal, D., & Singer, G. H. (2004). Marital adjustment in parents of children with disabilities: A historical review and meta-analysis. *Research and Practice for Persons With Severe Disabilities, 29,* 95–103.

Seltzer, M. M., Krauss, M. W., Orsmond, G. I., & Vestal, C. (2000). Families of adolescents and adults with autism: Uncharted territory. In L. M. Glidden (Ed.), *International review of research on mental retardation* (Vol. 23, pp. 267–294). San Diego, CA: Academic Press.

Seltzer, M. M., Krauss, M. W., Shattuck, P. T., Orsmond, G., Swe, A., & Lord, C. (2003). The symptoms of autism spectrum disorders in adolescence and adulthood. *Journal of Autism and Developmental Disorders, 33,* 565–581.

Shapiro, A. F., Gottman, J. M., & Carrere, S. (2000). The baby and the marriage: Identifying factors that buffer against decline in

marital satisfaction after the first baby arrives. *Journal of Family Psychology, 14,* 59–70.
Shiono, P. H., & Quinn, L. S. (1994). Epidemiology of divorce. *Future of Children, 4,* 15–28.
Smith, L. E., Hong, J., Seltzer, M. M., Greenberg, J. S., Almeida, D. M., & Bishop, S. L. (2010). Daily experiences among mothers of adolescents and adults with autism spectrum disorder. *Journal of Autism and Developmental Disorders, 40,* 167–178.
Solomon, E. (Producer), & Thierry, L. (Producer & Director). (2006). *Autism everyday* [Motion picture]. United States: Autism Speaks.
Sparrow, S. S., Carter, A. S., & Cicchetti, D. V. (1993). *Vineland Screener: Overview, reliability, validity, administration, and scoring.* New Haven, CT: Yale University Child Study Center.
Szatmari, P., Jones, M. B., Fisman, S., Tuff, L., Bartulucci, G., Mahoney, W. J., & Bryson, S. E. (1995). Parent and collateral relatives of children with pervasive developmental disorders: A family history study. *American Journal of Medical Genetics, 60,* 282–290.
Tzeng, J. M., & Mare, R. D. (1995). Labor market and socioeconomic effects on marital stability. *Social Science Research, 24,* 329–351.
Urbano, R. C., & Hodapp, R. M. (2007). Divorce in families of children with Down syndrome: A population-based study. *American Journal on Mental Retardation, 112,* 261–274.
Witt, W. P., Riley, A. W., & Coiro, M. J. (2003). Childhood functional status, family stressors, and psychological adjustment among school-aged children with disabilities in the United States. *Archives of Pediatric Adolescent Medicine, 157,* 687–695.
Wymbs, B. T., Pelham, W. E., Molina, B. S. G., Gnagy, E. M., Wilson, T. K., & Greenhouse, J. B. (2008). Rate and predictors of divorce among parents of youths with ADHD. *Journal of Consulting and Clinical Psychology, 76,* 735–744.

Received November 2, 2009
Revision received March 30, 2010
Accepted April 5, 2010 ■

Trends in the Prevalence of Developmental Disabilities in US Children, 1997–2008

WHAT'S KNOWN ON THIS SUBJECT: US data on the changes in the prevalence of developmental disabilities are scarce. Although there are a few studies on individual disabilities, data examining the impact of the full range of developmental disabilities are unavailable.

WHAT THIS STUDY ADDS: Developmental disabilities make a significant contribution to overall childhood health. We show the health disparities that exist for specific populations and how selected conditions have increased over the past 10 years.

AUTHORS: Coleen A. Boyle, PhD,[a] Sheree Boulet, PhD,[a] Laura A. Schieve, PhD,[a] Robin A. Cohen, PhD,[b] Stephen J. Blumberg, PhD,[b] Marshalyn Yeargin-Allsopp, MD,[a] Susanna Visser, MS,[a] and Michael D. Kogan, PhD[c]

[a]National Center on Birth Defects and Developmental Disabilities and [b]National Center for Health Statistics, Centers for Disease Control and Prevention, Atlanta, Georgia; and [c]Maternal and Child Health Bureau, Health Resources and Services Administration, Rockville, Maryland

KEY WORDS

developmental disabilities, prevalence, autism, attention deficit hyperactivity disorder

ABBREVIATIONS

NHIS—National Health Interview Survey
ADHD—attention deficit hyperactivity disorder

All authors made substantial intellectual contributions to the study, including the conception and design, acquisition of data, analysis, and interpretation. All authors participated actively in the drafting and revising of the manuscript. Finally, all authors approved the final version that was submitted for publication. Dr Coleen A. Boyle had full access to all the data and takes responsibility for the integrity of the data and accuracy of the data analysis and contributed to the study design and concept, analysis and interpretation of the data, drafting of the manuscript, critical review of the manuscript, and statistical analysis. Dr Sheree Boulet contributed to the study design and concept, acquisition of the data, analysis and interpretation of the data, and critical review of the manuscript. Dr Laura Schieve contributed to the study design and concept, analysis and interpretation of the data, drafting of the manuscript, and critical review of the manuscript. Dr Robin A. Cohen contributed to the acquisition of the data and analysis and interpretation of the data. Dr Stephen J. Blumberg contributed to the analysis and interpretation of the data, drafting of the manuscript, and critical review of the manuscript. Dr Marshalyn Yeargin-Allsopp contributed to the analysis and interpretation of the data, drafting of the manuscript, and critical review of the manuscript. Dr Susanna Visser contributed to the analysis and interpretation of the data, drafting of the manuscript, and critical review of the manuscript. Dr Michael D. Kogan contributed to the analysis and interpretation of the data, drafting of the manuscript, and critical review of the manuscript.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention or the Health Resources and Services Administration.

www.pediatrics.org/cgi/doi/10.1542/peds.2010-2989

doi:10.1542/peds.2010-2989

Accepted for publication Feb 25, 2011

Address correspondence to Coleen A. Boyle, PhD, National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, 1600 Clifton Rd, Atlanta, GA 30333. E-mail: cboyle@cdc.gov

(Continued on last page)

abstract

OBJECTIVE: To fill gaps in crucial data needed for health and educational planning, we determined the prevalence of developmental disabilities in US children and in selected populations for a recent 12-year period.

PARTICIPANTS AND METHODS: We used data on children aged 3 to 17 years from the 1997–2008 National Health Interview Surveys, which are ongoing nationally representative samples of US households. Parent-reported diagnoses of the following were included: attention deficit hyperactivity disorder; intellectual disability; cerebral palsy; autism; seizures; stuttering or stammering; moderate to profound hearing loss; blindness; learning disorders; and/or other developmental delays.

RESULTS: Boys had a higher prevalence overall and for a number of select disabilities compared with girls. Hispanic children had the lowest prevalence for a number of disabilities compared with non-Hispanic white and black children. Low income and public health insurance were associated with a higher prevalence of many disabilities. Prevalence of any developmental disability increased from 12.84% to 15.04% over 12 years. Autism, attention deficit hyperactivity disorder, and other developmental delays increased, whereas hearing loss showed a significant decline. These trends were found in all of the sociodemographic subgroups, except for autism in non-Hispanic black children.

CONCLUSIONS: Developmental disabilities are common and were reported in ~1 in 6 children in the United States in 2006–2008. The number of children with select developmental disabilities (autism, attention deficit hyperactivity disorder, and other developmental delays) has increased, requiring more health and education services. Additional study of the influence of risk-factor shifts, changes in acceptance, and benefits of early services is needed. *Pediatrics* 2011;127:1034–1042

Data on the prevalence of developmental disabilities have been used to describe the importance of these health problems and to assess the educational, medical, and social support needs for children with developmental disabilities and their families. Estimates of the prevalence of developmental disabilities in US children on the basis of the 1988 National Health Interview Survey (NHIS) indicated that 16.8% of children younger than 18 years of age had lifelong conditions arising in early childhood as a result of cognitive or physical impairment or a combination of the 2.[1] Findings from more recent surveys that used a more restrictive definition of developmental disabilities suggested that 13.2% of children had 1 or more developmental disabilities during 1997–2005 and 1.6% had 3 or more developmental disabilities.[2] These studies also documented the considerable impact of the disorders as measured by higher rates of health and special-education service use for children with developmental disabilities compared with children without developmental disabilities.

A number of factors may have influenced the prevalence of developmental disabilities over the past 10 to 15 years, including improved survival of the growing number of children born preterm or with birth defects or genetic disorders, such as spina bifida and Down syndrome,[3] whose improved survival may be offset by a disproportionate burden of neurologic and other impairments.[4,5] Other trends and medical practice changes that might contribute to a reduction of developmental disabilities in the population include increases in prenatal diagnosis and therapeutic abortion, older maternal age, new infant vaccines, and the expansion of newborn screening.[6,7] Finally, increased awareness and improved diagnosis, particularly for conditions with a behavioral phenotype, such as autism or attention deficit hyperactivity disorder (ADHD), may have contributed to changes over time.

Since 1997, the NHIS has routinely included questions on a broad array of developmental disabilities among children younger than 18 years of age. This survey, with population-based annual samples and consistent verbiage in individual disability condition questions, is ideal for monitoring trends in prevalence over time. We used data for a 12-year time period (1997–2008) to examine (1) the national prevalence of developmental disabilities according to major demographic and socioeconomic characteristics and (2) changes in the prevalence of developmental disabilities over time.

PARTICIPANTS AND METHODS

We used the Family Core and Sample Child Components of the NHIS from 1997 to 2008. The NHIS is an ongoing annual survey, conducted by the Centers for Disease Control and Prevention, National Center for Health Statistics, that uses a multistage probability sample to estimate the prevalence of a number of health conditions in the civilian noninstitutionalized population of the United States.[8,9] Demographic and health data on family members are obtained through an in-person interview with a knowledgeable adult family member. For the Sample Child component, more detailed data are obtained for 1 randomly selected child younger than 18 years of age. For more than 90% of the children included in the NHIS Sample Child component, the knowledgeable adult interviewed was a parent or legal guardian.

The current analysis was limited to children aged 3 to 17 years (total 1997–2008 unweighted sample size: 119 367). Children younger than 3 years of age were excluded because many developmental disabilities are not recognized or diagnosed before that age. The average household response rate for the NHIS was 88.3% (range of annual rates: 84.9–91.8%); the average conditional response rate for the sample child component was 91.2% (range: 85.6–93.7%).

The specific conditions assessed were as follows: ADHD; cerebral palsy; autism; seizures; stammering or stuttering; mental retardation; moderate to profound hearing loss; blindness; learning disorders; and other developmental delays (see Table 1 for the survey questions). The same set of questions were asked over the 11 survey years; the ex-

TABLE 1 The NHIS Questions on Developmental Disabilities, 1997–2008

Condition	Survey Question
ADHD/attention deficit disorder (ADD),[a] autism, cerebral palsy, mental retardation,[b] and other developmental delay	"Has a doctor or health professional ever told you that [survey child] had any of the following conditions?"
Seizures and stuttering or stammering	"During the past 12 months, has [survey child] had any of the following conditions?"
Moderate to profound hearing loss	"Which statement best describes [survey child's] hearing without a hearing aid: good, a little trouble, a lot of trouble, or deaf?"[c]
Blindness	"Is [survey child] blind or unable to see at all?"
Learning disability	"Has a representative from the school of a health professional ever told you that [survey child] has a learning disability?"

[a] NHIS shifted from asking about ADD in 1997–1999 to asking about ADD and ADHD in 2000 and later.

[b] Referred to as intellectual disability in the text and tables.

[c] Categories were revised in 2008 to the following: excellent; good; a little trouble; moderate trouble; a lot of trouble; and deaf. Moderate to profound hearing loss included the categories of deaf and a lot of trouble hearing for 1997–2007 and moderate trouble, a lot of trouble, and deaf for 2008.

ception was an expansion of the hearing-loss categories in 2008 (see Table 1 for details). Although the NHIS questionnaire used the term "mental retardation," to be more closely aligned to currently accepted terminology, we refer to this condition as "intellectual disability."[10] The time frame for the majority of the questions refers to whether the child was "ever" diagnosed with the condition; for seizures and stuttering or stammering the reference period was the "past 12 months," and moderate to profound hearing loss and blindness referred to the current status of the child. A child was considered to currently have a condition if there was an affirmative response, regardless of the time frame of the questions. There was substantial collinearity between learning disabilities and intellectual disabilities, and we therefore report learning disabilities as a consequence of the intellectual disability rather than a co-occurring condition. That is, children with reported intellectual disabilities and learning disabilities were only included in the intellectual disability category.

We examined the prevalence of any parent-reported developmental disabilities and of each individual developmental disability for the 12-year period combined and assessed how the estimates varied by a number of demographic and socioeconomic characteristics, including the child's age; gender and race/ethnicity; mother's education; total family income level from all sources, including supplemental security income (with income defined relative to the federal poverty level); and health insurance status (any public, private-only, no health insurance reported). Children covered by both private insurance and the state's Medicaid programs are included under "any public." We also assessed secular trends for each disability over 4 3-year time intervals (1997–1999; 2000–2002; 2003–2005; and 2006–2008). For the disabilities with statistically significant temporal trends, we conducted additional analyses to determine whether trends were uniform within the demographic and socioeconomic subgroups. Income stratification in this report is based on both reported and imputed income.[11]

Prevalence estimates were weighted using NHIS weights to represent the US noninstitutionalized population of children. Variance estimates were produced using Sudaan software to account for the complex NHIS sample design. χ^2 Tests were used to determine whether the prevalence estimates differed among the various groups being compared. Wald-F tests were used to assess linear trends over the 4-calendar-year time periods. All associations and differences described in the text were statistically significant at the $P < .05$ level. Human subject review was not required for this analysis of publicly available data.

RESULTS

Prevalence and Demographic Characteristics

The prevalence of any developmental disability in 1997–2008 was 13.87% and ranged from 0.13% for blindness to 6.69% for ADHD and 7.66% for learning disabilities (Table 2). In general, there was higher prevalence in older children for conditions likely to be first recognized or confirmed in the school years, including ADHD and learning disabilities. Little change across age groups was noted for cerebral palsy, moderate to profound hearing loss, and other developmental delays. There was a lower prevalence in older children for stuttering or stammering. Hispanic children had a lower prevalence of several disorders relative to non-Hispanic white and black children, including ADHD and learning disabilities; the prevalence of other developmental delays was higher only in comparison to non-Hispanic white children. Stuttering or stammering was reported more often in non-Hispanic black children than non-Hispanic white children. Boys had twice the prevalence of any developmental disability and excess prevalence for ADHD, autism, learning disabilities, stuttering or stammering, and other developmental delays, specifically.

There was a nearly twofold higher prevalence of any reported developmental disability among children insured by Medicaid relative to those insured by private insurance, and this pattern was statistically significant for ADHD, learning disabilities, intellectual disabilities, seizures, stuttering or stammering, and other developmental delays. Family incomes below the federal poverty level were associated with a higher prevalence of parent-reported developmental disabilities overall and learning disabilities, intellectual disabilities, stuttering or stammering, and other developmental delays, specifically. Lower maternal education (ie, any attainment less than a college degree) was associated with a higher prevalence of any developmental disabilities, learning disabilities, and stuttering or stammering.

Time Trends

For all developmental disabilities combined, there was a small, but statistically significant, linear increase in the prevalence over the 4 time periods, from 12.84% in 1997–1999 to 15.04% in 2006–2008 (Table 3). Of the individual disorders, ADHD and autism showed significant and successive increases over time. Other developmental delays, a catch-all category, also showed significant increases over the time period, but the increase was observed only between the most recent 2 intervals (from 2003–2005 to 2006–2008).

ADHD, because of its considerably higher prevalence, was chiefly responsible for the upward trend in the overall prevalence of developmental disabilities, with a 33% increase in prevalence from 1997–1999 to 2006–2008. Autism, however, showed, by far, the largest relative increase, with nearly a fourfold change from a prevalence of 0.19% in 1997–1999 to 0.74% in 2006–2008. Moderate to profound hearing loss was the only disorder to decline in prevalence, showing a 31% decrease from 1997–1999 to 2006–2008.

Although the magnitude of the change varied somewhat among the various descriptive factors (Table 4), in general, we observed upward trends in the parent-reported prevalence of ADHD and autism and a decrease for moderate to profound hearing loss. One exception was race/ethnicity and autism, with a lack of a significant increase in non-Hispanic black children.

DISCUSSION

Developmental disabilities affect a significant proportion of children in the United States. We found that 15% of children aged 3 to 17 years, or nearly 10 million children in 2006–2008, had a developmental disability on the basis of parent report. The 17% increase in prevalence over the 12-year period represents ~1.8 million more children with developmental disabilities in 2006–2008 than a decade earlier.

It is difficult to corroborate the overall prevalence reported in this study because of the lack of comparable studies using a similar grouping of conditions. In comparing the prevalence for individual disorders, however, we find good agreement for some of the prevalence estimates. A comparable high prevalence of ADHD recently was reported from the 2003–2007 National Survey of Children's Health, using a similar set of parent-reported survey questions.[12] Prevalence rates for au-

TABLE 2 Prevalence of Developmental Disabilities in Children Aged 3 to 17 Years, by Selected Demographic and Socioeconomic Factors, NHIS, 1997–2008

Condition	Total, %	Age, %, y		Race and Ethnicity,%			Gender,%		Maternal Education,%			Poverty Level,%	Health Insurance Coverage,%		
		3–10	11–17	Non-Hispanic White	Non-Hispanic Black	Hispanic	Boys	Girls	Less Than High School	High School/ Some College	College Graduate or Higher	<200%	≥200%	Private	Medicaid or CHIP	Uninsured
Any developmental disability	13.87	11.78	16.24[c]	14.99	14.77	10.65[d,e]	18.04[c]	9.50	13.89	14.78	10.88[g,h]	16.08	12.42[j]	12.10	20.28[k]	11.61
ADHD	6.69	4.72	8.93[c]	7.82	6.30	3.87[d,e]	9.51[c]	3.73	5.46	7.26[i]	5.35	7.20	6.36	6.01	9.55[k]	4.97[l]
Autism	0.47	0.56	0.37	0.52	0.41	0.32	0.74[c]	0.19	0.25	0.50	0.61	0.44	0.49	0.45	0.67	0.19
Blind/unable to see at all	0.13	0.10	0.16	0.12	0.13	0.15	0.16	0.10	0.16	0.13	0.07	0.16	0.11	0.10	0.17	0.17
Cerebral palsy[a]	0.39	0.36	0.37	0.39	0.36	0.33	0.36	0.37	0.33	0.35	0.42	0.41	0.34	0.61	0.60	0.33
Moderate to profound hearing loss	0.45	0.44	0.46	0.51	0.41	0.32	0.54	0.35	0.56	0.50	0.28	0.64	0.32	0.34	0.77	0.44
Learning disabilities	7.66	5.07	9.27[c]	7.58	7.62	5.50[d,e]	8.97[c]	5.01	8.06	7.50	4.85[g,h]	8.57	6.03[j]	5.94	10.87[k]	6.16[l]
Intellectual disabilities[b]	0.71	0.59	0.84	0.62	1.06	0.70	0.78	0.63	0.93	0.70	0.48	1.03	0.50[j]	0.44	1.68[k]	0.38[l]
Seizures in the past 12 months	0.67	0.72	0.61	0.66	0.91	0.61	0.73	0.62	0.73	0.75	0.45	0.91	0.52	0.49	1.31[k]	0.46[l]
Stuttered or stammered in the past 12 months	1.60	1.99	1.15[c]	1.27	2.63[f]	1.96	2.25[g]	0.91	2.57	1.59	0.96[g,h]	2.40	1.07[j]	1.08	3.09[k]	1.64[l]
Other developmental delay	3.65	3.86	3.41	3.97	3.62	2.64[d]	4.64[c]	2.61	3.19	3.91	3.32	4.39	3.16[j]	3.03	6.06[k]	2.42[l]

[a] We excluded cerebral palsy from the analysis for 2004–2007 because of the high likelihood of interviewer error arising from a questionnaire change in 2004.
[b] The survey question asked about mental retardation, but we refer to the condition as intellectual disability.
[c] $P < .05$, ages 3–10 vs 11–17 years.
[d] $P < .05$, non-Hispanic white versus Hispanic.
[e] $P < .05$, non-Hispanic black versus Hispanic.
[f] $P < .05$, non-Hispanic white versus non-Hispanic black.
[g] $P < .05$, less than high school versus college graduate.
[h] $P < .05$, high school versus college graduate.
[i] $P < .05$, less than high school versus high school graduate.
[j] $P < .05$, <200% versus ≥200% poverty level.
[k] $P < .05$, private insurance versus Medicaid.
[l] $P < .05$, Medicaid versus uninsured.

TABLE 3 Trends in Prevalence of Specific Developmental Disabilities in Children Aged 3 to 17 Years, NHIS, 1997–2008

Disability	*n* (Unweighted)	All Years, %	1997–1999, %	2000–2002, %	2003–2005, %	2006–2008, %	Percent Change 1997–1999 versus 2006–2008[c]
Any developmental disability	15956	13.87	12.84	13.70	13.88	15.04	17.1[d]
ADHD	7652	6.69	5.69	6.71	6.77	7.57	33.0[d]
Autism	537	0.47	0.19	0.35	0.59	0.74	289.5[d]
Blind/unable to see at all	160	0.13	0.11	0.15	0.12	0.13	18.2
Cerebral palsy	305	0.39	0.39	0.43	[b]	[b]	[b]
Moderate to profound hearing loss	533	0.45	0.55	0.44	0.42	0.38	30.9
Learning disability	8154	7.04	6.86	7.24	6.82	7.24	5.5
Intellectual disability[a]	868	0.71	0.68	0.73	0.75	0.67	−1.5
Seizures, past 12 months	792	0.67	0.66	0.65	0.66	0.72	9.1
Stuttered or stammered, past 12 months	1924	1.60	1.63	1.40	1.69	1.68	3.1
Other developmental delay	3978	3.65	3.40	3.28	3.67	4.24	24.7[d]

Source: Centers for Disease Control and Prevention, National Center for Health Statistics, NHIS.

[a] Survey question asked about mental retardation, but we refer to the condition as intellectual disability.

[b] We excluded cerebral palsy from the analysis for 2004–2007 because of the high likelihood of interviewer error arising from a questionnaire change in 2004.

[c] Percent change between 1997–1999 and 2006–2008.

[d] Test of linear trend over 4 time periods, $P < .05$.

tism, cerebral palsy, seizures, blindness, and stuttering or stammering are comparable with those from several population-based prevalence studies using varied study methods.[13–18] This is particularly relevant for seizures, where the nomenclature, as endorsed by the International League Against Epilepsy, for recurrent seizures is epilepsy and not seizures or seizure disorder.[18] The prevalence of moderate to profound hearing loss was considerably higher, whereas the prevalence of intellectual disabilities was ~50% lower than findings from a population-based surveillance program that requires auditory test results for moderate to profound hearing loss and cognitive test results for intellectual disabilities.[17] A number of factors may have influenced these discordant findings, including a more restrictive case definition in the records-based surveillance program for moderate to profound hearing loss (ie, bilateral measured loss of 40 dB or greater) than that used in the NHIS analysis. In the case of intellectual disabilities, and particularly mild intellectual disabilities, because testing often is done in the context of educational placement, the parent or guardian may never have been told that their child's test results suggested functioning in the intellectual disabilities range. Also, since 1997, federal law has allowed for state and local education agencies to extend the use of the less-specific "developmental delay" category up to 9 years of age, enabling many children to not require a more specific education classification, such as intellectual disability.[19] Some of these children may have been identified in the NHIS by the question "other developmental delay," as suggested by the high and increasing prevalence for this category.[20] Although it is not clear what specific functional problems children with other developmental delays have, Boulet et al[2] showed that 76% have a co-occurring developmental disability and that learning disabilities and ADHD were the most frequent co-occurring conditions.

The 17% increase in all developmental disabilities over the 12 years was caused in large part by shifts in the prevalence of ADHD and autism. Increases in autism during the mid-1990s to late 1990s and continuing through the late 2000s have been noted in a number of studies[14,19,21–23] using varying definitions of autism and study designs, ranging from administrative educational and service system data to retrospective studies of successive birth cohorts of children. Although data on trends in ADHD are less available, they support a similar increase.[23,24] A Danish study[23] reported that trends in the birth cohort prevalence of several neuropsychiatric disorders, including autism and hyperkinetic disorder (*International Classification of Diseases 10 Revision* classification that is closely aligned with the hyperactivity component of ADHD) increased significantly for children born in 1990 through 1999. A US-based study[24] reported significant increases in the prevalence of office-based visits for ADHD during 1991–1998. Finally, an upward trend in prevalence, using US education data, was found for the "other health impaired" education category, which, since 1991, is the education category used for children with ADHD.[19,25] Decreases in the prevalence of moderate to profound hearing loss over the 12-year period have not been reported previously. Trend data from service records over a shorter time frame showed little to no change.[17] Nationally, the number of infants identi-

fied with congenital moderate to profound hearing loss from state newborn-screening programs has increased dramatically with the expansion of universal screening[26]; however, it is unlikely that this program would have impacted the prevalence for this survey. The lower prevalence of moderate to profound hearing loss from the NHIS was limited to 2006–2008; in 2008, there was a modification in the moderate to profound hearing loss categories, which makes it difficult to determine whether this lower trend continues. More data are needed to better understand this finding.

Factors responsible for increases in autism and ADHD are numerous. Availability of services and in how the service system classifies children with behavioral disorders has progressed as we learn more about the advantages of earlier interventions. Improvements in clinical, parental, and societal recognition of and screening for these disorders have occurred. For example, we have national campaigns to increase awareness of autism,[27] and the American Academy of Pediatrics has incorporated ongoing monitoring of a child's development as a practice recommendation for pediatricians in 2007.[28] Another contributing factor may be the efficacy of medications and behavioral treatments for ADHD.[29] There also has been an increase in the prevalence of known prenatal risk factors for these conditions. Examples include increases in the prevalence of preterm birth and the recognition of the full range of potential adverse developmental consequences of late preterm birth,[4,5] shifts toward older parental age, and increases in the prevalence of assisted reproductive technologies and possibly other hormonal infertility treatments and the consequent increase in multiple births, each of which is associated

TABLE 4 Prevalence of ADHD, Autism, and Moderate to Profound Hearing Loss in Children Ages 3 to 17 years, by Select Characteristics and Time Period, NHIS, 1997–2008

Characteristics	ADHD, %				Percentage Change 1997–1999 versus 2006–2008	Autism, %				Percentage change 1997–1999 versus 2006–2008	Moderate to Profound Hearing Loss, %				Percentage Change 1997–1999 versus 2006–2008
	1997–1999	2000–2002	2003–2005	2006–2008		1997–1999	2000–2002	2003–2005	2006–2008		1997–1999	2000–2002	2003–2005	2006–2008	
Age, y															
3–10	4.16	4.80	4.74	5.19	24.8	0.27	0.40	0.72	0.87	222.2	0.54	0.43	0.45	0.34	−37.04
11–17	7.51	8.90	8.98	10.24	36.4	0.11	0.31	0.45	0.59	436.4	0.57	0.46	0.37	0.43	−24.56
Gender															
Male	8.43	9.55	9.44	10.59	25.6	0.31	0.58	0.94	1.12	261.3	0.65	0.57	0.52	0.42	−35.38
Female	2.83	3.73	3.96	4.39	55.1	0.07	0.11	0.22	0.34	385.7	0.45	0.31	0.30	0.35	−22.22
Race/ethnicity															
Non Hispanic white	6.74	7.95	7.80	8.89	31.9	0.16	0.36	0.74	0.88	450.0[a]	0.64	0.45	0.50	0.44	−31.25
Non Hispanic black	4.40	6.08	6.86	7.81	77.5	0.33	0.36	0.51	0.44	33.3	0.39	0.49	0.40	0.35[c]	−10.26
Hispanic	3.13	3.65	4.10	4.39	40.3	0.17	0.25	0.32	0.47	176.5[a]	0.37	0.40	0.20	0.32	−13.51
Mother's education:															
Less than high school	5.08	5.42	5.89	5.44	7.1	0.19	0.16	0.33	0.33	73.7	0.68	0.57	0.47	0.51	−25.00
High school/some college	6.07	7.27	7.33	8.43	38.9	0.16	0.37	0.62	0.85	431.3	0.62	0.50	0.49	0.37	−40.32
College or greater	4.51	5.47	5.15	6.09	35.0	0.27	0.49	0.73	0.88	225.9	0.31	0.24	0.24[c]	0.32	3.23
% of poverty level															
<200	6.05	6.98	7.13	8.54	41.2	0.22	0.35	0.48	0.69	213.6	0.84	0.71	0.55	0.47	−44.05
≥200%	5.45	6.55	6.53	6.89	26.4	0.17	0.35	0.65	0.78	358.8	0.36	0.28	0.32	0.32	−11.11
Health insurance															
Private	5.23	6.18	6.25	6.46	23.5	0.16	0.31	0.63	0.73	356.3	0.41	0.34	0.31	0.31	−24.39
Medicaid or CHIP	8.81	9.40	8.88	10.64	20.8	0.36	0.59	0.59	0.94	161.1	1.29	0.86	0.62	0.55	−57.36
Uninsured	4.33	5.03	5.50	5.17	19.4	[b]	0.22[c]	0.26[c]	0.20[c]	[d]	0.44	0.32[c]	0.61[c]	0.41[c]	−7.3

[a] Test of linear trend over 4 time periods, $P < .05$.

[b] Estimates with an SE of higher than 50% are not shown.

[c] Estimates have a relative SE of more than 30% and less than or equal to 50% and should be used with caution because they do not meet the standards of reliability or precision.

[d] Not calculated because of imprecise estimates.

with adverse developmental outcomes.[30] Finally, given that the shift in developmental disability prevalence over time seems to be focused on conditions that are based on an emotional or behavioral phenotype, a societal shift in the acceptance and destigmatization of such conditions in young children also may play a role.[31]

Several of our findings regarding the descriptive characteristics of children with developmental disabilities were noteworthy. Others studies have reported lower prevalence estimates for autism and ADHD in Hispanics,[12,14,32,33] although findings from more recent studies suggest that the gaps may be closing.[13] Rather than these patterns reflecting true differences, they are more likely the result of language barriers, lack of access to services, and health insurance coverage. The predominance of boys with developmental disabilities also was remarkable. Although the increased gender ratio for selected developmental disabilities is well described, this study showed the pattern present for nearly all developmental disabilities. Some of this is certainly biological, such as X-linked genetic disorders that result in intellectual disabilities and other functional limitations. Others have described a cultural factor related to greater incentive for case finding in boys compared with girls.[34] Alternatively, there may be gender-specific presentations of some of the disorders, particularly for conditions with an exclusively behavior phenotype (ADHD and autism) that favor the identification of boys over girls. ADHD is a good example, in that girls tend to exhibit less of the impulsivity associated with the disorder and therefore maybe be less likely to come to clinical attention.[35]

Regarding socioeconomic inequities, public health insurance coverage seemed to be associated with a higher prevalence of developmental disabilities; low family income and low maternal education had similar but less significant impacts. Larson and Halfon[36] showed a similar inverse socioeconomic gradient with family income and the prevalence of ADHD, learning disabilities, and speech problems but not autism. Some of the impact with public insurance is likely reflecting eligibility for Medicaid for children with disabilities.

The strengths of the NHIS are important to highlight. The survey has a nationally representative sample that allows for generalizability to the US population of 3- to 17-year-old children. The same set of questions was asked of parents in each survey year. As a consequence, this is the only study able to examine, in detail, trends in these disorders. The response rate for the NHIS remained at exemplary high levels over the 11 years, despite the challenges of door-to-door surveys, limiting our concerns about the bias resulting from selectivity and nonresponse.

Limitations also are important to consider. Parent report of medical conditions is not without error. Inaccurate reporting can result from parental distress and the stigma associated with some of the conditions; the questions may be misunderstood or there may be variations in professional terminology used for developmental disabilities; for example, autism can be referred to by more broad or umbrella terms, such as autism spectrum disorders. Also, specific terms fall out of accepted use (mental retardation versus intellectual disability and seizure disorder versus epilepsy). A few studies[33,37,38] have examined the validity of parent report for selected developmental disabilities. Some, but not all, of the conditions seem to have high validity (see Boulet et al[2] for more detail.) Ongoing survey research is needed to maintain the validity of the survey questions, while balancing the benefits of historical information to compare overtime. Finally, although we assumed that many of these conditions are chronic, in fact, a condition may resolve to the point where parents or health care providers may no longer consider the child as having the disorder. Recent evidence[13,39] of this was found for autism, and a longitudinal study showed considerable changes in diagnoses over time for children with physical and emotional or behavior diagnoses. Finally, some children included in the stuttering or stammering or seizures categories may have had transient conditions, resulting in an overestimation of the prevalence of these conditions.

CONCLUSIONS

We found that the number of children with developmental disabilities has increased over the decade. These findings have a direct bearing on the need for health, education, and social services, including the need for more specialized health services (mental health services, medical specialists, therapists, and allied health professionals). Also, the consequent burden on families and caregivers will need to be considered. Finally, more detailed study of the influence of risk factor shifts, changes in acceptance, and benefits of early services is needed to better understand why these shifts have occurred.

ACKNOWLEDGMENTS

The National Health Interview Study is supported by the Centers for Disease Control and Prevention, Atlanta, Georgia.

REFERENCES

1. Boyle CA, Decoufle P, Yeargin-Allsopp M. Prevalence and health impact of developmental disabilities in US children. *Pediatrics*. 1994;93(3):399–403
2. Boulet SL, Boyle CA, Schieve LA. Health care use and health and functional impact of developmental disabilities among US children, 1997–2005. *Arch Pediatr Adolesc Med*. 2009;163(1):19–26
3. Shin M, Besser LM, Kucik JE, Lu C, Siffel C, Correa A. Prevalence of Down syndrome among children and adults in 10 regions in the United States. *Pediatrics*. 2009;124(6):1565–1571
4. Boulet SL, Schieve LA, Boyle CA. Birth weight and health and developmental outcomes in US children, 1997–2005. *Matern Child Health J*. 2009[Epub ahead of print]
5. Petrini JR, Dias T, McCormick MC, Massolo ML, Green NS, Escobar GJ. Increased risk of adverse neurological development for late preterm infants. *J Pediatr*. 2009;154(2):169–176
6. Brosco JP, Mattingly M, Sanders LM. Impact of specific medical interventions on reducing the prevalence of mental retardation. *Arch Pediatr Adolesc Med*. 2006;160(3):302–309
7. Wilcken B, Haas M, Joy P, et al. Expanded newborn screening: outcome in screened and unscreened patients at age 6 years. *Pediatrics*. 2009;124(2). Available at: www.pediatrics.org/cgi/content/full/124/2/e241
8. National Center for Health Statistics, National Health Interview Survey. About the National Health Interview Survey [article online], 2009. Available at: www.cdc.gov/nchs/nhis/about_nhis.htm#sample_design Accessed April 22, 2011
9. National Center for Health Statistics. National Health Interview Survey: research for the 1995–2004 redesign. *Vital Health Stat*. 1999;(126):1–119
10. Schalock RL, Luckasson RA, Shogren KA. Perspectives: the renaming of mental retardation: understanding the change to the term intellectual disability. *Intellectual Dev Disabilities*. 2007;45(2):116–124
11. Schenker N, Raghunathan TE, Chiu PL, Makuc D, Zhang G, Cohen AJ. Multiple imputation of family income and personal earnings in the National Health Interview Survey: methods and examples [article online], 2009. National Center for Health Statistics, Atlanta, GA. Available at: www.cdc.gov/nchs/data/nhis/tecdoc3.pdf Accessed April 22, 2011
12. Centers for Disease Control and Prevention. Increasing prevalence of parent-reported attention-deficit/hyperactivity disorder among children: United States, 2003–2007. *MMWR* 2010;59(44):1439–1443
13. Kogan MD, Blumberg SJ, Schieve LA, et al. The prevalence of parent-reported diagnosis of autism spectrum disorder among children in the United States, 2007. *Pediatrics*. 2009;124(5):1395–1403
14. Autism and Other Developmental Disabilities Monitoring Network. Prevalence of autism spectrum disorders: Autism and Developmental Disabilities Monitoring Network, United States, 2006. *MMWR*. 2009;58(SS-10):2–21
15. Yeargin-Allsopp M, Van Naarden-Braun K, Doernberg N, Benedict R, Kirby R, Durkin M. Prevalence of cerebral palsy in 8-year-old children in three areas of the United States in 2002: a multisite collaboration. *Pediatrics*. 2008;121(3):547–554
16. Murphy CC, Trevathan E, Yeargin-Allsopp M. Prevalence of epilepsy and epileptic seizures in 10-year-old children: results from the Metropolitan Atlanta Developmental Disabilities Study. *Epilepsia*. 1995;36(9):866–872
17. Karapurkar-Bhasin, T, Brocksen S, Avchen RN, Van Naarden-Braun K. Prevalence of four developmental disabilities among children age 8 years: Metropolitan Atlanta Developmental Disabilities Surveillance Program, 1996 and 2000. *MMWR*. 2006;55(SS01):1–9
18. International League Against Epilepsy. Revised terminology and concepts for organization of the epilepsies: report of the Commission on Classification and Terminology [article online], 2009. Available at: www.ilae-epilepsy.org/visitors/centre/ctf/ctfoverview.cfm Accessed April 22, 2011
19. Newschaffer CJ, Falb, MD, Gurney JG. National autism prevalence trends from United States special education data. *Pediatrics*. 115(3). www.pediatrics.org/cgi/content/full/115/3/e277
20. Larson SA, Lakin KC, Doljanac D. (2005). Problems in defining mental retardation and developmental disability: using the National Health Interview Survey [article online], 2005. Vol 7, No 1. DD Data Brief, University of Minnesota-Minneapolis, Institute on Community Integration. Available at: http://rtc.umn.edu/docs/dddb7-1.pdf. Accessed April 22, 2011
21. Gurney JG, Fritz MS, Ness KK, Sievers P, Newschaffer CJ, Shapiro EG. Analysis of prevalence trends of autism spectrum disorder in Minnesota. *Arch Pediatr Adolesc Med*. 2003;157(7):622–627
22. Schechter R, Grether JK. Continuing increases in autism reported to California's developmental services system: Mercury in retrograde. *Arch Gen Psychiatry*. 2008;65(1):15–16
23. Atladóttir HO, Parner ET, Schendel D, Dalsgaard S, Thomsen PH, Thorsen P. Time trends in reported diagnoses of childhood neuropsychiatric disorders. *Arch Pediatr Adolesc Med*. 2007;161(2):193–198
24. Robinson LM, Skaer TL, Sclar DA, Galin RS. Is attention deficit hyperactivity disorder increasing among girls in the US? *CNS Drugs*. 2002;16(2):129–137
25. US Department of Education, Office of Special Education Programs. Clarification of policy to address the needs of children with ADD within general and/or special education [article online], 1999. Available at: www.ed.gov/policy/speced/leg/idea/brief6.html?esp=0. Accessed April 22, 2011
26. Gaffney M, Eichwald J, Grosse SD, Mason CA. Identifying infants with hearing loss: United States, 1999–2007. *MMWR*. 2010;59(8):220–223
27. Daniel KL, Prue C, Taylor MK, Thomas J, Scales M. 'Learn the Signs. Act Early: ' a campaign to help every child reach his or her full potential. *Public Health Reports*. 2009;123 (Suppl 1):e11–e16
28. American Academy of Pediatrics Council on Children with Disabilities. Identifying infants and young children with developmental disorders in the medical home: an algorithm for developmental surveillance and screening. *Pediatrics*. 2006;118(1):405–420
29. MTA Cooperative Group. National Institute of Mental Health multimodal treatment study of ADHD: 24-month outcomes of treatment strategies for attention-deficit/hyperactivity disorder. *Pediatrics*. 2004;113(4):754–761
30. Schieve LA, Devine O, Boyle CA, Petrini JR, Warner L. Estimation of the contribution of non-assisted reproductive technology ovulation stimulation fertility treatments in US singleton and multiple births. *Am J Epidemiol*. 2009;170(11):1396–1407 Dec 1
31. Grinker RR, Yeargin-Allsopp M, Boyle C. Culture and autism spectrum disorders: the impact on prevalence and recognition. In: Amaral D, Geschwin D, Dawson G, Eds. *Autism Spectrum Disorders*. New York, NY: Oxford University Press, In press
32. Froehlich TE, Lanphear BP, Epstein JN, Barbaresi WJ, Katusic SK, Kahn RS. Prevalence, recognition, and treatment of attention-deficit/hyperactivity disorder. *Arch Pediatr Adolesc Med*. 2007;161(9):857–864

33. Schieve LA, Rice C, Boyle C, Visser SN. Mental health in the United States: parental report of diagnosed autism in children aged 4–17 years: United States, 2003–2004. *MMWR*. 2006;55(17):481–486

34. Rowland AS, Lesesne CA, Abramowitz AJ. The epidemiology of attention-deficity/hyperactivity disorder (ADHD): a public health view. *Ment Retard Dev Disabil Res Rev*. 2002;8(3):162–70

35. deHaas PA. Attention styles and peer relationships of hyperactive and normal boys and girls. *J Abnorm Child Psychol*. 1986; 14(3):457–467

36. Larson K, Halfon N. Family income gradients in the health and health care access of US children. *Matern Child Health J*. 2010; 14(3): 332–342

37. Ackland MJ, Wade RQ. Health status of Victorian special school children. *J Paediatr Child Health*. 1995;31(5): 423–427

38. Miller JE, Gaboda D, Davis D. Early childhood illness: comparability of maternal reports and medical records. *Vital Health Stat 2*. 2001;(131):1–10

39. Van Cleave J, Gortmaker SL, Perrin JM. Dynamic of obesity and chronic health conditions among children and youth. *JAMA*. 2010;303(7):623–630

(Continued from first page)

PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).

FINANCIAL DISCLOSURE: *The authors have indicated that they have no personal financial relationships relevant to this article to disclose.*

NeuroMolecular Medicine (2024) 26:20
https://doi.org/10.1007/s12017-024-08781-6

REVIEW

Unraveling the Endocannabinoid System: Exploring Its Therapeutic Potential in Autism Spectrum Disorder

Ankit Jana[1] · Arnab Nath[2] · Palash Sen[3] · Swikriti Kundu[4] · Badrah S. Alghamdi[5,6] · Turki S. Abujamel[7,8] · Muhammad Saboor[9] · Chan Woon-Khiong[1] · Athanasios Alexiou[10,11,12,13] · Marios Papadakis[14] · Mohammad Zubair Alam[5,15] · Ghulam Md Ashraf[9]

Received: 8 December 2023 / Accepted: 4 March 2024

Abstract

The salient features of autism spectrum disorder (ASD) encompass persistent difficulties in social communication, as well as the presence of restricted and repetitive facets of behavior, hobbies, or pursuits, which are often accompanied with cognitive limitations. Over the past few decades, a sizable number of studies have been conducted to enhance our understanding of the pathophysiology of ASD. Preclinical rat models have proven to be extremely valuable in simulating and analyzing the roles of a wide range of established environmental and genetic factors. Recent research has also demonstrated the significant involvement of the endocannabinoid system (ECS) in the pathogenesis of several neuropsychiatric diseases, including ASD. In fact, the ECS has the potential to regulate a multitude of metabolic and cellular pathways associated with autism, including the immune system. Moreover, the ECS has emerged as a promising target for intervention with high predictive validity. Particularly noteworthy are resent preclinical studies in rodents, which describe the onset of ASD-like symptoms after various genetic or pharmacological interventions targeting the ECS, providing encouraging evidence for further exploration in this area.

Keywords Autism spectrum disorder · Endocannabinoid system · Anandamide (AEA) · 2-Arachidonoylglycerol (2-AG)

Abbreviations

2-AG	2-Arachidonoylglycerol
3D	Three dimensional
AEA	Anandamide
ASD	Autism spectrum disorder
ATP	Adenosine triphosphate
BDNF	Brain-derived neurotrophic factor
cDNA	Complementary DNA
CNR2	CB2 receptor genes
CNS	Central nervous system
DAGL-α	Diacylglycerol lipase alpha
eCBs	Endocannabinoids
ECS	Endocannabinoid system
FAAH	Fatty acid amide hydrolase
FXS	Fragile X syndrome
GPR55	G-protein-coupled receptor 55
IL-1	Interleukin 1
IL-12	Interleukin 12
IL-17	Interleukin 17
IL-1β	Interleukin 1β
IL-6	Interleukin 6
LPS	Lipopolysaccharide
MAGL	Monoacyl-glycerol lipase
MECP-2	Methyl-CpG binding protein 2
mGluR-LTD	Group I metabotropic glutamate receptor-dependent long-term depression
MIF	Macrophage inhibitory factor
mRNA	Messenger RNA
mTOR	Mammalian target of rapamycin
NAPE-PLD	N-acyl phosphatidylethanolamine-specific phospholipase D
NK cells	Natural killer cells
NLGNs	Neuroligins
OEA	Oleoylethanolamide
PCR	Polymerase chain reaction
PEA	Palmitoylethanolamide
PET	Position emission tomography
PND	Post-natal day
PNS	Peripheral nervous system
PPAR	Peroxisome proliferator-activated receptors
RTT	Rett syndrome
TGF-β	Transforming growth factor beta

Extended author information available on the last page of the article

Published online: 14 May 2024

THC	9-Tetrahydrocannabinol
THIK-1	Tandem-pore domain halothane-inhibited K^+ channel 1
TLR	Toll-like receptor
TNF-α	Tumor necrosis factor-alpha
VPA	Valproic acid

Introduction

Autism spectrum disorder (ASD) refers to a group of neurodevelopmental disorders that involve significant difficulties in communication and social interaction, as well as the presence of restricted, repetitive, and stereotyped patterns of behavior. Along with these defining features, ASD is commonly associated with a rage of comorbidities, including aggression, hyperactivity, seizures, depression, sleep disturbances, gastrointestinal problems, and immunological malfunction. The global incidence rate of ASD is estimated to be approximately 1%, with a male-to-female ratio of approximately 3:1 (Werling & Geschwind, 2013). Despite its significant public health impact and high prevalence rates, the pathogenesis of ASD remains poorly understood, in large part due to ASD's complicated genetic and environmental interactions. Recent literature suggests that ASD is characterized by impairments in synaptic function, which are believed to contribute to the core symptoms of the disorder. Consequent to the observed synaptic impairments in ASD, the endocannabinoid system (ECS) has gained significant attention as a potential contributor to the initiation and/or progression of the disorder. This is because the ECS has the ability to modulate a variety of synaptic mechanisms, including neurotransmission, synaptic currents, inhibition (E/I balance), and neuroplasticity. Moreover, the ECS has been implicated in several processes that are frequently affected in individuals with ASD, such as social communication, motor control, repetitive behaviors, emotional control, as well as learning, and memory (Zou et al., 2021).

ECS comprises cannabinoid receptors CB1, found as a neuronal target of the psychoactive ingredient of *Cannabis sativa*, 9-tetrahydrocannabinol (THC), and CB2, and their endogenous lipid ligands, i.e., the endocannabinoids (eCBs). These eCBS include anandamide (AEA) and 2-arachidonoylglycerol (2-AG), which are synthesized on demand and function as retrograde neurotransmitters (Pascucci et al., 2020; Su et al., 2021). It is noteworthy that the ECS provides a critical link between the immune system and the central nervous system (CNS). CB2 receptors are primarily found on immune cells and modulate the immune system, whereas CB1 receptors are found abundantly in the CNS (particularly in the hippocampus, cerebral cortex, basal ganglia, and cerebellum), and peripheral nervous system (PNS).

The involvement of the ECS in ASD extends beyond its influence on synaptic function and neuroplasticity. Indeed, emotional and behavioral responses to social and environmental stimuli as well as modulation of learning, memory, seizure susceptibility, and circadian rhythm, are also thought to be regulated by the ECS in ASD (Marco & Laviola, 2012; Marsicano & Lutz, 2006; Rubino et al., 2008; Trezza & Vanderschuren, 2008; Trezza et al., 2012). This highlights the broad impact that the ECS has on various physiological and behavioral processes that are disrupted in individuals with ASD. In addition to preclinical studies, human neuroimaging research has also uncovered the relationships between polymorphisms in the CB1 receptor gene, *CNR1*, and social reward sensitivity, suggesting that variations in CB1 receptors could contribute to ASD-related irregularities in social reward processing. Furthermore, postmortem analysis of the brains diagnosed with ASD has revealed lower CB1 receptor expression, adding further support to the notion that the ECS plays a key role in the pathogenesis of ASD (Aishworiya et al., 2022; Baron-Cohen, 2004; Chakrabarti et al., 2006; Domschke et al., 2008). Regardless of the fact that these data imply the involvement of the ECS in ASD, there is still a dearth of research exploring the role of ECS in ASD, and our knowledge of the EC signaling in ASD remains limited.

This review focuses on studies investigating ECS alterations and the effects of pharmacological modulation of the ECS in animal models of ASD. In addition, the potential of the ECS as a therapeutic target for ASD is discussed.

Genetic Model of ASD and ECS

Variations in the genes encoding CB1 receptors have a significant impact on social behavior in individuals. The observed reduction in CB1 receptors levels among ASD patients suggests a direct association between them. However, studies have shown that CB1 receptor ligand AEA is present in relatively lower amounts in ASD children, whereas the 2-AG level remains unchanged (Pietropaolo et al., 2020).

The CB2 receptor was first identified in 1993 through cDNA-based polymerase chain reaction (PCR) clone of the human promyelocytic leukemic line HL60, using degenerative primers (Munro et al., 1993). CB2 receptors belong to the G-protein-coupled receptor family and are composed of an internal C-terminal, three extracellular and three intracellular loops, seven transmembrane domains, and an external N-terminal. CB2 receptors show approximately 44% amino acid sequence similarity to CB1 receptors with hydrophobic domains 1, 2, 5, 6, 7, and the extracellular domain of both receptors sharing at least 50% similarity in amino acid sequence (Matsuda, 1997). The *CNR2* gene is highly conserved across different taxa. The human *CNR2*

gene consists of a single translated exon flanked by 5' and 3' untranslated regions and a single untranslated exon (Sipe et al., 2005). However, transcriptional products of mammalian CB2 receptor genes (CNR2) vary among species. The mouse (23 kb) and rat (20 kb) CB2 receptor genes are almost four times shorter than the human CB2 receptor gene (90 kb). The mouse CB2 receptor gene is transcribed into two mRNAs from three exons, whereas the rat CB2 receptor gene can be spliced into four mRNAs from three exons (Cording & Bateup, 2023; Onaivi et al., 2013). Compared to human CB2 receptors, the amino acid sequence homology is lower between human and mouse CB2 receptors (82%) than between human and rat CB2 receptors (93%). Although polymorphism of the *CNR2* gene is not well studied, but it may be associated with depression in humans. However, Karsak et al. reported that *CNR2* gene polymorphism correlates with osteoporosis and other autoimmune diseases (Karsak et al., 2005).

Further studies in the Japanese population showed that Q63R polymorphism of the *CNR2* gene is linked with alcoholism (Ishiguro et al., 2007) and depression (Onaivi et al., 2013). *CNR2* gene expression in peripheral immune cells prevents inflammation and neuronal damage and exerts specific changes in the central nervous system. Activated glial cells, NK cells, and monocytes show the highest levels of CB2 receptors indicating that CB2 receptors may be a key player in cytokines release and immune cell migration during different pathophysiological conditions. CB2 receptors have been found to be upregulated during inflammation in other brain-associated cells and have been shown to play a vital role in reducing depression in rodents (Morcuende et. al 2022, Garcia-Gutierrezet. al 2018, Onaivi et. al 2008). Furthermore, the expression level of the *CNR2* gene has been shown to increase in the hippocampus of offspring exposed to VPA (Onaivi, 2006). The evidence presented suggests that overexpression of *CNR2* gene could be a potential therapeutic approach for treating inflammation and depression in autistic children. *CNR2* and *FAAH* genes are closely linked in mice and humans. It has been shown that anandamide-deactivating enzyme FAAH inhibition can ameliorate social disabilities in ASD-related models BTBR and $fmr1^{-/-}$ mice (Wei et al., 2016). It is likely that bidirectional modulation of *CNR2* and *FAAH* genes would likely increase social interaction and reduce anxiety and neuroinflammatory responses in autistic children.

In addition, Fragile X Syndrome (FXS) is one of the significant monogenetic reasons for ASD. FXS occurs due to mutation in the *Fmr1* gene on the X chromosome, which leads to reduction or absence of the FMRP protein (Zou et al., 2021). FMRP is instrumental for the normal development of synapses in the brain, and its absence or reduction can cause various symptoms such as developmental delay, anxiety, intellectual and physical disabilities, and repetitive behaviors among others (Garber et al., 2008). FXS also leads to ASD in at least 30% of cases, and the Fmr1 knockout mouse is considered a model system for FXS (Hagerman et al., 2010; Kazdoba et al., 2014). Patients suffering from FXS have an impaired endocannabinoid signaling system (Zhang & Alger, 2010). Moreover, studies have shown that modulation of either CB1 or CB2 receptors in the Fmr1 knockout mouse can improve some behavioral symptoms associated with ASD (Arnau Busquets-Garcia et al., 2013). JZL184 increases CB1 receptors through the 2-AG signaling pathway, and its application in Fmr1 knockout mouse led to decrease in behavioral abnormalities (Fig. 1) (Arnau Busquets-Garcia et al., 2013; Jung et al., 2012). In addition, the deletion of the *CB1* receptor gene (CN1R) or pharmacological blockage of CB1 receptors resulted in reduced cognitive and seizure-related neurological problems in Fmr1 knockout mouse. However, rimonabant, a promising CB1 targeting drug, has been associated with severe adverse effects (Maria Gomis-González et al., 2016). Modulation of AEA levels may be a potential therapeutic approach. FAAH inhibitors increase the level of AEA, and their application improves cognitive and social behavioral problems in Fmr1 knockout mice (Fig. 1) (Qin et al., 2015). Furthermore, treatment with the CB2 receptor agonist AM630 has shown to ameliorate anxiety and audiogenic seizure behaviors in the Fmr1 knockout mouse model (Fig. 1). Therefore, drugs that can alter the efficiency of CB2 receptors may be a potential therapeutic target to cure ASD-related behavioral traits (Busquets-Garcia et al., 2013).

Neuroligins (NLGNs) are a group of postsynaptic cell adhesion molecules. They control the maturity and function of excitatory and inhibitory synapses in the mammalian brain (Jamain et al., 2008; Südhof, 2008). Mutations of *NLGN3* and *NLGN4* in humans are associated with seizures, X-linked intellectual disability, and autistic behavior. In particular, the $Arg_{451}Cys$ (R451C) missense mutation of *NLGN3* has been linked with ASD in humans. Similarly, *NLGN3* mutant mice with the $R_{451}C$ mutation show impaired social communication, increased synaptic inhibition in the somatosensory cortex, and excitatory transmission in the hippocampus (Etherton et al., 2011). *NLGN3* mutant mice model not only expresses partial characteristics of ASD condition, but it also provides sufficient information about synaptic gene regulation and ASD (Radyushkin et al., 2009). Studies on NLGN3R451C knock-in and NLGN3 knockout mouse models have shown that disruption of tonic EC signaling mediated by CB1 receptors in the hippocampus and the somatosensory cortex causes autistic behaviors (Zamberletti et al., 2017). Although CB2 receptors do not have a direct role in controlling NLGNs associated ASD-like phenotypes, a combination of drugs modulating both CB1 and CB2 receptors may be a possible pharmacological approach to mitigate ASD-related symptoms. Alteration in CB2

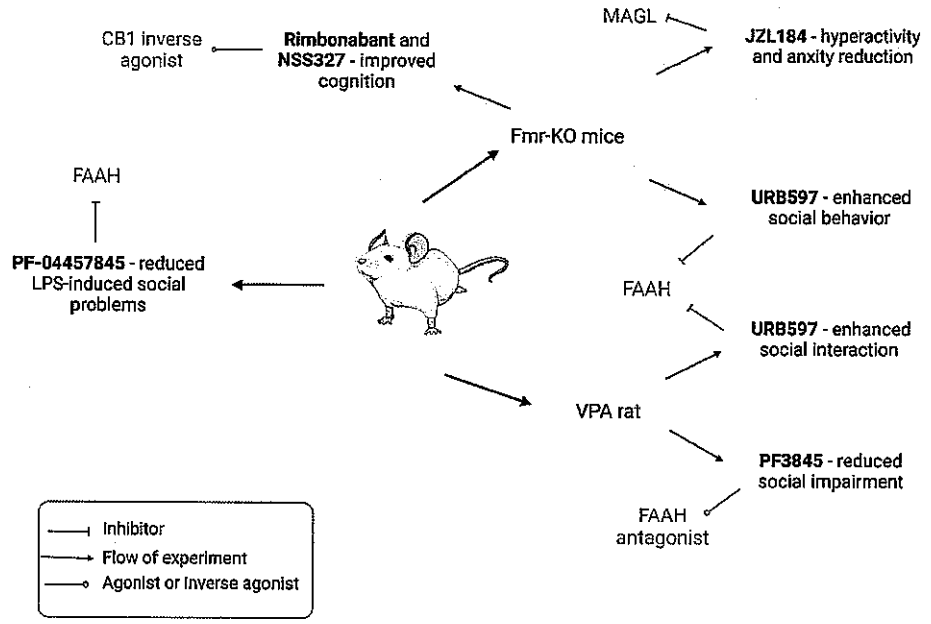


Fig. 1 Simplified representation of the endocannabinoid (EC) system upon nerve stimulus. The endocannabinoids (AEA and 2-AG) trigger the CB1 receptors of presynaptic neurons. 2-AG is generated through hydrolysis of DAG by the DAGLα and DAGLβ enzymes, whereas AEA is synthesized through the action of NAPE-PLD enzyme. Membrane depolarization or nerve stimulation elevates the intracellular Ca^{2+} level that induces the 2-AG and AEA production in postsynaptic neurons. Retrograde attachment of AEA and 2-AG to CB1 receptors initiates downstream pathways that lowers Ca^{2+}, reduces neurotransmitter release and leads to endocannabinoid degradation via MAGL and FAAH. Rimonabant and NESS3027 are two potential inhibitors of CB1 receptors. CB2 receptors are preferentially found in immune cells and reduce IL-1 expression. AM630 blocks CB2 receptors

receptors activity and AEA metabolism have been observed in blood monocyte-derived macrophages and peripheral blood mononuclear cells of ASD patients (Siniscalco et al., 2013). This evidence suggests that CB2 and other endocannabinoid signaling compounds may play a critical role in influencing ASD-related symptoms. Nevertheless, zebrafish and humans share similarities in the endocannabinoid pathway (Bailone et al., 2022), including CB1, CB2 receptors, as well as key enzymes of the endocannabinoid system, such as prostaglandin-endoperoxide synthase 2, fatty acid amide hydrolase, and transient receptor potential Cation Channel 1A (Elphick, 2012; Klee et al., 2012; Lam et al., 2006). Studies using the zebrafish model have established that the CB2 inverse agonist JTE-907 acts as an anxiogenic agent, while the non-selective CB agonist WIN 55,212 has anxiolytic effects (Hasumi et al., 2020; Prasad et al., 2020).

Role of ECS in Pathophysiology of ASD

Uncovering the etiopathogenesis of ASD is extremely challenging because this ailment arises from a complex interplay of multiple genetic and environmental factors that act through a multitude of complicated disease mechanisms, such as imbalances between neuronal excitation and inhibition and hypo- and/or hyper-connectivity (Pardo & Eberhart, 2007). As mentioned earlier, the genetics of ASD are extremely diverse, involving hundreds of ASD susceptibility genes (Roux et al., 2019). The intricacy appears to preclude any simple characterization of pathophysiological mechanisms that can explain the interactions and permutative effects of polygenic mutations, as well as the role of environmental impact (McOmish et al., 2014). However, successful understanding of the etiopathology of ASD can immediately lead to the discovery of new therapeutic options that can treat the root cause of ASD. Currently, existing therapeutic interventions for ASD patients only target peripheral symptoms such as anxiety, irritability, aggression, and seizures, and they are treated with anxiolytics, antipsychotics, and anticonvulsants, in that order. These symptom-focused treatments do not address the root cause of ASD, and they are linked with severe side effects that are particularly undesirable in children. To solve the pressing need for better treatments, animal research is focused on identifying new molecular targets for potent interventions by dissecting prevalent ASD etiopathological pathways.

The ECS has demonstrated pathogenetic significance and potential as a novel therapeutic target in our search for shared contributing factors for ASD.

Firstly, CB1Rs are extensively expressed in the brain (Mackie, 2005). Second, the ECS has emerged as an essential modulator of neuronal function. Endocannabinoid signaling affects synaptogenesis and neural interconnectedness throughout development. Impairment of these pathways underlies the pathophysiology of autism spectrum disorder. As shown in Fig. 2, CB1 receptors are present presynaptically in both GABAergic and glutamatergic neurons. They are triggered by endogenous ligands such as AEA and 2-AG (Berghuis et al., 2007; FREUND et al., 2003). After membrane depolarization or excitation of metabotropic receptors, endogenous cannabinoids including AEA and 2-AG are generated at the postsynaptic location. This generation is caused by calcium elevation, which can induce plasma membrane lipid reconfiguration. The biosynthetic enzyme N-acyl phosphatidylethanolamine-specific phospholipase D (NAPE-PLD) produces AEA, while diacylglycerol lipase alpha (DAGL-a) produces 2-AG (FREUND et al., 2003). These endogenous cannabinoids retrogradely attach to presynaptic CB1 receptors, activating multiple intracellular pathways that lower intracellular calcium levels and restrict neurotransmitter release. Endogenous cannabinoids are eliminated through a reuptake mechanism. AEA is decomposed by fatty acid amide hydrolase (FAAH), and 2-AG by monoacyl-glycerol lipase (MAGL).

In recent times, behavioral conditions including depression, autism, and schizophrenia have been linked to dopamine abnormalities. Evidence from neurochemistry demonstrates that activation of CB1R expression on GABA neurons in the ventral tegmental area (VTA) reduces GABAergic transmission, which in turn increases dopaminergic neurotransmission in the nucleus accumbens (NAc) (Sperlágh et al., 2009). Clarifying the connection between ECS and DA in ASD may aid in improving our knowledge of the etiopathogenesis of the disorder and may lead to the development of new treatment approaches, as dopamine signaling anomalies have been linked to ASD in both animal models (Pascucci et al., 2020) and autistic individuals (Su et al., 2021).

Neuroimmunology of ASD and ECS

Microglia and macrophage are considered to be the key immune cells in repairing CNS injuries and infections, as they mediate phagocytosis of pathogens and initiate neuroinflammatory responses by releasing cytokines such as IL-1,

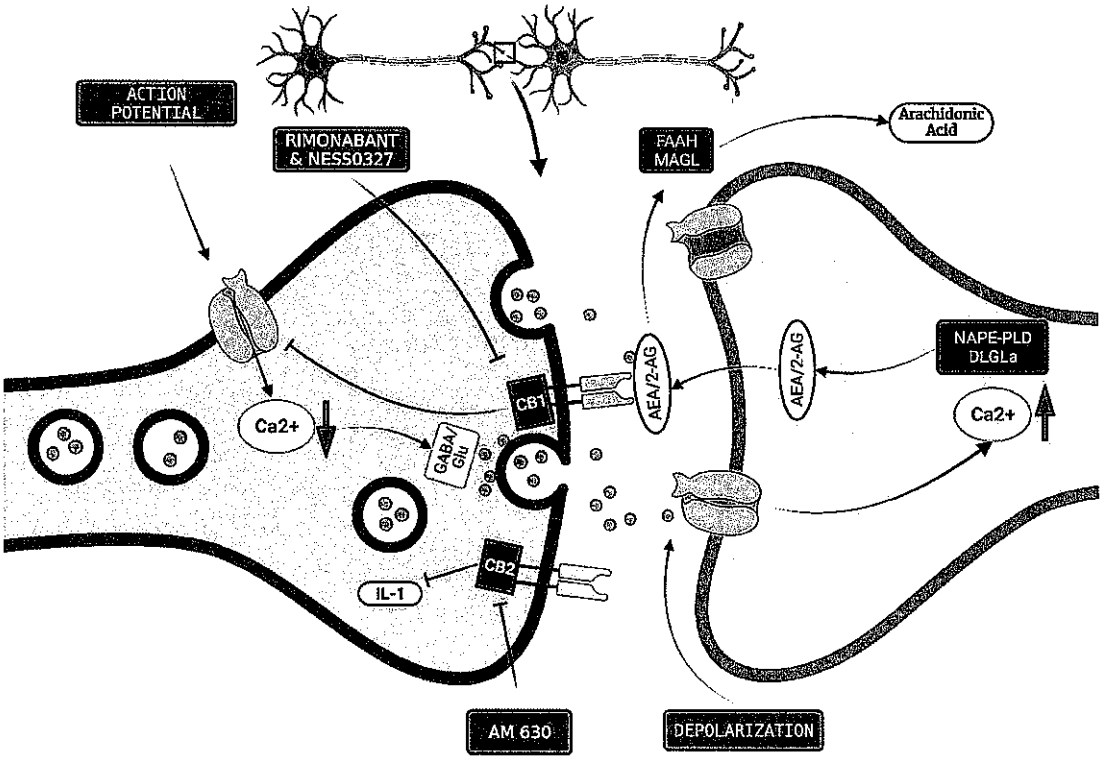


Fig. 2 Role of Endocannabinoid System in Pathophysiology of ASD. CB1 and CB2 receptors can work as a potential therapeutic target of ASD. Rimonabant & NESS0327 targets CB1; whereas AM630 targets CB2

IL-6, TNFα, etc. (Janda et al., 2018; Yang et al., 2010). In recent times, neuroimmunologists have been largely focusing on microglia, the resident immune population of brain parenchyma, which is classified as mononuclear phagocytes, including monocyte-derived cells, dendritic cells, peripheral and CNS associated macrophages (Gomez Perdiguero et al., 2013; Prinz et al., 2011). Microglia are derived from a common pool immune progenitors found in the fetal yolk sac, which also give rises to astrocytes and oligodendrocytes (Ginhoux et al., 2010, 2013). After entering the CNS at embryonic day 9.5, microglia arrive in the CNS before astrocytes and even before the commencement of true cortical neurogenesis, which begins at approximately embryonic day 12 (Hughes et al., 2023; Miller & Gauthier, 2007).

Microglial Involvement and Biology of ASD

Positron emission tomography (PET) and post-mortem analyses have both showed high levels of neuroinflammation and increased microglia activation in the brains of individuals with ASD, indicating the microglial involvement in ASD (Morgan et al., 2010; Vargas et al., 2005). Recently, a distinct microglial signature has been observed from large-scale transcriptomic data analysis from post-mortem cerebral cortex (Suzuki et al., 2013). Changes in synaptic density have also been observed in post-mortem ASD brain tissues (Hutsler & Zhang, 2010), and in ASD mouse models (Comery et al., 1997; Hughes et al., 2023; Tang et al., 2014; Wang et al., 2017). These changes may be due to defects in developmental synaptic pruning (Hansel, 2019). Indeed, current evidence suggests that microglia may contribute to ASD progression through dysregulation of synaptic pruning (Di Marco et al., 2016; Lenz & Nelson, 2018). This hypothesis is supported by the finding that inhibition of microglia autophagy increases synaptic density and reduces sociability in mice (Kim et al., 2017). Studies on the mouse model of Rett syndrome (RTT), a syndromic form of ASD caused by mutations in the methyl-CpG binding protein 2 (MECP-2) encoding gene, provide additional support for the involvement of microglia in ASD pathogenesis (Lombardi et al., 2015). One model of RTT showed that neuronal loss of MECP-2 caused increased synaptic engulfment by microglia in subsequent stages of disease, although microglia themselves did not exhibit any loss (Schafer et al., 2016). This suggests that neuronal loss of MECP-2 alone is sufficient to induce aberrant microglial activity.

In ASD, aggregated alpha-synuclein released from dying dopaminergic neurons activates microglia, leading to high production of proinflammatory cytokines and reactive oxygen species (ROS) which is one of the hallmarks of ASD. Transforming growth factor beta (TGF-β) is one of the factors involved in ASD. Lower levels of TGF-β have been observed in ASD children with high behavioral scores. In addition, macrophage inhibitory factor (MIF), which plays a role in the neural and endocrine systems, is also associated with ASD (Fingerle-Rowson & Bucala, 2001). Two polymorphisms in the promoter region of MIF linked with autism have been observed in genotypic studies of 1000 families (Grigorenko et al., 2008). Differences in NK cell activity have also been observed in ASD patients with some studies showing reduced cytotoxic activity of NK cells in ASD children compared to control. Toll-like receptors (TLRs) expressed by monocytes also act as markers of ASD. TLR-2 and TLR-4 stimulation of monocytes produce proinflammatory cytokines in ASD individuals compared to controls. However, TLR-9 stimulation showed decreased production of proinflammatory cytokines in ASD compared with non-ASD patients, suggesting that monocytes from ASD children have different response in stimulating innate immunity compared with controls. Recent studies on post-mortem brain and spinal cord samples from 11 individual with ASD have revealed high activation of microglia and astroglia, as well as increased levels of cytokines monocyte chemotactic protein-1 (MCP-1) and TGF-beta compared to control (Vargas et al., 2005). After measuring the cytokine levels in brain samples of individuals with ASD in comparison to age and sex-matched non-ASD individuals, there was a significant increase in proinflammatory and Th1 cytokines. These studies provide a clear insight into the immune status of ASD, and due to its anti-inflammatory activity, the ECS can be considered a promising tool for modulating microglial involvement in ASD.

Microglial-Endocannabinoid Signaling and ASD

Preclinical evidence supporting a role of ECS signaling in ASD comes from studies in rodent models of MIA and neuroinflammation (Hughes et al., 2023; Salloum-Asfar et al., 2023). For example, the production of MIA-based IL-17 in response to the innate immune stimulator polyinosine:polycytidylic acid [poly(I:C)] induces abnormal cortex development and ASD-like sociability deficits in mouse offspring (Gunn et al., 2016). Recent in vivo and in vitro studies suggest that ECS plays an outstanding role in communication between the nervous and immune systems during neuronal damage and inflammation in the CNS. Some studies have reported the proinflammatory role of cannabis in protecting against the activation of microglia with a complex mechanism (Bailone et al., 2022; Killestein et al., 2003; Maestroni, 2004). Moreover, proinflammatory cytokines such as IL-6, IL-12, and TNF-α can cause neuroinflammation and neurodegeneration (Wang et al., 2015). The activation of ECS has been identified as one of the mechanisms that protect against the detrimental effects

of these proinflammatory cytokines. During inflammation, 2-AG, which is a ligand of endocannabinoid receptor, is released from various immune cells and induces neuroprotection through several mechanisms by binding to endocannabinoid receptor (Zou & Kumar, 2018). Microglial cells and astrocytes produce 2-AG in response to intracellular Ca^{+2} and glutamate receptor stimulation, which stimulates purinergic P2X7 receptor (Carrier et al., 2004; Hu et al., 2022). The ECS, specially CB2 receptor, mediates T and B lymphocytes proliferation, apoptosis, macrophage-mediated killing of sensitized cells, production of inflammatory cytokines by the inhibiting cyclic AMP/ Protein kinase A (PKA) pathways, migration of B cells, and cytokines induction. Dendritic cells also have the capability to undergo cannabinoid-induced apoptosis due to their immunosuppressive properties (Do et al., 2004; Hu et al., 2022). So, ECS can be a crucial target for ASD therapies due to its anti-inflammatory and immunosuppressive effects. Targeting the ES receptor can lead to side effects like inhibition of learning and memory, hence the ES CB2 receptor, which is present in microglia, is being targeted nowadays. High levels of mRNA and protein of the CB2 receptor have been observed in the blood of autistic children, suggesting its essential role in ASD (Hu et al., 2022; Siniscalco et al., 2013). Various pharmacological molecules can be used to reduce microglia-mediated neurodegeneration and inflammation and beta-amyloid induced neurotoxicity by modulating various ECS receptors like CB1, CB2 and unknown receptors. In the future, we should focus on altered eCB signaling in microglia and the mechanism by which it yields protective and detrimental effects in CNS to provide improved therapies for ASD patients(Kibret et al., 2023).

CBS as a Potential Therapeutic Target of ASD

Several animal models of ASD have shown variations in ECS functionality through various techniques. The Fmr1-KO mouse model of FXS, a monogenic developmental condition linked to ASD, has been investigated the most regarding the ECS in relation to ASD models. Since FXS also lacks therapies, researchers have also explored the ECS for potential medications. In Fmr1-KO mice, aberrations and imbalances in EC signaling have been observed, indicating that their correction via 2-AG, AEA, and cannabinoid CB1 and CB2 receptors may have medical benefits. First, the link between hippocampus mGluR activation and CB mobilization was strengthened in Fmr1-KO mice, while CB1R expression was unaffected (Zamberletti et al., 2017). In addition, an increase in striatal diacylglycerol lipase activity was observed (Maccarrone et al., 2010). Furthermore, the use of the drug JZL184 (which inhibits the breakdown of 2-AG by MAGL) to enhance 2-AG signaling corrected hyperactivity and anxiety in Fmr1-KO mice (Jung et al., 2012). In addition, it has been demonstrated that modulating AEA signaling can improve certain behavioral characteristics of Fmr1-deficient animals. In a study, a single injection of the FAAH blocker URB597 improved unpleasant memory and anxiety-like behavior in Fmr1-deficient mice without impairing their social behavior (Qin et al., 2015). In contrast, Wei et al. discovered that acute injection of URB597 to inhibit FAAH completely corrected the social deficit in Fmr1 deletion mice, indicating that boosting AEA activity at CB1 receptors may exhibit a prosocial behavior effect in mouse models of ASD (Wei et al., 2016). Furthermore, activation of either CB1 or CB2 receptors was found to alleviate certain behavioral symptoms of Fmr1-deficient animals. In the murine model, genetic and pharmacological inhibition of CB1 receptors with the CB1 receptor antagonist/inverse agonist rimonabant reversed cognitive impairments, epilepsy susceptibility, and nociceptive desensitization. Biochemically, CB1 receptor inhibition in the hippocampus of Fmr1 mutant mice corrected the overactivation of mTOR signaling and dendritic spine formation. Intriguingly, treatment with the CB2 inverse agonist AM630 had no effect on anxiety-like behavior or audiogenic seizure susceptibility, indicating that CB1 and CB2 receptors play distinct roles in the behavioral symptoms of FXS (Busquets-Garcia et al., 2013). A recent study by Gomis-Gonzales et al. validated the favorable effect of blocking CB1 receptors on the cognitive function of Fmr1 knockout mice. The authors demonstrated that low doses of rimonabant and the neutral antagonist NESS0327 prevented cognitive abnormalities in Fmr1 knockout mice, as determined by the novel object recognition test. Interestingly, the cognitive benefits of rimonabant were associated with the restoration of mGluR-LTD in the hippocampus of Fmr1-deficient animals (Gomis-González et al., 2016).

In addition, the VPA rat model has been widely used to assess the potential implications of the ECS in ASD. Rats treated with a single injection of VPA on gestational day 12.5 (GD 12.5) exhibited decreased mRNA expression of the enzyme primarily responsible for producing 2-AG DAGL, in the cerebellum, and enhanced activity of the 2-AG-catabolizing enzyme, MAGL in the hippocampus (Kerr et al., 2013). Gene expression of CB1 and CB2 receptors were unaffected; however, rats prenatally exposed to VPA showed altered expression of phosphorylated CB1 receptor in the amygdala, hippocampus, and dorsal striatum, with no alterations in the prefrontal cortex, cerebellum, and nucleus accumbens (Mangiatordi et al., 2023; Servadio et al., 2016). Modifications were also observed in the expression of other receptor targets for ECs, namely PPAR and GPR55 in the frontal cortex and PPAR and GPR55 in the hippocampus (Kerr et al., 2013). AEA and its congeners, oleoylethanolamide (OEA), and palmitoylethanolamide (PEA) were increased in the hippocampus of VPA-exposed rats immediately after

social exposure, indicating that prenatal VPA exposure may have altered AEA signaling in response to interpersonal stimuli (Kerr et al., 2013). Rats exposed to VPA exhibited alterations in AEA metabolism from early life to adulthood. In fact, decreased expression of NAPE-PLD and increased expression of FAAH were observed in whole brains of rats treated with VPA (Servadio et al., 2016).

It is worth noting that increasing AEA signaling by inhibiting its degradation has been shown to alleviate the behavioral phenotype resulting from prenatal VPA exposure. Specifically, a systemic injection of the FAAH antagonist PF3845 at a dose of 10 mg/kg was found to ameliorate the social impairment observed in male mice exposed to VPA (Kerr et al., 2016). In comparison, PF3845 had no effect on the social behavior of female mice exposed to VPA, suggesting that FAAH inhibition may induce sexually dimorphic behaviors in VPA-exposed female mice (Kerr et al., 2016). Likewise, URB597 treatment improved the interaction problems of VPA-exposed pups in the homing test and reversed their social deficiencies in the three-chamber and social play behavior tests (Mechoulam, 2023; Servadio et al., 2016).

Moreover, recently Schiavi et al. examined the role of endocannabinoid neurotransmission in autism-like feautures in Fmr1-Δexon 8 rats. Their study revealed reduced anandamide in the hippocampus and elevated 2-arachidonoylglycerol (2-AG) in the amygdala of these rats. Increasing anandamide levels lessened cognitive abnormalities, while blocking amygdalar 2-AG transmission improved sociability in the rats, as demonstrated by behavioral tests (Schiavi et al., 2023). Considering the pivotal role of endocannabinoids in the etiopathology of ASD described in this article, it is not astonishing that researchers have investigated the therapeutic potential of certain phytocannabinoids, such as cannabidiol (Hill et al., 2023; Parrella et al., 2023; Shani Poleg et al., 2019). Although cannabidiol has only a weak affinity for CB1 receptors, it has been found to inhibit FAAH, which is responsible for the breakdown of AEA (Cristino et al., 2020; Parrella et al., 2023). This is thought to be particularly beneficial for individuals with ASD, who have been shown to have lower levels of AEA (Aishworiya et al., 2022; Aran et al., 2019). According to preliminary findings, cannabidiol has reduced symptoms of hyperactivity, self-injurious behaviors, anxiety, and sleep problems in ASD children. Recent clinical trials have not only demonstrated the effectiveness of cannabidiol in treating ASD symptoms, but also cognitive symptoms in individuals with FXS, without any adverse effects (Heussler et al., 2019; Tartaglia et al., 2019).

Although post-natal LPS injection is not widely accepted as a model of ASD, additional studies suggest that suppressing FAAH may be a treatment option for diseases characterized by reduced social behavior. In mice exposed postnatally to LPS, disruptions to the ECS have been described (Doenni et al., 2016; Mondal et al., 2023). Early-life inflammation caused by a single LPS administration on post-natal day (PND) 14 impaired both male and female adolescent social play and non-play behavior. Interpersonal impairments caused by LPS were associated with decreased CB1 receptor binding, higher AEA levels, and, interestingly, elevated FAAH activity in the amygdala. Prior to the social interaction test, oral administration of 1 mg/kg of the FAAH inhibitor PF-04457845 restored LPS-induced abnormalities in social behavior. A similar improvement was noticed following direct administration of PF-04457845 into the basolateral amygdala, suggesting that altered AEA signaling in this brain area play an important role in transmitting LPS-induced social deficits in at least female mice (Doenni et al., 2016; Shamabadi et al., 2024).

Medical Cannabinoid and Risks

Agonists and antagonists of CB1 and CB2 have the potential to act as drug targets for ASD. However, there are several challenges in designing CB2 receptors-modulating drugs to alleviate ASD symptoms. CB2 receptors, like other lipid-binding receptors, bind to multiple non-specific ligands, making it difficult to design specific agonist and antagonist ligands for CB2 receptors due to off-target effects. The high abundance of CB1 receptors and other lipid-based endocannabinoid receptors also leads to more non-specific binding of CB2 ligands with CB1 receptors, triggering different downstream signaling pathways. Therefore, specific agonists or antagonists targeting CB2 receptors need to be synthesized and undergo multiple in vitro, in vivo, and clinical trials to minimize potential side effects (Atwood & Mackie, 2010). Different agonists of CB2 receptors can target other downstream regulatory molecules to modify their function. CB2 agonists display distinct of functional selectivity (Atwood et al., 2012a, 2012b; Mechoulam, 2023; Pinapati et al., 2024). For instance, aminoalkylindoles (e.g., WIN55,212-2, AM1241, etc.), which are common CB2 receptor agonists, cannot block calcium channels and do not have any role in CB2 receptor internalization. However, these molecules can activate the MAP kinase pathway and stimulate beta-arrestin2 (Shoemaker et al., 2005). They can recruit beta-arrestin2 to the plasma membrane and initiate different downstream signaling pathways. While blocking calcium channels and receptor internalization are crucial components of regulating any signaling pathway, aminoalkylindoles fail to do so (Nguyen et al., 2012). Therefore, standard CB2 receptor agonists can only control a portion of the signaling pathway. Similarly, inverse agonists and antagonists of CB2 receptors can also exhibit similar functional selectivity. Inverse agonists are drugs that selectively couple receptors to one type of downstream signaling molecule while reducing their association with other signaling molecules.

Different inverse agonists can target different signaling molecules, so it is important to specify which molecules are being regulated and how they affect ASD before using these drugs as potential therapeutics. For example, commonly used CB2 receptor inverse agonists like SR144258 selectively target CB2 receptor internalization, whereas other like AM630 have a neutral effect (Atwood et al., 2012a, 2012b; Oka et al., 2005). It is important to specify the regulatory molecules and their impact on ASD for every agonist and antagonist before using them as potential therapeutics. Further research, animal trials and clinical trials are needed to focus on these areas. Furthermore, functional efficacy is also an essential parameter for the application of any drugs, as the density of receptors and downstream signaling molecules determine the practical impact of ligands. Moreover, functional efficacy varies in different cellular and physiological conditions in GPCR targeting drugs (Efron & Taylor, 2023; Strange, 2002). In the case of in vitro studies, transfected cells of CB2 receptors are used to evaluate the functional efficacy of ligands. However, the density of CB2 receptors is usually low in physiological conditions. Therefore, it is important to note that any agonists that work effectively in vitro may only act as partial agonists in physiological conditions. On the other hand, antagonists may behave as a reverse agonists in different physiological conditions, which could be additional challenge to the effective function of drugs particularly in cases where there is a higher density of receptors or downstream signaling molecules (Fong & Heymsfield, 2009). However, the likelihood of this problem occurring in CB2 receptor antagonists is minimal due to their low physiological density. While drugs targeting CB1 receptors are commonly used to treat different brain pathophysiological diseases, drugs targeting CB2 receptors may have a less significant off-target effect due to their low density in normal physiological conditions. Beside this, using cannabinoid-based drugs to treat children and adolescent patients affected by ASD has raised many social and legal controversies (Bou Khalil, 2012). During childhood and adolescence, several critical brain development processes occur, and it is believed that cannabinoid-based drugs may have adverse effects on the brain (Poleg et al., 2019) or induce cannabis addiction. Some clinical data also support these arguments (Bailone et al., 2022; Parrella et al., 2023; Shani Poleg et al., 2019; Salloum-Asfar et al., 2023). For instance, a preclinical rodent study found that chronic application of CB2 receptors agonist WIN 55,212-2 during puberty resulted in severe behavioral disturbance in adulthood (Schneider & Koch, 2003; Schneider et al., 2008).

From the cell biological and neuroimmunological perspective, targeting CB2 receptors with agonists and antagonists could offer new therapeutic options for ameliorating ASD-related symptoms. Promising results have been observed in preclinical and in vitro studies. However, the available clinical trials data is disappointing, with many drugs being withdrawn or failing to reach their primary pharmacological targets. This disparity between preclinical and clinical data highlights the need for further pharmacological studies to determine the clinical and functional efficacy of CB2 receptor-based drugs. To develop possible drugs, humanoid rodent models or 3D organoid models could be used for in vitro studies, while considering all aspects of CB2 receptor pharmacological properties. With careful research and development, CB2-based therapeutics may soon become available in clinics, providing a potential cure for ASD patients.

Conclusion & Perspectives

Clinical and preclinical evidence clearly supports the role of the ECS in the etiopathogenesis of ASD and its potential for medication development. According to Geschwind's "many genes, similar pathways" concept (Geschwind, 2008), evidence from ASD-related mouse lines and pharmacological interventions targeting the ECS in wild-type animals suggests that an imbalance in ECS signaling is a possible common etiopathological route of this complex condition. This is consistent with the ECS's substantial modulatory effect on neural functioning and cognitive maturation. However, this field remains unexplored, and many researchers have emphasized the need to understand how ECS failure contributes to aberrant brain maturation. The use of neurons derived from induced pluripotent stem cells is anticipated to provide new insights, as it has already contributed to our understanding of the etiology of various neuropsychiatric disorders, in order to distinguish the significance of ECS in abnormal brain growth from fully developed synaptic functionality (Wen et al., 2014; Yeh & Levine, 2017).

Although preclinical data suggest that modifying the ECS through the pharmaceutical therapies may be useful for alleviating ASD symptoms, no definitive conclusions can be drawn due to the early stage of research. Evidence suggests that increasing AEA signaling by inhibiting its disintegration promotes prosocial behavior in several mouse models of ASD. Furthermore, acute or chronic inhibition of CB1 receptors has been shown to have positive effects on cognitive impairments in animal models of FXS. Interestingly, medication was systematically administered in most investigations. However, changes in the ECS documented in mouse models of ASD seem to vary depending on the specific brain area studied, suggesting a potential diverse contribution to ASD-like manifestations. If so, it is unlikely that any prospective treatment method would rely on a single targeted molecule.

In this review, we aim to demonstrate that the ECS's role in ASD is a near foregone conclusion, based on the vast amount of data presented here. However, we do not intend to imply that the ECS alone can explain the etiopathology

of ASD. On the contrary, we, along with other experts in the field, are convinced that any comprehensive understanding of ASD must incorporate parallel pathogenic elements. This complex neurodevelopmental disorder is essentially caused by intricate interactions between parallel systems that regulate brain development. The ECS may provide a clue to the identity other major players and the complexity of the situation. Accordingly, drug design should seek new molecular pathways for multi-target pharmacology.

Acknowledgements This research work was funded by the Institutional Fund Projects under grant no. (IFFPP-24-22). Therefore, authors gratefully acknowledge technical and financial support from Ministry of Education and Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, Saudi Arabia.

Author Contributions Ankit Jana: Investigation, Writing—original draft, Writing—review & editing, Visualization, Conceptualization. Arnab Nath: Investigation, Writing—original draft, Writing—review & editing. Palash Sen: Investigation, Writing—original draft. Swikriti Kundu: Writing—review & editing, Artwork. Badrah S. Alghamdi: Artwork, Turki S. Abujamel: Writing—review & editing. Muhammad Saboor: Writing—review & editing. Chan Woon Khiong: Writing—review & editing. Athanasios Alexiou: Writing—review & editing. Marios Papadakis: Conceptualization, Supervision. Mohammad Zubair Alam: Writing-review & editing. Ghulam Md Ashraf: Conceptualization, Supervision.

Funding Open Access funding enabled and organized by Projekt DEAL. This research work was funded by the Institutional Fund Projects under Grant No. (IFFPP-24-22). Therefore, authors gratefully acknowledge technical and financial support from Ministry of Education and Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, Saudi Arabia. Open Access funding enabled and organized by Projekt DEAL. This work was supported by the University of Witten-Herdecke Germany.

Data Availability No datasets were generated or analyzed during the current study.

Declarations

Competing Interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical Approval Not applicable.

Consent for Publication Not applicable.

References

Aishworiya, R., Valica, T., Hagerman, R., & Restrepo, B. (2022). An update on psychopharmacological treatment of autism spectrum disorder. *Neurotherapeutics, 19*(1), 248–262. https://doi.org/10.1007/s13311-022-01183-1

Aran, A., Eylon, M., Harel, M., Polianski, L., Nemirovski, A., Tepper, S., Schnapp, A., Cassuto, H., Wattad, N., & Tam, J. (2019). Lower circulating endocannabinoid levels in children with autism spectrum disorder. *Molecular Autism, 10*(1), 1–11.

Atwood, B. K., & Mackie, K. (2010). CB2: A cannabinoid receptor with an identity crisis. *British Journal of Pharmacology, 160*(3), 467–479.

Atwood, B. K., Straiker, A., & Mackie, K. (2012a). CB2: Therapeutic target-in-waiting. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 38*(1), 16–20. https://doi.org/10.1016/j.pnpbp.2011.12.001

Atwood, B. K., Wager-Miller, J., Haskins, C., Straiker, A., & Mackie, K. (2012b). Functional selectivity in CB2 cannabinoid receptor signaling and regulation: Implications for the therapeutic potential of CB2 ligands. *Molecular Pharmacology, 81*(2), 250–263.

Bailone, R. L., Fukushima, H. C. S., De Aguiar, L. K., & Borra, R. C. (2022). The endocannabinoid system in Zebrafish and its potential to study the effects of Cannabis in humans. *Laboratory Animal Research, 38*(1), 1–12.

Baron-Cohen, S. (2004). Autism: Research into causes and intervention. *Pediatric Rehabilitation, 7*(2), 73–78.

Berghuis, P., Rajnicek, A. M., Morozov, Y. M., Ross, R. A., Mulder, J., Urbán, G. M., Monory, K., Marsicano, G., Matteoli, M., Canty, A., Irving, A. J., Katona, I., Yanagawa, Y., Rakic, P., Lutz, B., Mackie, K., & Harkany, T. (2007). Hardwiring the brain: Endocannabinoids shape neuronal connectivity. *Science, 316*(5828), 1212–1216. https://doi.org/10.1126/science.1137406

Bou Khalil, R. (2012). Would some cannabinoids ameliorate symptoms of autism? *European Child & Adolescent Psychiatry, 21*(4), 237–238.

Busquets-Garcia, A., Gomis-González, M., Guegan, T., Agustín-Pavón, C., Pastor, A., Mato, S., Pérez-Samartín, A., Matute, C., De La Torre, R., & Dierssen, M. (2013). Targeting the endocannabinoid system in the treatment of fragile X syndrome. *Nature Medicine, 19*(5), 603–607.

Carrier, E. J., Kearn, C. S., Barkmeier, A. J., Breese, N. M., Yang, W., Nithipatikom, K., Pfister, S. L., Campbell, W. B., & Hillard, C. J. (2004). Cultured rat microglial cells synthesize the endocannabinoid 2-arachidonylglycerol, which increases proliferation via a CB2 receptor-dependent mechanism. *Molecular Pharmacology, 65*(4), 999–1007. https://doi.org/10.1124/mol.65.4.999

Chakrabarti, B., Kent, L., Suckling, J., Bullmore, E., & Baron-Cohen, S. (2006). Variations in the human cannabinoid receptor (CNR1) gene modulate striatal responses to happy faces. *European Journal of Neuroscience, 23*(7), 1944–1948.

Comery, T. A., Harris, J. B., Willems, P. J., Oostra, B. A., Irwin, S. A., Weiler, I. J., & Greenough, W. T. (1997). Abnormal dendritic spines in fragile X knockout mice: Maturation and pruning deficits. *Proceedings of the National Academy of Sciences USA, 94*(10), 5401–5404.

Cording, K. R., & Bateup, H. S. (2023). Altered motor learning and coordination in mouse models of autism spectrum disorder. *Frontiers in Cellular Neuroscience, 17*, 1270489. https://doi.org/10.3389/fncel.2023.1270489

Cristino, L., Bisogno, T., & Di Marzo, V. (2020). Cannabinoids and the expanded endocannabinoid system in neurological disorders. *Nature Reviews Neurology, 16*(1), 9–29. https://doi.org/10.1038/s41582-019-0284-z

Di Marco, B., Bonaccorso, C. M., Aloisi, E., Antoni, S., & Catania, M. V. (2016). Neuro-inflammatory mechanisms in developmental disorders associated with intellectual disability and autism spectrum disorder: A neuro-immune perspective. *CNS & Neurological Disorders-Drug Targets, 15*(4), 448–463.

Do, Y., McKallip, R. J., Nagarkatti, M., & Nagarkatti, P. S. (2004). Activation through cannabinoid receptors 1 and 2 on dendritic cells triggers NF-kappaB-dependent apoptosis: Novel role for endogenous and exogenous cannabinoids in immunoregulation. *The Journal of Immunology, 173*(4), 2373–2382. https://doi.org/10.4049/jimmunol.173.4.2373

Doenni, V. M., Gray, J. M., Song, C. M., Patel, S., Hill, M. N., & Pittman, Q. J. (2016). Deficient adolescent social behavior following early-life inflammation is ameliorated by augmentation of anandamide signaling. *Brain, Behavior, and Immunity, 58*, 237–247. https://doi.org/10.1016/j.bbi.2016.07.152

Domschke, K., Dannlowski, U., Ohrmann, P., Lawford, B., Bauer, J., Kugel, H., Heindel, W., Young, R., Morris, P., & Arolt, V. (2008). Cannabinoid receptor 1 (CNR1) gene: Impact on antidepressant treatment response and emotion processing in major depression. *European Neuropsychopharmacology, 18*(10), 751–759.

Efron, D., & Taylor, K. (2023). Medicinal Cannabis for paediatric developmental, behavioural and mental health disorders. *International Journal of Environmental Research and Public Health, 20*(8), 5430.

Elphick, M. R. (2012). The evolution and comparative neurobiology of endocannabinoid signalling. *Philosophical Transactions of the Royal Society b: Biological Sciences, 367*(1607), 3201–3215.

Etherton, M., Földy, C., Sharma, M., Tabuchi, K., Liu, X., Shamloo, M., Malenka, R. C., & Südhof, T. C. (2011). Autism-linked neuroligin-3 R451C mutation differentially alters hippocampal and cortical synaptic function. *Proceedings of the National Academy of Sciences USA, 108*(33), 13764–13769.

Fingerle-Rowson, G. R., & Bucala, R. (2001). Neuroendocrine properties of macrophage migration inhibitory factor (MIF). *Immunology and Cell Biology, 79*(4), 368–375. https://doi.org/10.1046/j.1440-1711.2001.01024.x

Fong, T. M., & Heymsfield, S. B. (2009). Cannabinoid-1 receptor inverse agonists: Current understanding of mechanism of action and unanswered questions. *International Journal of Obesity, 33*(9), 947–955. https://doi.org/10.1038/ijo.2009.132

Freund, T. F., Katona, I., & Piomelli, D. (2003). Role of endogenous cannabinoids in synaptic signaling. *Physiological Reviews, 83*(3), 1017–1066. https://doi.org/10.1152/physrev.00004.2003

Garber, K. B., Visootsak, J., & Warren, S. T. (2008). Fragile X syndrome. *European Journal of Human Genetics, 16*(6), 666–672.

Garcia-Gutierrez, M. S., Navarrete, F., Navarro, G., Reyes-Resina, I., Franco, R., Lanciego, J. L., Giner, S., Manzanares, J. (2018). Alterations in gene and protein expression of cannabinoid cb2 and gpr55 receptors in the dorsolateral prefrontal cortex of suicide victims. *Neurotherapeutics*. (2018) *15*, 796–806. https://doi.org/10.1007/s13311-018-0610-y

Geschwind, D. H. (2008). Autism: Many genes, common pathways? *Cell, 135*(3), 391–395. https://doi.org/10.1016/j.cell.2008.10.016

Ginhoux, F., Greter, M., Leboeuf, M., Nandi, S., See, P., Gokhan, S., Mehler, M. F., Conway, S. J., Ng, L. G., & Stanley, E. R. (2010). Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science, 330*(6005), 841–845.

Ginhoux, F., Lim, S., Hoeffel, G., Low, D., & Huber, T. (2013). Origin and differentiation of microglia. *Frontiers in Cellular Neuroscience, 2013*(7), 45.

Gomez Perdiguero, E., Schulz, C., & Geissmann, F. (2013). Development and homeostasis of "resident" myeloid cells: The case of the microglia. *Glia, 61*(1), 112–120. https://doi.org/10.1002/glia.22393

Gomis-González, M., Busquets-Garcia, A., Matute, C., Maldonado, R., Mato, S., & Ozaita, A. (2016). Possible therapeutic doses of cannabinoid type 1 receptor antagonist reverses key alterations in fragile X syndrome mouse model. *Genes (basel)*. https://doi.org/10.3390/genes7090056

Grigorenko, E. L., Han, S. S., Yrigollen, C. M., Leng, L., Mizue, Y., Anderson, G. M., Mulder, E. J., de Bildt, A., Minderaa, R. B., Volkmar, F. R., Chang, J. T., & Bucala, R. (2008). Macrophage migration inhibitory factor and autism spectrum disorders. *Pediatrics, 122*(2), e438-445. https://doi.org/10.1542/peds.2007-3604

Gunn, J. K., Rosales, C. B., Center, K. E., Nuñez, A., Gibson, S. J., Christ, C., & Ehiri, J. E. (2016). Prenatal exposure to cannabis and maternal and child health outcomes: A systematic review and meta-analysis. *British Medical Journal Open, 6*(4), e009986. https://doi.org/10.1136/bmjopen-2015-009986

Hagerman, R., Hoem, G., & Hagerman, P. (2010). Fragile X and autism: Intertwined at the molecular level leading to targeted treatments. *Molecular Autism, 1*(1), 1–14.

Hansel, C. (2019). Deregulation of synaptic plasticity in autism. *Neuroscience Letters, 688*, 58–61. https://doi.org/10.1016/j.neulet.2018.02.003

Hasumi, A., Maeda, H., & Yoshida, K.-I. (2020). Analyzing cannabinoid-induced abnormal behavior in a zebrafish model. *PLoS ONE, 15*(10), e0236606.

Heussler, H., Cohen, J., Silove, N., Tich, N., Bonn-Miller, M. O., Du, W., O'Neill, C., & Sebree, T. (2019). A phase 1/2, open-label assessment of the safety, tolerability, and efficacy of transdermal cannabidiol (ZYN002) for the treatment of pediatric fragile X syndrome. *Journal of Neurodevelopmental Disorders, 11*(1), 1–9.

Hill, M. N., Haney, M., Hillard, C. J., Karhson, D. S., & Vecchiarelli, H. A. (2023). The endocannabinoid system as a putative target for the development of novel drugs for the treatment of psychiatric illnesses. *Psychological Medicine, 53*(15), 7006–7024.

Hu, C., Li, H., Li, J., Luo, X., & Hao, Y. (2022). Microglia: Synaptic modulator in autism spectrum disorder. *Front Psychiatry, 13*, 958661. https://doi.org/10.3389/fpsyt.2022.958661

Hughes, H., Moreno, R., & Ashwood, P. (2023). Innate immune dysfunction and neuroinflammation in autism spectrum disorder (ASD). *Brain, Behavior, and Immunity, 108*, 245–254.

Hutsler, J. J., & Zhang, H. (2010). Increased dendritic spine densities on cortical projection neurons in autism spectrum disorders. *Brain Research, 1309*, 83–94.

Ishiguro, H., Iwasaki, S., Teasenfitz, L., Higuchi, S., Horiuchi, Y., Saito, T., Arinami, T., & Onaivi, E. (2007). Involvement of cannabinoid CB2 receptor in alcohol preference in mice and alcoholism in humans. *The Pharmacogenomics Journal, 7*(6), 380–385.

Jamain, S., Radyushkin, K., Hammerschmidt, K., Granon, S., Boretius, S., Varoqueaux, F., Ramanantsoa, N., Gallego, J., Ronnenberg, A., & Winter, D. (2008). Reduced social interaction and ultrasonic communication in a mouse model of monogenic heritable autism. *Proceedings of the National Academy of Sciences USA, 105*(5), 1710–1715.

Janda, E., Boi, L., & Carta, A. R. (2018). Microglial phagocytosis and its regulation: A therapeutic target in Parkinson's disease? *Frontiers in Molecular Neuroscience, 11*, 144.

Jung, K.-M., Sepers, M., Henstridge, C. M., Lassalle, O., Neuhofer, D., Martin, H., Ginger, M., Frick, A., DiPatrizio, N. V., & Mackie, K. (2012). Uncoupling of the endocannabinoid signalling complex in a mouse model of fragile X syndrome. *Nature Communications, 3*(1), 1–11.

Karsak, M., Cohen-Solal, M., Freudenberg, J., Ostertag, A., Morieux, C., Kornak, U., Essig, J., Erxlebe, E., Bab, I., & Kubisch, C. (2005). Cannabinoid receptor type 2 gene is associated with human osteoporosis. *Human Molecular Genetics, 14*(22), 3389–3396.

Kazdoba, T. M., Leach, P. T., Silverman, J. L., & Crawley, J. N. (2014). Modeling fragile X syndrome in the Fmr1 knockout mouse. *Intractable & Rare Diseases Research, 3*(4), 118–133.

Kerr, D. M., Downey, L., Conboy, M., Finn, D. P., & Roche, M. (2013). Alterations in the endocannabinoid system in the rat valproic acid model of autism. *Behavioural Brain Research, 249*, 124–132. https://doi.org/10.1016/j.bbr.2013.04.043

Kerr, D. M., Gilmartin, A., & Roche, M. (2016). Pharmacological inhibition of fatty acid amide hydrolase attenuates social behavioural deficits in male rats prenatally exposed to valproic acid. *Pharmacological Research, 113*(Pt A), 228–235. https://doi.org/10.1016/j.phrs.2016.08.033

Kibret, B. G., Canseco-Alba, A., Onaivi, E. S., & Engidawork, E. (2023). Crosstalk between the endocannabinoid and mid-brain dopaminergic systems: Implication in dopamine dysregulation. *Frontiers in Behavioral Neuroscience, 17*, 1137957.

Killestein, J., Hoogervorst, E. L., Reif, M., Blauw, B., Smits, M., Uitdehaag, B. M., Nagelkerken, L., & Polman, C. H. (2003). Immunomodulatory effects of orally administered cannabinoids in multiple sclerosis. *Journal of Neuroimmunology, 137*(1–2), 140–143. https://doi.org/10.1016/s0165-5728(03)00045-6

Kim, H. J., Cho, M. H., Shim, W. H., Kim, J. K., Jeon, E. Y., Kim, D. H., & Yoon, S. Y. (2017). Deficient autophagy in microglia impairs synaptic pruning and causes social behavioral defects. *Molecular Psychiatry, 22*(11), 1576–1584. https://doi.org/10.1038/mp.2016.103

Klee, E. W., Schneider, H., Clark, K. J., Cousin, M. A., Ebbert, J. O., Hooten, W. M., Karpyak, V. M., Warner, D. O., & Ekker, S. C. (2012). Zebrafish: A model for the study of addiction genetics. *Human Genetics, 131*(6), 977–1008.

Lam, C., Rastegar, S., & Strähle, U. (2006). Distribution of cannabinoid receptor 1 in the CNS of zebrafish. *Neuroscience, 138*(1), 83–95.

Lenz, K. M., & Nelson, L. H. (2018). Microglia and beyond: innate immune cells as regulators of brain development and behavioral function. *Frontiers in Immunology, 9*, 698. https://doi.org/10.3389/fimmu.2018.00698

Lombardi, L. M., Baker, S. A., & Zoghbi, H. Y. (2015). MECP2 disorders: From the clinic to mice and back. *The Journal of Clinical Investigation, 125*(8), 2914–2923. https://doi.org/10.1172/jci78167

Maccarrone, M., Rossi, S., Bari, M., De Chiara, V., Rapino, C., Musella, A., Bernardi, G., Bagni, C., & Centonze, D. (2010). Abnormal mGlu 5 receptor/endocannabinoid coupling in mice lacking FMRP and BC1 RNA. *Neuropsychopharmacology, 35*(7), 1500–1509. https://doi.org/10.1038/npp.2010.19

Mackie, K. (2005). Distribution of cannabinoid receptors in the central and peripheral nervous system. In R. G. Pertwee (Ed.), *Cannabinoids* (pp. 299–325). Springer.

Maestroni, G. J. (2004). The endogenous cannabinoid 2-arachidonoyl glycerol as in vivo chemoattractant for dendritic cells and adjuvant for Th1 response to a soluble protein. *The FASEB Journal, 18*(15), 1914–1916. https://doi.org/10.1096/fj.04-2190fje

Mangiatordi, G. F., Cavalluzzi, M. M., Delre, P., Lamanna, G., Lumuscio, M. C., Saviano, M., Majoral, J.-P., Mignani, S., Duranti, A., & Lentini, G. (2023). Endocannabinoid degradation enzyme inhibitors as potential antipsychotics: A medicinal chemistry perspective. *Biomedicines, 11*(2), 469.

Marco, E. M., & Laviola, G. (2012). The endocannabinoid system in the regulation of emotions throughout lifespan: A discussion on therapeutic perspectives. *Journal of Psychopharmacology, 26*(1), 150–163.

Marsicano, G., & Lutz, B. (2006). Neuromodulatory functions of the endocannabinoid system. *Journal of Endocrinological Investigation, 29*(3), 27.

Matsuda, L. A. (1997). Molecular aspects of cannabinoid receptors. *Critical Reviews™ in Neurobiology, 11*(2–3), 143.

McOmish, C. E., Burrows, E. L., & Hannan, A. J. (2014). Identifying novel interventional strategies for psychiatric disorders: Integrating genomics, 'enviromics' and gene–environment interactions in valid preclinical models. *British Journal of Pharmacology, 171*(20), 4719–4728. https://doi.org/10.1111/bph.12783

Mechoulam, R. (2023). A delightful trip along the pathway of cannabinoid and endocannabinoid chemistry and pharmacology. *Annual Review of Pharmacology and Toxicology, 63*, 1–13.

Miller, F. D., & Gauthier, A. S. (2007). Timing is everything: Making neurons versus glia in the developing cortex. *Neuron, 54*(3), 357–369. https://doi.org/10.1016/j.neuron.2007.04.019

Mondal, A., Sharma, R., Abiha, U., Ahmad, F., Karan, A., Jayaraj, R. L., & Sundar, V. (2023). A Spectrum of solutions: Unveiling non-pharmacological approaches to manage autism spectrum disorder. *Medicina, 59*(9), 1584.

Morcuende, A., García-Gutiérrez, M. S., Tambaro, S., Nieto, E., Manzanares, J., & Femenia, T. (2022). Immunomodulatory role of CB2 receptors in emotional and cognitive disorders. *Frontiers in Psychiatry, 13*, 866052.

Morgan, J. T., Chana, G., Pardo, C. A., Achim, C., Semendeferi, K., Buckwalter, J., Courchesne, E., & Everall, I. P. (2010). Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. *Biological Psychiatry, 68*(4), 368–376.

Munro, S., Thomas, K. L., & Abu-Shaar, M. (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature, 365*(6441), 61–65.

Nguyen, P. T., Schmid, C. L., Raehal, K. M., Selley, D. E., Bohn, L. M., & Sim-Selley, L. J. (2012). β-arrestin2 regulates cannabinoid CB1 receptor signaling and adaptation in a central nervous system region-dependent manner. *Biological Psychiatry, 71*(8), 714–724. https://doi.org/10.1016/j.biopsych.2011.11.027

Oka, S., Yanagimoto, S., Ikeda, S., Gokoh, M., Kishimoto, S., Waku, K., Ishima, Y., & Sugiura, T. (2005). Evidence for the involvement of the cannabinoid CB2 receptor and its endogenous ligand 2-arachidonoylglycerol in 12-O-tetradecanoylphorbol-13-acetate-induced acute inflammation in mouse ear. *Journal of Biological Chemistry, 280*(18), 18488–18497. https://doi.org/10.1074/jbc.M413260200

Onaivi, E. S. (2006). Neuropsychobiological evidence for the functional presence and expression of cannabinoid CB2 receptors in the brain. *Neuropsychobiology, 54*(4), 231–246.

Onaivi, E. S., Ishiguro, H., Gong, J. P., Patel, S., Meozzi, P. A., Myers, L., Perchuk, A., Mora, Z., Tagliaferro, P. A., Gardner, E., Brusco, A., Akinshola, B. E., Hope, B., Lujilde, J., Inada, T., Iwasaki, S., Macharia, D., Teasenfitz, L., Arinami, T., Uhl, G. R. (2008). Brain neuronal CB2 cannabinoid receptors in drug abuse and depression: from mice to human subjects. *PLoS One. 3*, e1640. https://doi.org/10.1371/journal.pone.0001640

Onaivi, E., Ishiguro, H., Sgro, S., & Leonard, C. (2013). Cannabinoid receptor gene variations in drug addiction and neuropsychiatric disorders. *Journal of Drug and Alcohol Research, 2*(1), 1–11.

Pardo, C. A., & Eberhart, C. G. (2007). The neurobiology of autism. *Brain Pathology, 17*(4), 434–447. https://doi.org/10.1111/j.1750-3639.2007.00102.x

Parrella, N.-F., Hill, A. T., Enticott, P. G., Barhoun, P., Bower, I. S., & Ford, T. C. (2023). A systematic review of cannabidiol trials in neurodevelopmental disorders. *Pharmacology Biochemistry and Behavior, 230*, 173607. https://doi.org/10.1016/j.pbb.2023.173607

Pascucci, T., Colamartino, M., Fiori, E., Sacco, R., Coviello, A., Ventura, R., Puglisi-Allegra, S., Turriziani, L., & Persico, A. M. (2020). P-cresol alters brain dopamine metabolism and exacerbates autism-like behaviors in the BTBR mouse. *Brain Sciences, 10*(4), 233.

Pietropaolo, S., Bellocchio, L., Bouzón-Arnáiz, I., & Yee, B. K. (2020). The role of the endocannabinoid system in autism spectrum disorders: Evidence from mouse studies. *Progress in Molecular Biology and Translational Science, 173*, 183–208.

Pinapati, K. K., Vidya, S., Khan, M. F., Mandal, D., & Banerjee, S. (2024). Gut bacteria, endocannabinoid system, and marijuana addiction: Novel therapeutic implications. *Health Sciences Review, 10*, 100144.

Poleg, S., Golubchik, P., Offen, D., & Weizman, A. (2019). Cannabidiol as a suggested candidate for treatment of autism spectrum disorder. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 89*, 90–96. https://doi.org/10.1016/j.pnpbp.2018.08.030

Prasad, A., Crowe, M., Burton, G., & McGrew, L. (2020). Anxiolytic effects of cannabinoid receptor agonists in the Zebrafish species, Danio Rerio. *The FASEB Journal, 34*(S1), 1–1.

Prinz, M., Priller, J., Sisodia, S. S., & Ransohoff, R. M. (2011). Heterogeneity of CNS myeloid cells and their roles in neurodegeneration. *Nature Neuroscience, 14*(10), 1227–1235. https://doi.org/10.1038/nn.2923

Qin, M., Zeidler, Z., Moulton, K., Krych, L., Xia, Z., & Smith, C. B. (2015). Endocannabinoid-mediated improvement on a test of aversive memory in a mouse model of fragile X syndrome. *Behavioural Brain Research, 291*, 164–171.

Radyushkin, K., Hammerschmidt, K., Boretius, S., Varoqueaux, F., El-Kordi, A., Ronnenberg, A., Winter, D., Frahm, J., Fischer, J., & Brose, N. (2009). Neuroligin-3-deficient mice: Model of a monogenic heritable form of autism with an olfactory deficit. *Genes, Brain and Behavior, 8*(4), 416–425.

Roux, S., Bailly, Y., & Bossu, J. L. (2019). Regional and sex-dependent alterations in Purkinje cell density in the valproate mouse model of autism. *NeuroReport, 30*(2), 82–88. https://doi.org/10.1097/wnr.0000000000001164

Rubino, T., Realini, N., Castiglioni, C., Guidali, C., Vigano, D., Marras, E., Petrosino, S., Perletti, G., Maccarrone, M., & Di Marzo, V. (2008). Role in anxiety behavior of the endocannabinoid system in the prefrontal cortex. *Cerebral Cortex, 18*(6), 1292–1301.

Salloum-Asfar, S., Elsayed, A. K., & Abdulla, S. A. (2023). Chapter 6—Potential approaches and recent advances in biomarker discovery in autism spectrum disorders. In A. S. El-Baz & J. S. Suri (Eds.), *Neural engineering techniques for autism spectrum disorder* (pp. 121–145). Academic Press.

Schafer, D. P., Heller, C. T., Gunner, G., Heller, M., Gordon, C., Hammond, T., Wolf, Y., Jung, S., & Stevens, B. (2016). Microglia contribute to circuit defects in Mecp2 null mice independent of microglia-specific loss of Mecp2 expression. *eLife*. https://doi.org/10.7554/eLife.15224

Schiavi, S., Manduca, A., Carbone, E., Buzzelli, V., Rava, A., Feo, A., Ascone, F., Morena, M., Campolongo, P., Hill, M. N., & Trezza, V. (2023). Anandamide and 2-arachidonoylglycerol differentially modulate autistic-like traits in a genetic model of autism based on FMR1 deletion in rats. *Neuropsychopharmacology, 48*(6), 897–907. https://doi.org/10.1038/s41386-022-01454-7

Schneider, M., & Koch, M. (2003). Chronic pubertal, but not adult chronic cannabinoid treatment impairs sensorimotor gating, recognition memory, and the performance in a progressive ratio task in adult rats. *Neuropsychopharmacology, 28*(10), 1760–1769.

Schneider, M., Schömig, E., & Leweke, F. M. (2008). PRECLINICAL STUDY: Acute and chronic cannabinoid treatment differentially affects recognition memory and social behavior in pubertal and adult rats. *Addiction Biology, 13*(3–4), 345–357.

Servadio, M., Melancia, F., Manduca, A., di Masi, A., Schiavi, S., Cartocci, V., Pallottini, V., Campolongo, P., Ascenzi, P., & Trezza, V. (2016). Targeting anandamide metabolism rescues core and associated autistic-like symptoms in rats prenatally exposed to valproic acid. *Translational Psychiatry, 6*(9), e902. https://doi.org/10.1038/tp.2016.182

Shamabadi, A., Karimi, H., Arabzadeh Bahri, R., Motavaselian, M., & Akhondzadeh, S. (2024). Emerging drugs for the treatment of irritability associated with autism spectrum disorder. *Expert Opinion on Emerging Drugs*. https://doi.org/10.1080/14728214.2024.2313650

Shoemaker, J. L., Ruckle, M. B., Mayeux, P. R., & Prather, P. L. (2005). Agonist-directed trafficking of response by endocannabinoids acting at CB2 receptors. *Journal of Pharmacology and Experimental Therapeutics, 315*(2), 828–838.

Siniscalco, D., Sapone, A., Giordano, C., Cirillo, A., de Magistris, L., Rossi, F., Fasano, A., Bradstreet, J. J., Maione, S., & Antonucci, N. (2013). Cannabinoid receptor type 2, but not type 1, is up-regulated in peripheral blood mononuclear cells of children affected by autistic disorders. *Journal of Autism and Developmental Disorders, 43*(11), 2686–2695. https://doi.org/10.1007/s10803-013-1824-9

Sipe, J. C., Arbour, N., Gerber, A., & Beutler, E. (2005). Reduced endocannabinoid immune modulation by a common cannabinoid 2 (CB2) receptor gene polymorphism: Possible risk for autoimmune disorders. *Journal of Leukocyte Biology, 78*(1), 231–238.

Sperlágh, B., Windisch, K., Andó, R. D., & Vizi, E. S. (2009). Neurochemical evidence that stimulation of CB1 cannabinoid receptors on GABAergic nerve terminals activates the dopaminergic reward system by increasing dopamine release in the rat nucleus accumbens. *Neurochemistry International, 54*(7), 452–457.

Strange, P. G. (2002). Mechanisms of inverse agonism at G-protein-coupled receptors. *Trends in Pharmacological Sciences, 23*(2), 89–95.

Su, T., Yan, Y., Li, Q., Ye, J., & Pei, L. (2021). Endocannabinoid system unlocks the puzzle of autism treatment via microglia. *Frontiers in Psychiatry, 12*, 734837.

Südhof, T. C. (2008). Neuroligins and neurexins link synaptic function to cognitive disease. *Nature, 455*(7215), 903–911.

Suzuki, K., Sugihara, G., Ouchi, Y., Nakamura, K., Futatsubashi, M., Takebayashi, K., Yoshihara, Y., Omata, K., Matsumoto, K., & Tsuchiya, K. J. (2013). Microglial activation in young adults with autism spectrum disorder. *JAMA Psychiatry, 70*(1), 49–58.

Tang, G., Gudsnuk, K., Kuo, S.-H., Cotrina, M. L., Rosoklija, G., Sosunov, A., Sonders, M. S., Kanter, E., Castagna, C., & Yamamoto, A. (2014). Loss of mTOR-dependent macroautophagy causes autistic-like synaptic pruning deficits. *Neuron, 83*(5), 1131–1143.

Tartaglia, N., Bonn-Miller, M., & Hagerman, R. (2019). Treatment of fragile X syndrome with cannabidiol: A case series study and brief review of the literature. *Cannabis and Cannabinoid Research, 4*(1), 3–9.

Trezza, V., Damsteegt, R., Manduca, A., Petrosino, S., Van Kerkhof, L. W., Pasterkamp, R. J., Zhou, Y., Campolongo, P., Cuomo, V., & Di Marzo, V. (2012). Endocannabinoids in amygdala and nucleus accumbens mediate social play reward in adolescent rats. *Journal of Neuroscience, 32*(43), 14899–14908.

Trezza, V., & Vanderschuren, L. J. (2008). Bidirectional cannabinoid modulation of social behavior in adolescent rats. *Psychopharmacology (berl), 197*(2), 217–227.

Vargas, D. L., Nascimbene, C., Krishnan, C., Zimmerman, A. W., & Pardo, C. A. (2005). Neuroglial activation and neuroinflammation in the brain of patients with autism. *Annals of Neurology, 57*(1), 67–81. https://doi.org/10.1002/ana.20315

Wang, M., Li, H., Takumi, T., Qiu, Z., Xu, X., Yu, X., & Bian, W.-J. (2017). Distinct defects in spine formation or pruning in two gene duplication mouse models of autism. *Neuroscience Bulletin, 33*(2), 143–152.

Wang, W. Y., Tan, M. S., Yu, J. T., & Tan, L. (2015). Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease. *Ann Transl Med, 3*(10), 136. https://doi.org/10.3978/j.issn.2305-5839.2015.03.49

Wei, D., Dinh, D., Lee, D., Li, D., Anguren, A., Moreno-Sanz, G., & Piomelli, D. (2016). Enhancement of anandamide-mediated endocannabinoid signaling corrects autism-related social impairment. *Cannabis and Cannabinoid Research, 1*(1), 81–89. https://doi.org/10.1089/can.2015.0008

Wen, Z., Nguyen, H. N., Guo, Z., Lalli, M. A., Wang, X., Su, Y., Kim, N.-S., Yoon, K.-J., Shin, J., & Zhang, C. (2014). Synaptic dysregulation in a human iPS cell model of mental disorders. *Nature, 515*(7527), 414–418.

Werling, D. M., & Geschwind, D. H. (2013). Sex differences in autism spectrum disorders. *Current Opinion in Neurology, 26*(2), 146.

Yang, I., Han, S. J., Kaur, G., Crane, C., & Parsa, A. T. (2010). The role of microglia in central nervous system immunity and glioma immunology. *Journal of Clinical Neuroscience, 17*(1), 6–10.

Yeh, M. L., & Levine, E. S. (2017). Perspectives on the role of endocannabinoids in autism spectrum disorders. *OBM Neurobiology, 1*(2), 5.

Zamberletti, E., Gabaglio, M., & Parolaro, D. (2017). The endocannabinoid system and autism spectrum disorders: Insights from animal models. *International Journal of Molecular Sciences, 18*(9), 1916.

Zhang, L., & Alger, B. E. (2010). Enhanced endocannabinoid signaling elevates neuronal excitability in fragile X syndrome. *Journal of Neuroscience, 30*(16), 5724–5729.

Zou, M., Liu, Y., Xie, S., Wang, L., Li, D., Li, L., Wang, F., Zhang, Y., Xia, W., & Sun, C. (2021). Alterations of the endocannabinoid system and its therapeutic potential in autism spectrum disorder. *Open Biology, 11*(2), 200306.

Zou, S., & Kumar, U. (2018). Cannabinoid receptors and the endocannabinoid system: Signaling and function in the central nervous system. *International Journal of Molecular Sciences*. https://doi.org/10.3390/ijms19030833

Authors and Affiliations

Ankit Jana[1] · Arnab Nath[2] · Palash Sen[3] · Swikriti Kundu[4] · Badrah S. Alghamdi[5,6] · Turki S. Abujamel[7,8] · Muhammad Saboor[9] · Chan Woon-Khiong[1] · Athanasios Alexiou[10,11,12,13] · Marios Papadakis[14] · Mohammad Zubair Alam[5,15] · Ghulam Md Ashraf[9]

✉ Marios Papadakis
drmariospapadakis@gmail.com

✉ Ghulam Md Ashraf
gashraf@sharjah.ac.ae

Ankit Jana
ankitjana99@gmail.com

Arnab Nath
natharnab572@gmail.com

Palash Sen
Palashsen933@gmail.com

Swikriti Kundu
swikritikundu26@gmail.com

Badrah S. Alghamdi
basalghamdi@kau.edu.sa

Turki S. Abujamel
tabujamel@kau.edu.sa

Muhammad Saboor
m.ahmed@sharjah.ac.ae

Chan Woon-Khiong
dbscwk@nus.edu.sg

Athanasios Alexiou
athanasios.th.alexiou@gmail.com

Mohammad Zubair Alam
mzalam@kau.edu.sa

[1] Department of Biological Sciences, National University of Singapore, Singapore 117558, Singapore

[2] Department of Developmental Biology and Genetics, Indian Institute of Science, Bangalore, Karnataka 560012, India

[3] School of Biosciences, Indian Institute of Technology, Kharagpur, West Bengal 721302, India

[4] Siksha Bhavana, Visva-Bharati University, Bolpur, West Bengal 731235, India

[5] Pre-Clinical Research Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia

[6] Department of Physiology, Neuroscience Unit, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia

[7] Vaccines and Immunotherapy Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia

[8] Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia

[9] Department of Medical Laboratory Sciences, College of Health Sciences, and Research Institute for Medical and Health Sciences, University of Sharjah, P.O. Box 27272, Sharjah, United Arab Emirates

[10] University Centre for Research & Development, Chandigarh University, Chandigarh-Ludhiana Highway, Mohali, Punjab, India

[11] Department of Research & Development, Funogen, Athens, Greece

[12] Department of Research & Development, AFNP Med, 1030 Vienna, Austria

[13] Department of Science and Engineering, Novel Global Community Educational Foundation, Hebersham, NSW 2770, Australia

[14] Department of Surgery II, University Hospital Witten-Herdecke, University of Witten-Herdecke, Heusnerstrasse 40, 42283 Wuppertal, Germany

[15] Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia

Cannabis and Cannabinoid Research
Volume X, Number X, 2022

DOI: 10.1089/can.2022.0112

Use of Medical Cannabis in Patients with Gilles de la Tourette's Syndrome in a Real-World Setting

Dana Barchel,[1,*] Orit Stolar,[2] Tomer Ziv-Baran,[3] Itai Gueta,[4] Matitiahu Berkovitch,[1] Elkana Kohn,[1] and Lihi Bar-Lev Schleider[5]

Abstract

Objective: Tourette's syndrome (TS) is a neurodevelopmental disorder characterized by vocal and motor tics and other comorbidities. Clinical recommendations for the use of medical cannabis are established, yet further guidance is needed. The aim of this study was to describe the experience of patients with TS with medical cannabis.

Materials and Methods: TS patients were recruited from a registry of patients ("Tikun Olam" company). Questionnaires were answered before and after 6 months of treatment. Patients were divided into two groups: (A) patients who responded and (B) patients who did not respond to the follow-up questionnaire. In group A, an analysis was made to evaluate the presence and frequency of motor and vocal tics. The patients' general mood, employment status, quality of life, and comorbidities were also included in the analysis.

Results: Seventy patients were identified. The tetrahydrocannabinol and cannabidiol mean daily dose was 123 and 50.5 mg, respectively. In group A, a statistically significant improvement was identified in quality of life ($p < 0.005$), employment status ($p = 0.027$), and in the reduction of the number of medications ($p < 0.005$). Sixty-seven percent and 89% of patients with obsessive-compulsive disorder and anxiety comorbidities, respectively, reported an improvement. No statistically significant improvement was identified in motor tics ($p = 0.375$), vocal tics ($p > 0.999$), tics frequency ($p = 0.062$), or general mood ($p = 0.129$). The most frequent adverse effects were dizziness ($n = 4$) and increased appetite ($n = 3$).

Conclusion: Subjective reports from TS patients suggest that medical cannabis may improve their quality of life and comorbidities. More studies are needed to evaluate the efficacy and safety of medical cannabis.
Registry in the MOH: https://www.moh.gov.sg/ (Trial number: 0185-19-ASF)

Keywords: Tourette's syndrome; medical cannabis; quality of life; obsessive compulsive disorder; motor and vocal tics

Introduction

Gilles de la Tourette's syndrome (Tourette's syndrome [TS]) is a neurodevelopmental disorder characterized by vocal and motor tics that cause distress and functional impairment.[1] Based upon the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), TS can be diagnosed by the presence of two or more motor tics and at least one vocal tic, occurring multiple times a day, nearly every day, for longer than a year, with onset before the age of 18.[2] Tics are described as sudden movements or vocalizations, which are repetitive and stereotypical, and may include eye blinking, jerks of the head, shoulders and torso, facial grimaces, humming noises, throat clearing, sniffing, grunting or

[1]Clinical Pharmacology and Toxicology Unit, Shamir (Assaf Harofeh) Medical Center, Zerifin, Israel; affiliated to Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel.
[2]Autistic Spectrum Disorder Clinic, Shamir (Assaf Harofeh) Medical Center, Zerifin, Israel; affiliated to Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel.
[3]Department of Epidemiology and Preventive Medicine, School of Public Health, Tel-Aviv University, Tel-Aviv, Israel.
[4]Internal Medicine A, Sheba Medical Center, The Institute of Clinical Pharmacology and Toxicology, Sheba Medical Center, Tel Hashomer, Israel; Sackler School of Medicine, Tel-Aviv University, Israel.
[5]Research Department, Tikun Olam—Cannbit Pharmaceuticals, Beer-Sheva, Israel.

*Address correspondence to: Dana Barchel, MD, Clinical Pharmacology and Toxicology Unit, Shamir (Assaf Harofeh) Medical Center, Zerifin 70300, Israel, E-mail: danab@shamir.gov.il, barchel6@hotmail.com

squealing and calling out a word or phrase.[1] Dysfunctional neurobiological and psychological processes contribute to the development and continuance of tics; however, emotional status (i.e., anxiety, frustration) and behavioral principles play a role in tic exacerbation.[3]

Tics can be associated with a poorer quality of life and up to 88% of patients reported that tics have a negative effect on their social skills, relationships and difficulties at school.[4] Comorbid disorders, in particular obsessive–compulsive disorder (OCD), attention deficit and hyperactivity disorder (ADHD), and depression and anxiety disorders are reported in most patients.[5] These comorbidities compound to worsen social difficulties and quality of life measures in TS patients.[5]

The pathophysiology of TS is still incompletely understood. Studies demonstrated a loss of inhibition resulting from abnormalities in brain, specifically the GABA and dopaminergic pathways.[6] Based on the fact that the endocannabinoid system plays a paramount role in basal ganglia function by modulating the activity of key neurotransmitters, including dopamine, glutamate, and GABA and thereby, influences different motor responses,[7] a "cannabinoid hypothesis" has been suggested in TS.[8]

Dopamine plays an important role in TS. Dopamine-blocking drugs are used to reduce tics, dopamine agonists are known to enhance tics, and the level of the dopamine metabolite, homovanillic acid, has been found to be reduced in the cerebrospinal fluid of TS patients.[8] Neuroimaging studies using various methods of single-photon emission computed tomography (SPECT) have demonstrated increased dopamine transporter binding and different dopamine D receptor binding in monozygotic twins discordant for TS.[9,10] Specific binding of [123I]AM281 to CB1 receptors using SPECT was also detected in TS patients when evaluating the treatment of tetrahydrocannabinol (THC).[11] Berding et al conducted a pilot [123I]AM281 SPECT study of CB1 receptor binding in TS patients before and during 9-THC therapy, imaging the central cannabinoid CB1 receptors *in vivo*, but found no significant difference in tracer binding to CB1 receptor values.[11]

On the other hand, in a recent study, elevated endocannabinoid levels in TS patients were found, possibly reflecting secondary changes to compensate for alterations in other neurotransmitter systems such as the dopaminergic system or possibly representing the primary cause of TS.[12]

The treatment of TS includes both behavioral interventions and pharmacological treatments. Some of the conventional drugs, including nondopaminergic agents and typical and atypical antipsychotics, are associated with intolerable adverse effects.[13] Clinical recommendations were published in 2019, which included treatment with cannabis-based medication in otherwise treatment-resistant adults with TS as an official recommendation.[14] In 2021, the treatment guidelines of the European Society for the Study of Tourette's syndrome were updated with similar recommendations.[15]

As of today, the experiences with medical cannabis in TS patients is limited and further research is needed.[14,15] In a literature review we found that the first reports of the beneficial effects of cannabis for TS were published in the late 80s and 90s and described TS patients who reported previous use of marijuana with improvements in TS symptoms.[13,16] Treatment of TS patients with THC was first described by Müller-Vahl et al in the late 90s, followed by two studies: a randomized crossover trial and a 6-week randomized trial, demonstrating a reduction in both tics and OCD behaviors.[17–19] More recent publications include a case report of a successful treatment with vaporized cannabis[20] and two publications of a single center's experience with TS patients that describe results from a telephone interview[21] and an online survey.[22]

The use of medical cannabis in children is rapidly growing. The current knowledge on the longterm side-effects of cannabinoids is based mainly on follow-up data from cannabis users.[23] Exposing children and young adults to medical cannabis early in life is inadvisable, because it may cause chronic adverse effects on brain development that increases the risk of psychosis.[24] There are case reports in children with TS who were treated successfully with medical cannabis as a last-resort therapy in which no adverse events were reported.[20,25,26] Hasan et al described a 15-year-old boy with refractory TS and ADHD that was successfully treated with D9-THC.[23,25] Szejko et al published a case report of a 7-year-old boy diagnosed with TS and ADHD who failed on conventional treatment but had significant improvements in tic reduction, ADHD symptoms, mood, stress, and quality of life when treatment was augmented with oral THC.[26] In another publication, Szejko et al describe a case of a 12-year-old boy diagnosed with TS who was successfully treated with a combination of vaporized medicinal cannabis and oral pure THC.[20]

In this study, we aimed to describe the real-life experiences and to assess the long-term effects of patients with TS using medical cannabis by collecting data from reports from a single medical center.

Materials and Methods

TS patients were identified from a medical cannabis clinic's patient registry. Diagnosis was made by a neurologist, according to the DSM-5 criteria.[2] Patients received a license for the use of medical cannabis under the indication of TS from the Israeli Ministry of Health. Before dispensing cannabis to patients, they were instructed by an experienced nurse practitioner on proper administration. The cannabis preparation (oil solution or inflorescence) was made by an approved supplier in Israel (Tikun Olam, Inc., Israel). Each patient completed a questionnaire before treatment and 6 months after treatment according to the standard treatment process at the clinic. Inclusion criteria included patients diagnosed with TS who were treated with medical cannabis for the TS indication.

Patients under 9 years of age were excluded. In most studies, TS starts at a mean age of around 6.7 years (range 1–17), beginning first with motor tics, followed by vocal tics at around age 9. This is why the age threshold chosen in our study was 9 years old.[27] Patients were divided in two groups: group A patients answered the questionnaire before treatment and after 6 months of active treatment and group B patients answered the questionnaire before treatment but did not respond to the 6-month follow-up questionnaire.

We analyzed and compared the baseline characteristics of patients with and without the follow-up questionnaire, the presence of vocal and motor tics, tics frequency, quality of life, general mood, employment status, and number of medications of conventional treatment. The number of medications was analyzed as a continuous variable. All other variables were categorical. Information about the types of conventional drugs used and the drugs that were discontinued was not fully reported and, therefore, was not included in the analysis. Vocal and motor tics were categorized as either present or absent based on the reports of participants. Tic frequency was scaled as daily or weekly. Quality of life was measured on a 5-point Likert scale from very low to very high. This was rated by the patient as part of a self-assessment.[28] General mood was categorized as positive, neutral, or negative.

In group A, a subanalysis of the changes after 6 months was made for all seven parameters and the comorbidities, OCD and ADHD. Reports of adverse events were recorded from the questionnaires and coded using the Medical Dictionary for Regulatory Activities.[29] The study was approved by the local Research Ethics Committee. The need for informed consent form was removed, due to the retrospective nature of the data analysis.

Statistical analysis

Categorical variables were described as frequency and percentage. Continuous variables were evaluated for normal distributions using histogram and Q-Q plot. They were reported as median and interquartile range. Chi-square test and Fishers' exact test or Mann–Whitney tests were applied to compare categorical and continuous variables, respectively, between those who continued the treatment at 6 months and those who stopped and between those who participated in the follow-up study and those who did not. The McNemar and Wilcoxon signed-rank tests were used to compare categorical and continuous variables between the two time points. All statistical tests were two sided and $p < 0.05$ was considered statistically significant. SPSS (IBM SPSS Statistics for Windows, version 25; IBM corp., Armonk, NY, USA, 2017).

Results

Eighty patients with the diagnosis of TS were included in the study. Of them, 70 patients met inclusion criteria. Ten patients did not meet inclusion criteria due to medical cannabis treatment for other indications and were excluded from the study. Out of 70 patients, 57 were males (81.4%) and 13 were females (18.6%). Median age was 31 years (range 9–64); seven patients were under 18 years of age. The use of conventional drug therapies for TS was reported by 28 patients. The most common drug groups were selective serotonin reuptake inhibitors (SSRI's), atypical antipsychotics, and monoamine depletors. Group A included 57 patients who completed the 6-month questionnaire, three of which were under 18 years of age (two patients were 16 and one patient was 15). Group B included 13 patients who did not complete the 6-month questionnaire. In the comparison of baseline characteristics and categorical variables between groups A and B, no significant differences were found (Table 1).

In group A ($n = 57$) there were 47 males (82%) and 10 females (18%). Of them, 46 patients answered the sections on cannabis consumption, product type, and daily dose. THC and cannabidiol average daily doses were 123 and 50.5 mg, respectively. Sixty-nine percent of participants used products with a high THC dose (Table 2). Forty patients (70%) used more than one cannabis product.

Table 1. A Comparison of Baseline Characteristics Between Groups A (Responded to Follow-Up) and B (Lost to Follow-Up)

Baseline characteristics at time zero	Group A ($n=57$)	Group B ($n=13$)	p-Value
Gender (male), *n* (%)	47 (82.5)	10 (77)	0.697
Median age (years)	31	30.5	
Presence of motor tics (%)	51 (96.2)	12 (92.3)	0.740
Presence of vocal tics (%)	37 (67)	10 (77)	0.634
Tics frequency (daily)	49	12	0.488
General mood (positive, %)	22 (42.3)	4 (33.3)	0.195
Employment status (employed, %)	27 (48.2)	8 (61.5)	0.236
On current medication (%)	38 (69)	6 (46.2)	>0.999

Six months after initiation of the medical cannabis treatment, patients responded to the same questionnaire again (group A; Table 3).

Vocal tics

Information on 48 (84%) patients who had vocal tics was available. Twenty-six (54.2%) patients reported no change after 6 months of treatment. Five (10.4%) patients reported an improvement, and four (8.3%) patients reported worsening. Thirteen (27%) patients reported no presence of vocal tics before or after treatment. The improvements were not statistically significant ($p>0.999$).

Motor tics

Information on 49 (86%) patients who had motor tics was available. Forty-four (89.8%) patients reported no change after 6 months of treatment. Four (8.7%) patients reported an improvement and one (2.2%) reported worsening. The improvements were not statistically significant ($p=0.375$).

Table 2. List of Medical Cannabis Products Used and Description of THC and Cannabidiol Concentrations for Each Product

Product name	No. of patients	Daily average consumption (in grams)	Daily average mg of THC	Daily average mg of CBD
Erez flowers	35	0.41	74.52	–
Alaska flowers	23	0.47	84.26	–
Midnight flowers	14	0.54	65.25	58.50
Avidekel flowers	4	0.41	4.13	74.25
Avidekel oil 15% CBD	12	0.32	3.16	47.45
Erez oil 15% THC	11	0.37	55.64	–

CBD, cannabidiol; THC, delta-9 tetrahydrocannabinol.

Table 3. The *p* Values for 6 Months Improvement Evaluation of Group A (Patients in Follow-Up)

Categorical variables	p-Value
Quality of life	<0.005
General mood (positive)	0.129
Employment status	0.027
Presence of motor tics	0.375
Presence of vocal tics	0.999
Tics frequency (daily)	0.062

Tics frequency

Information on 45 (79%) patients was available. Thirty-seven (82%) patients reported no change after 6 months of treatment. Six (13.3%) patients reported an improvement and two (4.4%) patients reported worsening. The improvement was not statistically significant ($p=0.062$).

OCD and anxiety disorder in group A

Of the 57 patients, 9 (15.7%) patients reported OCD as a comorbidity. Of those nine, six (67%) patients reported an improvement, two (22%) patients reported no change, and one (11%) patient reported worsening of OCD. Nineteen of 57 (33.3%) patients reported an anxiety disorder. Of those 19, 17 (89%) patients reported an improvement, and 2 (11%) patients reported no change.

Quality of life

Information on 43 patients was available. Thirty (69.8%) patients reported an improvement, 11 (25.6%) patients reported no change, and 2 (4.6%) patients reported worsening. The improvement was statistically significant ($p<0.005$).

General mood

Information on 44 patients was available. Twenty-five (56.8%) patients reported no change. Thirteen (29.3%) patients reported an improvement, and six (13.7%) patients reported worsening. The improvement was not statistically significant ($p=0.129$).

Employment status

Information on 43 patients was available. Twenty-nine (67.5%) reported no change. Eight (18.6%) patients reported a change of status, from unemployed to employed or student. The change was statistically significant ($p<0.05$).

Table 4. Adverse Events Reported in the Study

Adverse events	Number of reports
Dizziness	4
Increased appetite	3
Fatigue	3
Dry mouth	3
Decreased memory and concentration	2
Fear	2
Illuminations	1
Headache	1
Lightheadedness	1
Restlessness	1
Nausea	1
Itching	1

Dizziness, increased appetite, fatigue, and dry mouth were the most frequently reported.

Number of medications

Twenty-eight patients reported the use of conventional drugs for TS during the study period, using medical cannabis as a supplemental treatment. The average number of medications was 2 (range 0–7) before treatment and 0.5 (0–1) after 6 months of treatment. The decrease in medications was statistically significant ($p < 0.005$).

Adverse events were relatively minor. The most frequent adverse events reported (Table 4) were dizziness ($n = 4$), increased appetite ($n = 3$), fatigue ($n = 3$), and dry mouth ($n = 3$).

Discussion

A statistically significant improvement in quality of life ($p < 0.005$), employment status ($p = 0.027$), and reduction in the number of medications ($p < 0.005$) was found, with a statistically significant number of patients reporting improvements in OCD and anxiety symptoms after 6 months of treatment (group A). No statistically significant improvement was observed in motor ($p = 0.375$) and vocal ($p > 0.999$) tics.

The majority of TS patients experience poor quality of life and social and relationship difficulties. School performance and employment status are also reported to be affected by TS.[5] In 2008, Cavanna et al published a health-related measure specifically for TS cohorts, called the Gilles de la Tourette Syndrome-Quality of Life Scale.[30] In 2013, Cavanna et al conducted a survey to evaluate TS patients' quality of life, reviewing 13 studies that investigated health-related quality of life in adults and/or young people with TS.[31] They concluded that the severity of tics and the presence of comorbidities, particularly OCD and anxiety disorder, are associated with a poorer quality of life[31,32]; however, conventional treatment typically only slightly reduces these TS symptoms.[25]

Our study showed a statistically significant improvement in patients' quality of life ($p < 0.005$), measured from 1 to 5 on a 5-point Likert scale (very low to very high) reported by the patient. These findings coincide with the improvements in OCD and anxiety disorder comorbidities.

A statistically significant reduction in the number of conventional medications used was observed in our study ($p < 0.005$). Patients reported that this reduction was made in consultation with their primary care physician. This may suggest that medical cannabis contributes to an overall improvement in TS symptoms with less potential side effects.

Müller-Vahl et al studied the efficacy of THC treatment for TS patients, focusing on how the treatment improved motor and vocal tics.[18,19] Tic severity was measured according to the Shapiro Tourette's Syndrome Severity Scale, The Tourette's Syndrome Global Scale, and the Yale Global Tic Severity Scale (YGTSS).[33] OCD was assessed using a self-rating scale (Tourette's syndrome symptom list [TSSL]). Patients reported improvements in OCD; however, TSSL scores were only collected after administration of a daily single dose of THC for 2 days, at the first 24 h postadministration.[18] In a follow-up 6-week randomized study, Müller-Vahl et al found an improvement in motor and vocal tics.[19] In that study, TS patients were treated for over 6 weeks with up to 10 mg/day of THC and tics were rated at six visits according to the same scales.

Results showed an improvement in motor and vocal tic severity.[19] Our study did not find a statistically significant improvement in motor or vocal tics or in tic frequency; however, these results are limited in that the method of evaluation we utilized lacks the sensitivity of the scales used in other literature (i.e., YGTSS).[34] In other scales, the assessment is performed by rating tic severity according to number, frequency, intensity, complexity, and interference of tics.

The information collected from this cohort of patients sheds light on the impact of prolonged cannabis treatment's role in improving TS comorbidities, quality of life, social integration, and employment status of TS patients. Our study has some limitations. The sample size was small with no control group, the tics assessments were not performed using objective measures, and the information on conventional medications used or stopped was missing.

While the use of medical cannabis for the TS indication is increasing, the effect of medical cannabis on TS patients needs to be further studied. Most TS patient's reports on quality-of-life improvements are documented in social media networks and were not able to be appropriately quantified in this study. We did show that quality of life was improved in two-thirds of participants, likely due to the reduced anxiety and OCD symptoms. Our findings suggest that medical cannabis may be an effective and safe option to improve comorbidities and quality of life in TS patients. Medical cannabis effectiveness should be further evaluated in large-scale randomized clinical trials.

Authors' Contributions

D.B.: conceptualization and investigation, data acquisition, formal analysis, and writing original draft (lead); O.S.: writing—review and editing (equal); T.Z.-B.: formal analysis (lead); M.B.: supervision, and writing—review and editing (equal); I.G.: supervision (supporting); E.K.: conceptualization, and data acquisition (equal); L.B.-L.S.: conceptualization, and review and editing (equal). All authors have contributed to the article.

Author Disclosure Statement

L.B.-L.S. is employed by Tikun Olam Cannbit Pharmaceuticals. Except for that there are no competing interests.

Funding Information

This research did not receive grants, sponsorship or funding from public, commercial, or nonprofit organizations.

References

1. Efron D, Dale RC. Tics and Tourette syndrome. J Paediatr Child Health 2018;54(10):1148–1153; doi: 10.1111/jpc.14165
2. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, 5th ed., (DSM-5). American Psychiatric Publishing: Washington, DC, USA; 2013.
3. Gagné JP. The psychology of Tourette disorder: Revisiting the past and moving toward a cognitively-oriented future. Clin Psychol Rev 2019;67: 11–21; doi: 10.1016/j.cpr.2018.09.005
4. Eddy MC, Cavanna AE, Gulisano M, et al. Clinical correlates of quality of life in Tourette syndrome. Mov Disord 2011;26(4):735–738; doi: 10.1002/mds.23434
5. Huisman-van Dijk HM, Matthijssen SJMA, Stockmann RTS, et al. Effects of comorbidity on Tourette's tic severity and quality of life. Acta Neurol Scand 2019;140(6):390–398; doi: 10.1111/ane.13155
6. Hallett M. Tourette syndrome: Update. Brain Dev 2015;37(7):651–655; doi: 10.1016/j.braindev.2014.11.005
7. Fernández-Ruiz J. The endocannabinoid system as a target for the treatment of motor dysfunction. Br J Pharmacol 2009;156(7):1029–1040; doi: 10.1111/j.1476-5381.2008.00088.x
8. Müller-Vahl K, Kolbe H, Schneider U, et al. Cannabinoids: Possible role in patho-physiology and therapy of Gilles de la Tourette syndrome. Acta Psychiatr Scand 1998;98(6):502–506; doi: 10.1111/j.1600-0447.1998.tb10127.x
9. Malison RT, McDougle CJ, van Dyck CH, et al. [123I]Beta-CIT SPECT imaging of striatal dopamine transporter binding in Tourette's disorder. Am J Psychiatry 1995;152(9):1359–1361; doi: 10.1176/ajp.152.9.1359
10. Wolf SS, Jones DW, Knable MB, et al. Tourette syndrome: Prediction of phenotypic variation in monozygotic twins by caudate nucleus D2 receptor binding. Science 1996;273(5279):1225–1227; doi: 10.1126/science.273.5279.1225
11. Berding G, Müller-Vahl K, Schneider U, et al. [123I]AM281 single-photon emission computed tomography imaging of central cannabinoid CB1 receptors before and after delta9-tetrahydrocannabinol therapy and whole-body scanning for assessment of radiation dose in Tourette patients. Biol Psychiatry 2004;55(9):904–915; doi: 10.1016/j.biopsych.2004.01.005
12. Müller-Vahl K, Bindila L, Lutz B, et al. Cerebrospinal fluid endocannabinoid levels in Gilles de la Tourette syndrome. Neuropsychopharmacology 2020;45(8):1323–1329; doi: 10.1038/s41386-020-0671-6
13. Quezada J, Keith A. Current approaches and new developments in the pharmacological management of Tourette syndrome. CNS Drugs 2018; 32(1):33–45; doi: 10.1007/s40263-017-0486-0
14. Pringsheim T, Okun MS, Müller-Vahl K, et al. Practice guideline recommendations summary: Treatment of tics in people with Tourette syndrome and chronic tic disorders. Neurology 2019;92(19):896–906; doi: 10.1212/WNL.0000000000007466
15. Müller-Vahl K, Szejko N, Verdellen C, et al. European clinical guidelines for Tourette syndrome and other tic disorders: Summary statement. Eur Child Adolesc Psychiatry 2022;31(3):377–382; doi: 10.1007/s00787-021-01832-4
16. Sandyk R, Awerbuch G. Marijuana and Tourette's syndrome. J Clin Psychopharmacol 1988;8(6):444–445; doi: 10.1097/00004714-198812000-00021
17. Müller-Vahl K, Schneider U, Kolbe H, et al. Treatment of Tourette's syndrome with delta-9-tetrahydrocannabinol. Am J Psychiatry 1999;156(3): 495; doi: 10.1176/ajp.156.3.495
18. Müller-Vahl K, Schneider U, Koblenz A, et al. Treatment of Tourette's syndrome with delta 9-tetrahydrocannabinol (THC): A randomized crossover trial. Pharmacopsychiatry 2002;35(2):57–61; doi: 10.1055/s-2002-25028
19. Müller-Vahl K, Schneider U, Theloe K, et al. Delta 9-tetrahydrocannabinol (THC) is effective in the treatment of tics in Tourette syndrome: A 6-week randomized trial. J Clin Psychiatry 2003;64(4):459–465; doi: 10.4088/jcp.v64n0417
20. Szejko N, Jakubovski E, Fremer C, et al. Vaporized cannabis is effective and well-tolerated in an adolescent with Tourette syndrome. Med Cannabis Cannabinoids 2019;2(1):60–63; doi: 10.1159/000496355
21. Thaler A, Arad S, Schleider LB, et al. Single center experience with medical cannabis in Gilles de la Tourette syndrome. Parkinsonism Relat Disord 2019;61:211–213; doi: 10.1016/j.parkreldis.2018.10.004
22. Milosev LM, Psathakis N, Szejko N, et al. Treatment of Gilles de la Tourette syndrome with cannabis-based medicine: Results from a retrospective analysis and online survey. Cannabis Cannabinoid Res 2019;4(4):265–274; doi: 10.1089/can.2018.0050
23. Aran A, Cayam-Rand D. Medical cannabis in children. Rambam Maimonides Med J 2020;11(1):e0003; doi: 10.5041/RMMJ.10386
24. Krebs MO, Kebir O, Jay TM. Exposure to cannabinoids can lead to persistent cognitive and psychiatric disorders. Eur J Pain 2019;23(7):1225–1233; doi: 10.1002/ejp.1377
25. Hasan A, Rothenberger A, Münchau A, et al. Oral delta 9-tetrahydrocannabinol improved refractory Gilles de la Tourette syndrome in an adolescent by increasing intracortical inhibition: A case report. J Clin Psychopharmacol 2010;30(2):190–192; doi: 10.1097/JCP.0b013e3181d236ec
26. Szejko N, Jakubovski E, Fremer C, et al. Delta-9-tetrahydrocannabinol for the treatment of a child with Tourette syndrome: Case report. Eur J Med Case Rep 2018;2(2):39–41; doi: 10.24911/ejmcr/2/11
27. Robertson MM. Diagnosing Tourette syndrome: Is it a common disorder? J Psychosom Res 2003;55(1):3–6; doi: 10.1016/s0022-3999(02)00580-9
28. Ventegodt S, Merrick J, Andersen NJ. Measurement of quality of life II. From the philosophy of life to science. Scientific World Journal 2003;3: 962–971; doi: 10.1100/tsw.2003.76

29. Brown EG, Wood L, Wood S. The medical dictionary for regulatory activities (MedDRA). Drug Saf 1999;20(2):109–117; doi: 10.2165/00002018-199920020-00002
30. Cavanna AE, Schrag A, Morley D, et al. The Gilles de la Tourette syndrome-quality of life scale (GTS-QOL): Development and validation. Neurology 2008;71(18):1410–1416; doi: 10.1212/01.wnl.0000327890.02893.61
31. Cavanna AE, Luoni C, Selvini C, et al. Parent and self-report health-related quality of life measures in young patients with Tourette syndrome. J Child Neurol 2013;28(10):1305–1308; doi: 10.1177/0883073812457462
32. Cavanna AE, David K, Bandera V, et al. Health-related quality of life in Gilles de la Tourette syndrome: A decade of research. Behav Neurol 2013; 27(1):83–93; doi: 10.3233/BEN-120296
33. Eapen V, Snedden C, Črnčec R, et al. Tourette syndrome, co-morbidities and quality of life. Aust N Z J Psychiatry 2016;50(1):82–93; doi: 10.1177/0004867415594429
34. Jeon S, Walkup JT, Woods DW, et al. Detecting a clinically meaningful change in tic severity in Tourette syndrome: A comparison of three methods. Contemp Clin Trials 2013;36(2):414–420; doi: 10.1016/j.cct.2013.08.012

Cite this article as: Barchel D, Stolar O, Ziv-Baran T, Gueta I, Berkovitch M, Kohn E, Bar-Lev Schleider L (2022) Use of medical cannabis in patients with Gilles de la Tourette's syndrome in a real world setting, *Cannabis and Cannabinoid Research* X:X, 1–7, DOI: 10.1089/can.2022.0112.

Abbreviations Used

ADHD = attention deficit and hyperactivity disorder
DSM-5 = Diagnostic and Statistical Manual of Mental Disorders
OCD = obsessive compulsive disorder
SPECT = single photon emission computed tomography
THC = tetrahydrocannabinol
TS = Tourette's syndrome
TSSL = Tourette's syndrome symptom list
YGTSS = Yale Global Tic Severity Scale

Received: 16 November 2022 | Accepted: 14 May 2023

DOI: 10.1002/aur.2958

RESEARCH ARTICLE

What is specific about employment status, workplace experiences and requirements in individuals with autism in Germany?

Julia Espelöer[1] | **Julia Proft**[1] | **Phyllis Kemmer**[2] | **Christine M. Falter-Wagner**[3] | **Kai Vogeley**[1]

[1]Department of Psychiatry, University Hospital Cologne, Cologne, Germany

[2]Department of Child and Adolescent Psychiatry, LVR-Clinic Bonn, Bonn, Germany

[3]Department of Psychiatry, Medical Faculty, LMU Munich, Munich, Germany

Correspondence
Julia Espelöer, Department of Psychiatry, University Hospital Cologne, Kerpener Straße 62, 50924 Cologne, Germany.
Email: julia.espeloeer@uk-koeln.de

Funding information
Deutsche Forschungsgemeinschaft; European Commission; Federal German Ministry of Education and Research; Landschaftsverband Rheinland

Abstract

The challenge of sustainably integrating highly educated individuals with ASD without intellectual disabilities in the first labor market is repeatedly described in literature. In a retrospective study, a group of 197 clinically late-diagnosed adults with ASD without intellectual disabilities was compared to a closely matched group of 501 individuals who did not meet the criteria for the diagnosis of ASD within a utilization population of the Cologne Autism Outpatient Clinic. Results indicated that the pronounced demand for reduction of social and interpersonal requirements at the workplace (including planned or limited contact with colleagues and customers) as well as the experience of difficulties following unexpected changes in the daily routine were specific for ASD. In addition, individuals with ASD reported greater difficulties in finding a suitable job and being able to live on their wages, taking age and educational qualification into account. Supported employment measures were provided significantly more frequently to individuals in the ASD group. In conclusion, impairments in social skills emerged as one of the main obstacles of workplace performance for individuals with ASD emphasizing the necessity to develop and apply ASD-specific support services.

Lay Summary

Individuals with autism spectrum disorder (ASD) without intellectual disabilities show high unemployment rates despite high level of education. In order to achieve a cooperative interaction between people with and without autism, barriers that people with autism face in their working life should be spelled out. We studied 698 persons who visited the Adult Autism Outpatient Clinic, in 197 the diagnosis was confirmed, in 501 cases the diagnosis was not confirmed. Results indicated that, while all individuals in the sample had requirements for adjustment of the working environment, individuals with ASD reported a specific need for reduction of social and interpersonal requirements and for structured daily routines at work. Further, individuals with ASD reported difficulties in finding a suitable employment with sufficient salary also influenced by age and school education. In addition, individuals with ASD in the tested sample were more often supported by sheltered training measures ('supported employment'). In conclusion, difficulties in dealing with social skills demands were one of the main factors challenging

CF and KV should be considered joint last authors.

workplace performance for individuals with ASD, which requires ASD-specific support services.

KEYWORDS
adults, autism, employment, experiences, workplace requirements

INTRODUCTION

The clinical diagnosis of an Autism spectrum disorder (ASD) is characterized according to ICD-10 by impairments in social interaction and communication as well as restrictive, inflexible behavior patterns (American Psychiatric Association, 2013). Behavioral, cognitive, social, and sensory characteristics occur in varying degrees of severity, and the diversity and classification of the different manifestations is becoming increasingly important (Bottema-Beutel et al., 2020; Buijsman et al., 2023; Bury et al., 2020; Happé, 1999; Kapp et al., 2013; Johnson & Joshi, 2016; Monk et al., 2022; Sarrett, 2017; Tepest, 2021). Despite growing public awareness of autism, the psychosocial outcomes of people with ASD lag behind those of non-disabled adults and people with other disabilities (Shattuck et al., 2012). Unemployment rates are alarmingly high in autism (Espelöer et al., 2022) and it is time to draw society's attention to the needs of people with autism in the working environment in order to enable them to join the labor market, from which all parties could benefit, considering special strengths people with autism could bring to the workforce (Johnson & Joshi, 2016; Sarrett, 2017). However, in the labor market, social skills are often required, which presents a major challenge for individuals with ASD (Arora, 2017; Fazekas, 2020; Riggio, 2020). Already the job application process is a challenge for individuals with ASD, requiring flexibility and adaptation to new routines, overcoming miscommunication as well as social interaction deficits (Baldwin et al., 2014; Hurlbutt & Chalmers, 2004; Müller et al., 2003; Sarrett, 2017). Accordingly, asking about challenges in working life, individuals with ASD report difficulties in understanding expectations of employers or colleagues, recognizing implicit as well as explicit statements, and completing work assignments to the correct degree and period. Difficulties are reported in dealing with customer contact and in adapting quickly to unfamiliar situations and demands. Social requirements in particular represent a barrier that often cannot be overcome and interpersonal difficulties are more likely to cause termination than professional problems (Espelöer et al., 2022; Hurlbutt & Chalmers, 2004; Müller et al., 2003; Proft et al., 2016).

When asking people with ASD about their specific requirements for their work environments, the needs for limited social interaction, clear communication, reduced sensory input, support in prioritization and sufficient time to learn new tasks were expressed (Kirchner & Dziobek, 2014; Müller et al., 2003; Proft et al., 2016). Furthermore, the possibility of flexibly structuring their daily working schedule according to individual needs as well as consistent, predictable and clearly defined work assignments appear to be important. Nevertheless, intellectual demands must not lose relevance (Baldwin et al., 2014; Müller et al., 2003). Hence, alarmingly increased unemployment rates of up to five-fold higher compared to the general population (Espelöer et al., 2022; Maslahati et al., 2022) or underemployment paired with over-education in individuals with ASD without intellectual disabilities are the results (Baldwin et al., 2014; Frank et al., 2018; Hurlbutt & Chalmers, 2004; Maslahati et al., 2022; Riedel et al., 2016). Roux et al. (2013) described lower wages for individuals with ASD compared to individuals with learning or language difficulties or other mental health problems. Additionally, individuals with ASD without intellectual disabilities often need specific support, but are at the same time at higher risk of being overlooked in support services compared to individuals with other disabilities because of preserved intellectual abilities (Baldwin et al., 2014; Chen et al., 2015; Espelöer et al., 2022; Kirchner & Dziobek, 2014; Lehnhardt et al., 2012; Roux et al., 2013; Shattuck et al., 2012; Vogeley et al., 2013). Especially in the transition period of the first two years after leaving school, support seems to be of great importance so that individuals with ASD can enter working life successfully (Chen et al., 2015; Shattuck et al., 2012).

The importance of social competences in the workplace poses a challenge for people with mental disorders, as various disorders are associated with impairments in social interaction and communication, which might cause challenges in finding and maintaining suitable workplaces (Arora, 2017; Brunello & Schlotter, 2011; Fazekas, 2020; Olesen et al., 2013; Riggio, 2020). Autism-specific social characteristics should receive special scrutiny because, in particular, the psychosocial outcomes of young adults with ASD lag behind compared to persons with other mental disorders or disabilities and comparable demographics (Chen et al., 2015; Howlin, 2013; Howlin & Moss, 2012; Roux et al., 2013; Shattuck et al., 2012).

To gain a better understanding of specific needs of late-diagnosed individuals with ASD, the current study includes a clinical comparison group of individuals referred for diagnostic assessment to our autism outpatient clinic, for whom a diagnosis of ASD was ruled out (see Bloch et al., 2021; Falter-Wagner et al., 2022). This group of persons, who share many features with persons

with ASD, represents a particularly informative comparison group, as a comparison allows differentiation of autism-specific needs for the working environment over and above needs across F-diagnoses with social interaction challenges. Indeed, our results show that comparing these two groups of patients with social interaction challenges, either with or without ASD, there were very specific needs reported by study participants with ASD. Only a thorough understanding of these specificities can lead to alignment of therapeutic support services and development of *specific* employment services for individuals with ASD different from those for individuals with social interaction difficulties without ASD. The current study sought to approach an autism-specific profile in working life. The (i) first aim was to present the educational, vocational, and employment situation of individuals with ASD in comparison with those of a close clinical comparison group in order to identify indications of specific differences of persons with ASD. To investigate this research question, the first part of a vocational questionnaire (Proft et al., 2016; see section *Instrument*) was used referred to as 'professional development'. The (ii) second aim was to identify specific characteristics regarding workplace experiences and needs of the ASD group by investigating differences between these two groups. Here, the second part of the vocational questionnaire 'specific workplace characteristics' was used.

METHOD

Participants

Data were obtained retrospectively from the clinical database of the Adult Autism Outpatient Clinic, Department of Psychiatry, of the University Hospital of Cologne, Germany, partially overlapping with the sample of a previous study that focused on unemployment rates (see Espelöer et al., 2022). 3520 individuals were referred for diagnostic assessment during the period of 2014 to 2021 and received a vocational questionnaire (Proft et al., 2016) before their first interview to assess their employment situation. 1877 questionnaires were returned corresponding to a response rate of 53.3%. 852 of 1877 individuals completed the diagnostic process. Data of persons presenting with questionnaires with more than five missing values were excluded from the current analysis. Further, we excluded data of individuals without completed school education, mentally disabled persons, and individuals diagnosed with other pervasive developmental disorders (F84.8, F84.9). In total, 698 individuals were included in the current analysis. The sample consists of two groups, 197 individuals were clinically diagnosed with ASD according to ICD-10 (WHO, 1992) criteria (ASD+ group), whereas in 501 individuals a diagnosis of ASD was ruled out (ASD- group). In the ASD group, 85.3% of individuals were diagnosed with F84.5 ($n = 168$), 9.6% with F84.0 ($n = 19$), and 5.1% with F84.1 ($n = 10$).

Individuals attended the outpatient clinic for diagnostic clarification, usually based on suspected ASD due to social emotional symptoms. Registration for diagnosis in the outpatient clinic is subject to referral and thus based on a prior assessment by a psychiatrist or clinical psychologist. All individuals included in the current study showed Autism-Spectrum Quotient (AQ, Baron-Cohen et al., 2001) values above the clinical cut-off of 32. Diagnostic procedures complied with the German guidelines on ASD (Association of the Scientific Medical Societies, 2016) and were based on consensus diagnoses, which involves the independent assessment of at least two experienced clinicians. The exclusive aim is to verify or reject the diagnosis of ASD, possible differential diagnoses cannot be systematically investigated in detail because of time limitations. Individuals in both groups reported social difficulties, individuals in the ASD- group, however, did not fulfill criteria for any diagnosis within the classification of pervasive developmental disorders (ICD-10 F84). IQ testing is not standard in the diagnostic process. Due to a high educational qualification level in our sample (see Table 2), we assume that cognitive disabilities did not influence our results (Ritchies et al. 2018).

The average age of the ASD+ sample was 36.5 years (19–67), of which 62 (31.5%) individuals identified as female, 135 (68.5%) identified as male. In the ASD- group, the average age was 39.5 (18–70), 188 (37.5%) individuals identified as female and 313 (62.5%) identified as male. Groups did not significantly differ in gender ($X^2(1) = 2.25$, $p = 0.133$, $N = 698$). A significant difference was found for age, individuals in the ASD- group were older than individuals in the ASD+ group ($U = 42{,}070$, $p = 0.002$) (see Table 1).

Instrument

The vocational questionnaire (Proft et al., 2016) was completed before starting the diagnostic procedure and before communicating diagnostic results. The questionnaire was designed based on statements regarding personal workplace experiences and wishes for an ideal workplace of individuals with ASD in a prior qualitative study (Proft et al., 2016). The vocational questionnaire (Proft et al., 2016) consists of the two parts 'professional development' and 'specific workplace characteristics'. The second part again comprises the two categories '*Wishes and requirements for an ideal workplace' (W)* and '*Workplace experiences' (E)*.

'Professional development'

The first part is referred to as 'professional development' and captures descriptive data about formal education level, occupational skill level, employment status,

TABLE 1 Group descriptive

	ASD + *N*	%		ASD-*N*	%		X^2	*Df*	*p*
Male	135	68.5		313	62.5				
Female	62	31.5		188	37.5		2.25	1	0.133
	M (Mdn)	***SD***	**Range**	***M (Mdn)***	***SD***	**Range**	***U***		
Age	36.5 (36.0)	11.7	19–67	39.5 (39.0)	11.6	18–70	42,070		0.002
School education[a]	2.24 (2.00)	1.18		1.97 (2.00)	1.06		42,914		0.009

Note: Descriptive statistics by group.
Group: ASD+ = diagnosed with autism spectrum disorder, ASD- = diagnosis of autism spectrum disorder ruled out; X^2 = Chi square test of association; *U* = Mann Whitney *U* test; *N* = sample size; *M* = mean value; Mdn = Median; SD = standard deviation.
*$p < 0.05$.
[a]Low values indicate higher educational qualifications (scored 1 to 5).

psychosocial situation, periods of unemployment, and frequency and reasons of terminations (The German translation of levels of formal qualification attached as Supplementary material and Espelöer et al., 2022).

'Specific workplace characteristics'

The second part 'specific workplace characteristics is composed of the two categories 'Wishes and requirements for an ideal workplace' (W) and 'Workplace experiences' (E). Items were reversely coded where required prior to calculations.

'Wishes and requirements for an ideal workplace' (W). Ratings were performed using a 5-point Likert scale ("very important", "important", "moderately important", slightly important", and "unimportant"). This category of the questionnaire originally consists of 44 items (e.g. "W_02 Few people in the working environment"; "W_35 Retreat possibilities during breaks and/or when overstrained in the daily work routine"). Initially, the questionnaire was designed to examine individuals with ASD. Two items that required an existing ASD diagnosis were excluded from the analyses ("Informing people in the working environment about the autistic condition"; "Increasing awareness of autistic disorders"). Forty-two items were included in the present analyses.

'Workplace experiences' (E). Ratings were performed using a 5-point Likert scale ("strongly agree", "agree", "undecided", "disagree", and "strongly disagree"). The category of the questionnaire originally consists of 39 items (e.g. "E_04 I do not have common topics of conversation with colleagues"; "E_13 Shifting appointments and changes in the daily schedule at short notice (e.g. due to unforeseen meetings) are problematic for me"). Initially, the questionnaire was designed to examine individuals with ASD. Seven items that required an existing ASD diagnosis were excluded from the analyses (e.g. "My employer mainly tries to meet my autistic needs through concrete interventions (e.g. individual office)"). Thirty-two items were included in the present analyses.

Basic statistics

'Professional development'

For group comparisons, Chi square tests of association and Mann Whitney U tests were used.

'Specific workplace characteristics'

The distribution of the dataset was checked using Little's Missing Completely at Random Test (Little, 1988). For both parts of the questionnaire (*'Wishes and requirements for an ideal workplace' (W)* and *'Workplace experiences' (E)*), we expected that values were missing by chance. Missing values were replaced by the procedure of multiple imputation if not more than five values were missing. Data did not meet the assumption of normality and thus non-parametric tests were used.

An exploratory factor analysis was performed in order to reduce the complexity of the data. The exploratory approach has to be taken into account when describing and interpreting results (Bender & Lange, 2001). The required assumptions for factor analysis were met: The Kaiser-Meyer-Olkin coefficient (Kaiser & Rice, 1974) showed high values greater than 0.8, respectively (E: KMO = 0.831, W: KMO = 0.883), all MSA-coefficients showed values greater 0.5, and results of the Bartlett's tests of sphericity (Bartlett, 1951) were significant (E: $X^2(496) = 6997$, $p < 0.001$, W: $X^2(861) = 8914$, $p < 0.001$). After generating factors, post hoc comparisons were performed using reliability analyses and non-parametric Mann–Whitney *U* tests. To account for influences of age and school education on the results we employed linear regressions.

The number of factors to be extracted was determined using Horn's parallel analysis. Here, the eigenvalue progression and the scree plot were examined (see Supplementary material). In addition to the statistical analysis, the practical content-related importance of the respective items were taken into account as well. Items with a main factor loading of at least 0.4 (Gaskin & Happell, 2014)

TABLE 2 School education

School education	Group		$X^2_{(1)}$	p	φ
	ASD+ %	ASD- %			
University entrance-level qualification	53.3	65.5	8.84	0.003*	0.113
General certificate of secondary education	27.9	24.4	0.897	0.344	0.036
Basic secondary education	18.8	10.1	9.66	0.002*	0.118

Note: Values in % by group.
Group: ASD+ = diagnosed with autism spectrum disorder, ASD- = diagnosis of autism spectrum disorder ruled out; X^2 = Chi square test of association; φ = effect size.
*$p < 0.05$; low values indicate greater impairments.

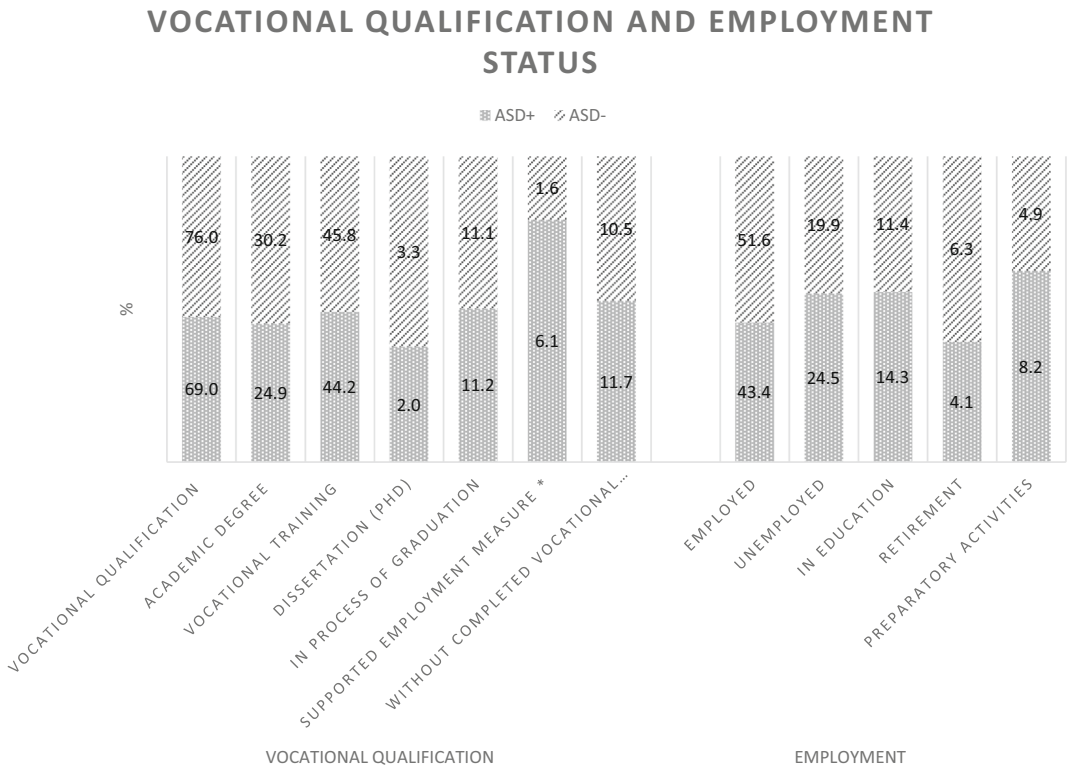


FIGURE 1 Vocational qualification and employment status by group, values in %. Group: ASD+ = diagnosed with autism spectrum disorder, ASD- = diagnosis of autism spectrum disorder ruled out. *$p < 0.05$; low values indicate greater impairments.

were included in the analysis. An item-rest correlation of ≥0.3 is suggested for an item to be included in a factor.

RESULTS[1]

'Professional development'

Education. Group comparisons show that individuals in the ASD- group were more highly educated compared to individuals in the ASD+ group ($U = 42{,}914$, $p = 0.009$). Significantly more individuals without ASD (ASD-) have achieved university entrance-level qualification ($X^2(1) = 8.84$, $p = 0.003$, $\varphi = 0.113$). In contrast, a significantly lower proportion of individuals without ASD (ASD-) reached basic secondary education ($X^2(1) = 9.66$, $p = 0.002$, $\varphi = 0.118$) (Table 2).

Vocational qualification, (Un)Employment, and Termination. Vocational qualifications following school education, comprising successfully completed academic degree and vocational training, did not significantly differ between groups, ($X^2(1) = 3.51$, $p = 0.061$, $\varphi = 0.072$). Supported employment measures[2] were provided significantly more frequently to individuals in the ASD+ group, ($X^2(1) = 9.78$, $p = 0.002$, $\varphi = 0.120$) (Figure 1). In the ASD- group an increased employment status was found, but differences were not significant,

[1]It is important to emphasize that exploratory results are reported herein (Bender & Lange, 2001).

[2]Supported employment measures describe a program of supportive vocational qualification aiming at integration into the labor market, e.g. through assistance in job search, job preparation, or communication with employers (Vogeley et al., 2013).

TABLE 3 'Workplace experiences'

Factor	*N*	Number of items	*S*	*E*	*α*	*r*	*M (SD)*
E_Factor 1: Social challenges	698	13	0.559	0.660	0.826	0.27	2.36 (0.642)
E_Factor 2: Job fit	698	4	−0.137	−1.02	0.768	0.45	3.03 (1.13)
E_Factor 3: Specific needs	698	3	−0.506	−0.197	0.576	0.30	3.81 (0.838)

Note: Descriptive statistics of factors of the questionnaire 'Workplace experiences'.
E = questionnaire 'Workplace experiences'; *N* = sample size; *S* = skewness; *E* = kurtosis; *α* = Cronbach's alpha (internal consistency); *r* = average inter-item correlation; *M* = mean value; *SD* = standard deviation.
Low values indicate greater impairments.

($X^2(1) = 3.82$, $p = 0.051$, $\varphi = 0.075$). Accordingly, a higher tendency was found for unemployment rates in the ASD+ group ($X^2(1) = 1.75$, $p = 0.186.$, $\varphi = 0.051$) (Figure 1).

The similarity of the groups is also evident in the duration of unemployment ($U = 41{,}085$, $p = 0.929$) as well as the frequency ($U = 39{,}303$, $p = 0.722$) and reason of termination. The reasons given for termination by both groups were mostly interpersonal followed by professional difficulties. No group differences were found with respect to proportion of termination initiated by the employer versus the employee.

'Specific workplace characteristics'

First, factor analyses of the categories '*Workplace experiences*' and '*Wishes and requirements for an ideal workplace*' were performed. In the following, group comparisons were presented for both categories, respectively (see section Group comparisons of the categories '*Workplace experiences*' and '*Wishes and requirements for an ideal workplace*').

'*Workplace experiences*'. Taking into account the result of the factor extraction by Horn's parallel analysis, the eigenvalue analysis, the scree plot, the content interpretation as well as the assumption of a minimum loading of 0.4 and a minimum number of three items per factor, a three-factor structure appeared to be most appropriate. A reduction from 32 to 20 items could be achieved by eliminating items with low main loadings. No relevant cross-loadings (>0.3) were obtained, so that the following three factors with a simple structure pattern were generated (see Supplementary material). E_Factor 1 '*Social challenges*' comprises difficulties in interpersonal interaction and communication, in understanding social rules, with teamwork, customer contact, flexibility, or lack of structure. E_Factor 2 '*Job fit*' comprises sufficiency of salary as well as difficulties in finding a suitable job. E_Factor 3 '*Specific needs*' refers to options to bring in one's own specific, individual interests, strengths, and requirements at the workplace.

Item E_03 was included in E_Factor 3 '*Specific needs*' despite a low item-rest correlation of 0.178. However, the item shows an acceptable factor loading of 0.406 and contributes content-related to the factor. The internal consistency of factors 1 and 2 were in the acceptable range of >0.7. Factor 3 did not meet sufficient but acceptable internal consistency with Cronbach's α of 0.576. The average inter-item correlation was in the acceptable range of 0.2 to 0.4 for factor 1 and factor 3. An increased value for factor 2 ($r = 0.45$) indicates a homogeneous factor containing items that measure the same characteristic (Table 3). For a factor with a low item count of three items, it is reasonable to tolerate mean inter-item correlations >0.4 in order to measure a specific domain (Bühner, 2011).

'*Wishes and requirements for an ideal workplace*'. When including the factor extraction by Horn's parallel analysis, the analysis of the eigenvalues, the scree plot, content related interpretations, the assumption of a minimum loading of 0.4 as well as a minimum number of three items per factor, a four-factor structure is suggested containing factors with a clear content based differentiation. A reduction from 42 to 25 items could be achieved by eliminating items with low main loadings so that the following four factors with a simple structure pattern were generated (see Supplementary material). W_Factor 1 '*Social challenges*' comprises specific requirements for social interaction and communication in the workplace such as reduced, specific, professional personal contact with colleagues, supervisors, and customers as well as structured daily schedules. W_Factor 2 '*Job fit*' comprises the need for sufficient salary as well as permanent employment. W_Factor 3 '*Specific needs*' comprises the need for individual support, strategies for dealing with excessive demands, and recognition of individual abilities. W_Factor 4 '*Individual work setting*' comprises the need for specific working conditions such as home office, individual office, or flexible work scheduling.

Only one relevant cross-loading (>0.3) was obtained for item W_27 on factor 1 (0.438) and factor 4 (0.502). With regard to content-related and statistical fit, the item

was assigned to factor 4. Item W_08 was eliminated despite a factor loading >0.4 (0.416) due to a low item-rest correlation of <0.3 (0.249) as well as an increased internal consistency of the factor when the item is dropped (Cronbach's $\alpha = 0.598$ vs. Cronbach's α 'if item dropped' $= 0.646$). Item W_20 was included in factor 4 despite a low item-rest correlation of 0.274. However, the item shows an acceptable factor loading of 0.448 and contributes content-related to the factor (see Supplementary material). The internal consistency of factor 1 and 3 were in the acceptable range of >0.7. Factor 2 with Cronbach's $\alpha = 0.646$ and factor 4 with Cronbach's $\alpha = 0.557$ did not meet sufficient internal consistency probably due to the small number of items. Both factors include only three items, which, however, show acceptable factor loadings >0.4 as well as content-related fit. The homogeneity of all factors was in the acceptable range of 0.2 to 0.4 (Table 4).

Group comparisons of the categories 'Workplace experiences' and 'Wishes and requirements for an ideal workplace'

'Workplace experiences'. Although individuals in the ASD+ group indicated worse experiences related to social requirements (E_Factor 1 *'Social challenges'*) than individuals in the ASD- group this difference was relevant only at a trend level: $U = 44{,}734$, $p = 0.054$, $r = 0.094$ (Table 5). Single item comparisons indicated greater impairment for individuals with ASD referred to item E_13 (*Shifting appointments and changes in the daily schedule at short notice (*e.g.*, due to unforeseen meetings) are problematic for me,* $U = 44{,}089$, $p = 0.021$, $r = 0.107$), E_04 (*I do not have common topics of conversation with colleagues,* $U = 43{,}927$, $p = 0.018$, $r = 0.110$), and E_11 (*I have problems with contacts to customers,* $U = 40{,}389$, $p < 0.001$, $r = 0.182$) (Table 6). The ASD+

TABLE 4 'Wishes and requirements for an ideal workplace'

Factor	*N*	Number of items	*S*	*E*	*α*	*r*	*M (SD)*
W_Factor 1: Social challenges	698	12	0.498	−0.062	0.863	0.35	2.35 (0.767)
W_Factor 2: Job fit	698	3	1.74	2.62	0.646	0.29	1.33 (0.516)
W_Factor 3: Specific needs	698	7	0.893	1.03	0.733	0.29	1.77 (0.606)
W_Factor 4: individual work setting	698	3	0.418	−0.358	0.557	0.29	2.38 (0.944)

Note: Descriptive statistics of factors of the questionnaire 'Wishes and requirements for an ideal workplace'.
W = questionnaire 'Wishes and requirements for an ideal workplace'; *N* = sample size; *S* = skewness; *E* = kurtosis; *α* = Cronbach's alpha (internal consistency); *r* = average inter-item correlation; *M* = mean value; *SD* = standard deviation.
Low values indicate greater impairments.

TABLE 5 Group comparisons of factors of the questionnaire 'Workplace experiences'

	ASD+(*N* = 197)			ASD-(*N* = 501)					
Factor	*M*	*SD*	*Mdn*	*M*	*SD*	*Mdn*	*U*	*p*	*r*
E_Factor 1: Social challenges	2.29	0.632	2.23	2.39	0.645	2.38	44,734	0.054	0.094
E_Factor 2: Job fit	2.85	1.07	3.00	3.10	1.14	3.25	42,795	0.006*	0.133
E_Factor 3: Specific needs	3.74	0.917	3.67	3.83	0.804	4.00	47,116	0.348	0.045

Note: Group comparisons of factors of the questionnaire 'Workplace experiences'.
Group: ASD+ = diagnosed with autism spectrum disorder, ASD- = diagnosis of autism spectrum disorder ruled out; *E* = questionnaire 'Workplace experiences'; *M* = mean value; *SD* = standard deviation; *Mdn* = median; *U* = Mann Whitney *U* test; *r* = effect size.
*$p < 0.05$; low values indicate greater impairments.

TABLE 6 Single item comparisons for E_Factor 1 'Social challenges' of the questionnaire 'Workplace experiences'

	ASD+(*N* = 197)		ASD-(*N* = 501)				
Items	*M*	*Mdn*	*M*	*Mdn*	*U*	*P*	*r*
E_13 Shifting appointments and changes in the daily schedule at short notice (e.g. due to unforeseen meetings) are problematic for me	1.92	2.00	2.11	2.00	44,089	0.021*	0.107
E_04 I do not have common topics of conversation with colleagues	2.45	2.00	2.65	3.00	43,927	0.018*	0.110
E_11 I have problems with contacts to customers	2.25	2.00	2.65	3.00	40,389	< 0.001*	0.182

Note: Single item comparisons for E_Factor 1 'Social challenges' of the questionnaire 'Workplace experiences'.
Group: ASD+ = diagnosed with autism spectrum disorder, ASD- = diagnosis of autism spectrum disorder ruled out; *E* = questionnaire 'Workplace experiences'; *M* = mean value; *Mdn* = median; *U* = Mann Whitney *U* test value; *r* = effect size.
(Only significant results are presented here; for the complete Table see Supplementary material).
*$p < 0.05$; low values indicate greater impairments.

sample specified significantly greater impairments with general conditions in the working environment (E_Factor 2 *'Job fit'*, $U = 42{,}795$, $p = 0.006$, $r = 0.133$) (Table 5), such as salary (E_38: *I can cover my living expenses with my salary*, $U = 42{,}214$, $p = 0.002$, $r = 0.145$) and finding a suitable job (E_21: *I have difficulties finding a suitable job*, $U = 42{,}885$, $p = 0.004$, $r = 0.013$) (Table 7). Including age and school education as covariates, an influence on the group effect was revealed ($R^2 = 0.0728$, $F(3{,}688) = 18.0$, $p < 0.001$). No interaction effect was found. A marginal group difference remains ($B = 0.159$, $t = 1.71$, $p = 0.087$), but age ($B = 0.017$, $t = 4.59$, $p < 0.001$) and school education ($B = -0.164$, $t = -4.30$, $p < 0.001$) needed to be included in the interpretation of the group effect. Results did not show group differences for E_Factor 3 *'Specific needs'* (Table 5).

'Wishes and requirements for an ideal workplace'. Individuals with ASD show significantly higher specific requirements for social interaction and communication on the factor level (W_Factor 1), attributing higher importance to the reduction of communication and interaction at work than individuals in the ASD- group, $U = 42{,}651$, $p = 0.005$, $r = 0.136$ (Table 8). The ASD+ sample attributes significantly higher importance to the following requirements than individuals in the ASD- group: W_15: *Personal contact with colleagues only for a short time window during the day for factual communication*, $U = 43{,}408$, $p = 0.011$, $r = 0.120$, W_02: *Few people in the working environment*, $U = 41{,}450$, $p < 0.001$, $r = 160$, W_12: *No contact with customers*, $U = 41{,}073$, $p < 0.001$, $r = 0.168$, W_34, *No business trips and/or field assignments*, $U = 41{,}547$, $p < 0.001$, $r = 0.158$, and W_10, *As few contacts as possible in the company*, $U = 43{,}949$, $p = 0.018$, $r = 0.109$ (Table 9).

Results did not show group differences for factor W_Factor 2 *'Job fit'*, W_Factor 3 *'Specific needs'*, and W_Factor 4 *'Individual work setting'* (Table 8). Only one single item comparison shows differences. Individuals with ASD indicated a greater importance for the following item: W_35 (item of W_Factor 3), *Retreat possibilities during breaks and/or when overstrained in the daily work routine*, $U = 45{,}245$, $p = 0.049$, $r = 0.083$ (Table 10).

DISCUSSION

Aim of the current study was to study both experiences on the one hand and requirements on the other hand concerning working life and employment status that are *specific* for individuals diagnosed with autism (ASD+) in comparison to individuals suffering from social difficulties to whom a diagnosis of ASD does not apply (ASD-). Presumably because of the shared social difficulties motivating referral for diagnostic evaluation of the whole population examined, we found in both groups comparable 'professional developments', including employment status, durations of unemployment, frequency of terminations including reasons thereof and vocational qualifications. Answering the first research aim, as an exception

TABLE 7 Single item comparisons for E_Factor 2 'Job fit' of the questionnaire 'Workplace experiences'

	ASD ($N = 197$)		CON ($N = 501$)				
Items	***M***	***Mdn***	***M***	***Mdn***	***U***	***P***	***r***
E_38[a] I can cover my living expenses with my salary	3.01	3.00	3.42	4.00	42,214	0.002*	0.145
E_21 I have difficulties finding a suitable job	1.92	1.00	2.25	2.00	42,885	0.004*	0.013

Note: Single item comparisons for E_factor 2 'Job fit' of the questionnaire 'Workplace experiences'.
Group: ASD+ = diagnosed with autism spectrum disorder, ASD- = diagnosis of autism spectrum disorder ruled out; *E* = questionnaire 'Workplace experiences'; *M* = mean value; *Mdn* = median; *U* = Mann Whitney *U* test value; *r* = effect size.
(Only significant results are presented here; for the complete Table see Supplementary material).
[a]Reverse coding.
*$p < 0.05$; low values indicate greater impairments.

TABLE 8 Group comparisons of factors of the questionnaire 'Wishes and requirements for an ideal workplace'

	ASD+($N = 197$)			ASD-($N = 501$)					
Factor	***M***	***SD***	***Mdn***	***M***	***SD***	***Mdn***	***U***	***p***	***r***
W_Factor 1: Social challenges	2.22	0.753	2.17	2.40	0.767	2.33	42,651	0.005*	0.136
W_Factor 2: Job fit	1.32	0.538	1.00	1.33	0.507	1.00	48,186	0.584	0.024
W_Factor 3: Specific needs	1.72	0.603	1.57	1.79	0.606	1.71	45,667	0.123	0.075
W_Factor 4: individual fit	2.38	0.967	2.33	2.38	0.936	2.33	49,026	0.893	0.007

Note: Group comparisons of factors of the questionnaire 'Wishes and requirements for an ideal workplace'.
Group: ASD+ = diagnosed with autism spectrum disorder, ASD- = diagnosis of autism spectrum disorder ruled out; W = questionnaire 'Wishes and requirements for an ideal workplace'; *M* = mean value; *SD* = standard deviation; *Mdn* = median; *U* = Mann Whitney U test; *r* = effect size.
*$p < 0.05$; low values indicate greater impairments.

TABLE 9 Single item comparisons for W_Factor 1 'Social challenges' of the questionnaire 'Wishes and requirements for an ideal workplace'

	ASD+($N = 197$)		ASD-($N = 501$)				
Items	***M***	***Mdn***	***M***	***Mdn***	***U***	***p***	***r***
W_15 Personal contact with colleagues only for a short time window during the day for factual communication	2.55	3.0	2.82	3.0	43,408	0.011*	0.120
W_02 Few people in the working environment	1.65	1.00	1.92	2.00	41,450	< 0.001*	0.160
W_12 No contact with customers	2.36	2.00	2.75	3.00	41,073	< 0.001*	0.168
W_34 No business trips and/or field assignments	2.18	2.00	2.55	3.00	41,547	< 0.001*	0.158
W_10 As few contacts as possible in the company	2.04	2.00	2.23	2.00	43,949	0.018*	0.109

Note: Single item comparisons for W_Factor 1 'Social challenges' of the questionnaire 'Wishes and requirements for an ideal workplace'.
Group: ASD+ = diagnosed with autism spectrum disorder, ASD- = diagnosis of autism spectrum disorder ruled out; W = questionnaire 'Wishes and requirements for an ideal workplace'; *M* = mean value; *Mdn* = median; *U* = Mann Whitney U test value; *r* = effect size.
(Only significant results are presented here; for the complete Table see Supplementary material).
*$p < 0.05$; low values indicate greater impairments.

TABLE 10 Single item comparisons for W_Factor 3 'Specific needs' of the questionnaire 'Wishes and requirements for an ideal workplace'

	ASD ($N = 197$)		CON ($N = 501$)				
Items	***M***	***Mdn***	***M***	***Mdn***	***U***	***P***	***r***
W_35 Retreat possibilities during breaks and/or when overstrained in the daily work routine	1.50	1.00	1.62	1.00	45,245	0.049*	0.083

Note: Single item comparisons for W_Factor 3 'Specific needs' of the questionnaire 'Wishes and requirements for an ideal workplace'.
Group: ASD+ = diagnosed with autism spectrum disorder, ASD- = diagnosis of autism spectrum disorder ruled out; W = questionnaire `Wishes and requirements for an ideal workplace`; *M* = mean value; *Mdn* = median; *U* = Mann Whitney U test value; *r* = effect size.
(Only significant results are presented here; for the complete Table see Supplementary material).
*$p < 0.05$; low values indicate greater impairments.

of these many similarities, supported employment measures were utilized significantly more frequently by individuals with ASD. Specific differences were hypothesized for the increased requirement for reduced interpersonal interactions and communication at work and the tendency toward greater difficulties in dealing with social demands in the ASD group, representing the second aim related to 'specific workplace characteristics'. Greater difficulty in finding a suitable workplace with sufficient salary was found in persons with ASD, especially among younger individuals and persons with lower level of education.

'Specific workplace characteristics'

'Workplace experiences'. Difficulties in dealing with social requirements became evident in both groups on the factor level, with a tendency toward greater impairments for individuals with ASD (E_Factor 1 *'Social challenges'*). However, evidence indicated that challenges with flexible scheduling, customer contact, and identifying shared interests and conversation topics with colleagues were specifically pronounced among individuals with ASD. Here, the so-called *hygiene factors* become apparent (Herzberg, 1972) as one key component of his two-factor motivation theory. The theory includes two independent, non-complementary factors and states that so-called *motivators* (e.g., achievement, recognition, responsibility) might increase job satisfaction, while dissatisfaction might be increased by a lack of so-called *hygiene factors* (e.g., salary, job security, supervision, relationships at work). Hence, autism-specific impairments in social skills as well as inflexible routines and ritualized behaviors significantly affect the successful integration and maintenance of employment and according to Herzberg (1972) as a lack of *hygiene factors* might increase dissatisfaction in working life.

Reports of workplace experiences indicate that individuals with ASD were more likely struggling to live on their wages than individuals with a ruled out ASD diagnosis. People in the ASD group also reported greater difficulties in finding suitable employment (E_Factor 2 *'Job fit'*). It turned out that age and educational qualifications were found to have an impact on salary and job fit with greater difficulties at younger ages and lower educational levels. It could be assumed that the level of school-leaving qualifications and thus the advanced age could affect the amount of salary and thus job security (Whittenburg et al. 2019). However, we find a lack of sufficient salary and job security as a dissatisfaction of *hygiene factors* might increase dissatisfaction in working life.

'Wishes and requirements for an ideal workplace'. Autism-specific qualitative impairments in processing social information intuitively as well as reduced competences in flexible planning and prioritization seem to require specific working conditions. In accordance with qualitative studies of Müller et al. (2003) as well as

Hurlbutt and Chalmers (2004), results of the current study suggested that the wish to reduce interpersonal demands for communication and interaction in the workplace represents a specific characteristic for individuals with ASD (W_Factor 1 *'Social challenges'*). More precisely, people with ASD, in contrast to non-autistic persons, attribute significantly greater importance on interactions that are brief and focus purely on content and on interactions with only a limited number of colleagues or preferably, if possible, only one specific contact person as well as not having customer contact. Again, it might be assumed that job dissatisfaction becomes apparent by the lack of *hygiene factors*.

The wish of individuals in the ASD group for reduction of social and sensory stimulation becomes evident in a variety of areas. The ASD group appears to place a higher importance on not traveling or doing fieldwork as part of their job duties than individuals with a ruled out ASD diagnosis (W_Factor 1 *'Social challenges'*). Overloads due to sensory overstimulation were frequently reported in individuals with ASD and have now been included as a diagnostic criterion for ASD in DSM-5 (American Psychiatric Association, 2013). This becomes also evident in the current study. The need for a retreat during breaks and in situations of excessive demands is suggested to be specific for people with ASD compared to people without ASD (W_Factor 3 *'Specific needs'*). The recognition of individual needs at the workplace, the importance of financial security, and the possibility of flexibly adapting framework conditions (home office, individual office, flexible work scheduling; comparable to *hygiene factors*) to one's own needs were similarly pronounced in both groups (W_Factor 2 *'Job fit'*). Both groups attribute similar importance to the general conditions of a secure job and a salary that is appropriate to the professional activity and ensures economic security. However, confirming previous research (Roux et al., 2013), individuals with ASD show greater difficulties in obtaining adequate salary and a suitable workplace, which was even aggravated with younger age and lower educational qualification.

It turns out that, in both groups, there is a marked discrepancy between individual wishes and requirements at work and experienced workplace conditions. Comparable to previous results (Hurlbutt & Chalmers, 2004; Müller et al., 2003), autism-specific needs and impairments in social competences and flexible behavior become apparent. Difficulties in dealing with customers and colleagues, the need for professional rather than personal exchange, and for minimizing continuously interactions with superiors and colleagues were found to be more pronounced in both factors (*'Workplace experiences'* and *'Wishes and requirements for an ideal workplace'*) in individuals with ASD than in individuals without ASD. Consistent with a qualitative study by Müller et al. (2003), both factors indicate that the opportunity to work alone and autonomously accommodates the need for stimulus reduction and retreat.

A more recent, empirically supported theoretical concept extending the two-factor motivation theory by Herzberg (1972) is the job demands-resources model (Demerouti et al., 2001;Demerouti & Bakker, 2011 ; Schaufeli, 2017), which differentiates the two components of *job demands* and *job resources* in order to understand the emergence of health impairments such as stress, and motivational processes such as work engagement, and the effect on job performance. *Job demands* refer to physical, psychological, social as well as organizational aspects of work (e.g., workload, conflicts with colleagues, financial insecurity) and may affect the employee's stress level, which in combination with reduced *job resources* may have a negative impact on mental health and job performance. *Job resources* (e.g., support from others, team climate, feedback, financial security, job control) have motivational qualities by enhancing job engagement and may protect against stress. It becomes apparent that the reduction of *job demands* (pronounced difficulties with social demands, need for reduced social and sensory stimulation, lack of financial security and job fit) does not automatically increase job engagement (Demerouti & Bakker, 2011; Schaufeli, 2017). Following the job demands-resources model, the current results suggest autism-specific employment support structures with attention to individual resources. Awareness of ASD-specific requirements at the workplace seems to be crucial. Employers may also benefit in this way if job performance can be increased by paying attention to the respective job resources and reducing job demands.

'Professional development'

Vocational qualification. What differentiates between groups is that individuals in the ASD group are supported significantly more often by supported employment programs suggesting a higher need of support services (Shattuck et al., 2012; Vogeley et al., 2013). Results might also indicate difficulties for individuals with ASD to successfully transit from school into vocational qualification (Müller et al. 2003). It can be assumed that the more structured environment of education, where social difficulties are met with higher acceptance and tolerance compared to the context of employment could be beneficial for people with ASD (Frank et al., 2018; Maslahati et al., 2022; Müller et al., 2008). Socially insecure or withdrawn behavior may lead to irritation in the workplace (Vogeley et al., 2013). Demands for social skills, adaptability, and flexible behavior seem to be increasing in working life. The likelihood of finding employment decreases due to impaired conversational skills (Roux et al., 2013). However, unfortunately there is a distinct lack of appropriate support services especially for individuals with ASD without intellectual disabilities

(Shattuck et al., 2012) resulting in the need for ongoing parental engagement (Baldwin et al., 2014).

In contrast to the differences described above, both groups were comparable in terms of their employment status and vocational qualifications. Individuals in both groups are comparatively often in process of graduation or without completed vocational qualification at the time of data collection. Individuals with ASD show decreased employment rates and, respectively, increased unemployment rates compared to individuals without ASD, however, results did not differ significantly.

Gap between educational level and employment status. Unemployment rates of 24.5% in the ASD group and 19.9% in the group of individuals without ASD are alarmingly high in both groups compared to those of the general German population (5.2%, Federal Employment Agency, 2022; see Espelöer et al., 2022 for a full discussion). Increased unemployment rates are frequently described in international as well as national literature, with rates ranging from 24% to 73% (Baldwin et al., 2014; Howlin, 2013; Howlin & Moss, 2012; Taylor & Seltzer, 2011; Vogeley et al., 2013) even in above-average educated individuals with ASD (Espelöer et al., 2022; Frank et al., 2018; Maslahati et al., 2022; Riedel et al., 2016). Although over half of the ASD group achieved a university entrance-level qualification (53.3%), this number was even higher in the comparison group of individuals without ASD (65.5%). Complementary to these results, higher rates of basic secondary education were achieved by the ASD group (18.8%) compared to the group of individuals without ASD (10.1%). Compared to the general German population with university entrance-level qualifications of 32.5%, an overqualification in individuals with ASD without intellectual disability becomes evident (see Espelöer et al., 2022 for a full discussion). However, an increased need for supported employment among individuals with ASD is reported. The risk of unemployment persists in both groups despite high levels of educational and vocational qualifications (Baldwin et al., 2014; Frank et al., 2018; Riedel et al., 2016). This discrepancy could be associated with Herzberg's (1972) intrinsic *motivators*, which might lead to decreased job satisfaction due to reduced recognition, responsibility, and development opportunities.

Termination and periods of unemployment. Difficulties in successfully participating in the working life are also reflected in repeated terminations (Espelöer et al., 2022; Frank et al., 2018). Limited abilities in soft skills and inflexible and rigid adherence to ritualistic and stereotyped behaviors could arguably hinder successful job retention. The majority of individuals in both groups had already experienced unemployment (ASD+: 68.5%, ASD-: 70.4%), with nearly half of individuals in the ASD group (47.5%) and 43.6% in the group of individuals with a ruled out ASD diagnosis experiencing long-term unemployment. In the general German population, 39.3% of unemployed persons were affected by long-term unemployment (Federal Employment Agency, 2022). Comparable to previous research (Frank et al., 2018), average periods of unemployment of 24.9 months in both groups were reported in the current study, too. Likewise, both groups similarly reported to have experienced job termination most common due to interpersonal difficulties rather than professional difficulties.

Strengths and limitations

We present data of a new and comprehensive questionnaire based on statements of individuals with ASD, which were collected in a prior qualitative study (Proft et al., 2016). Limiting the study, our convenience samples happen to differ in age and educational qualification given the posthoc database analysis. We did not adjust groups with respect to age and educational qualifications, but included both factors as covariates to avoid an artificial distortion of the population that visited the autism outpatient clinic in Cologne. This is also recommended for future studies. In the present study, we focused on persons with late-diagnosed ASD without intellectual disability. It is important to emphasize that the results cannot be generalized to all people within the autism spectrum. A large sample size as well as a sufficient response rate of 53.3% were achieved based on data from people who were motivated and able to fully complete the questionnaire.

We did not ask about the level of income and therefore could not include this point in the discussion of the results that people with ASD report greater difficulties in being able to make a living on their wages than individuals without ASD. This point should be included in future studies in order to be able to make precise objective statements. Differential diagnoses and comorbid disorders are common in adults with ASD (Strunz et al. 2014; Chen et al., 2015; Hudson et al. 2019) and have been discussed as relevant factors in research on the employment situation and level of functioning in individuals with ASD (Chen et al., 2015; Croen et al., 2015; Fombonne et al., 2021; Riedel et al., 2016). In the current study, self-reported diagnoses during lifetime provided by patients themselves were collected (see table in Supplementary material). A detailed clinical examination of differential diagnoses or comorbidities was not performed in the present study because the aim of the autism outpatient clinic is exclusively to confirm or reject the diagnosis of ASD. Level of functioning of individuals with ASD was not explicitly recorded but a relatively high level of functioning could be assumed on the basis of high educational qualification levels and late diagnoses of mainly F84.5 (85.3%). A more specific characterization of samples should be investigated as an important further aspect in future prospective studies.

CONCLUSION

The results of the current analyses described an autism-specific profile of a mismatch between social skills and social requirements in the workplace both with respect to past experiences as well as future requirements. Our results show that there are very high unemployment rates and sometimes unsurmountable challenges by the workplace posed on individuals with social interaction difficulties. There is no doubt that there is a marked need for improved support structures across these individuals. The current results suggest though that support structures should not rely on one-fits-all approaches. Instead, the results demonstrate an autism-specific profile characterized by pronounced need for reduction of social and interpersonal demands at work, for structured working conditions as well as the autism-specific difficulties with finding a suitable job ensuring economic security. Future studies should consider the effects of age and educational qualification on employment outcomes. As aspects could be described based on the exploratory approach that may influence successful integration into working life for people with interactional difficulties and specifically with ASD, these characteristics should be further investigated and corroborated in future prospective studies. Autism-specific employment support is urgently needed and awareness and knowledge about the specific requirements of individuals with ASD without intellectual disability at the workplace is potentially very helpful. Employers in turn can potentially benefit from the diverse skills set people with ASD can bring to the workplace if challenges for them are minimized.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation and analysis were performed by JE. The first draft of the manuscript was written by JE and all authors commented on previous versions of the manuscript. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved the final manuscript for publication. All authors had full access to data and CF verified the data.

ACKNOWLEDGMENTS

We thank all the participants who took part in this study. Open Access funding enabled and organized by Projekt DEAL.

FUNDING INFORMATION

The study was funded by a grant of the "Landschaftsverband Rheinland (LVR)" for a project that developed a supported employment program for adults with ASD in Germany. CF was supported by the DFG (*Deutsche Forschungsgemeinschaft*) [grant numbers FA 876/3–1, FA 876/5–1]. KV received funding from the Federal German Ministry of Education and Research (grant number: 16SV7244) and from the EC, Horizon 2020 Framework Program, FET Proactive (grant agreement id: 824128).

CONFLICT OF INTEREST STATEMENT

On behalf of all authors, the corresponding author states that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

Data availability The datasets generated and analyzed in the current study are not publicly available as they are retrieved from a clinical database of the Adult Autism Outpatient Clinic, Department of Psychiatry, of the University Hospital of Cologne, Germany. The authors will provide data and materials supporting the results or analyses presented in the current paper upon reasonable request.

ETHICS STATEMENT

The manuscript was submitted to the local Ethics Committee of the Medical Faculty, University of Cologne, which confirmed that the study is exempt from the requirement of ethical approval as under German law no separate ethics application to and statement of ethical approval by the local ethics committee are required for performing purely retrospective clinical database analyses.

ORCID

Julia Espelöer https://orcid.org/0000-0003-2847-0648

REFERENCES

American psychiatric association. (2013). Diagnostic and statistical manual of mental disorders (5th ed.). 10.1176/appi.books.9780890425596

Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften. (2016). Autismus-Spektrum-Stoerungen im Kindes-, Jugend- und Erwachsenenalter - Teil 1: Diagnostik - Interdisziplinare S3-Leitlinie der DGKJP und der DGPPN sowie der beteiligten Fachgesellschaften, Berufsverbande und Patientenorganisationen Langversion [Association of the scientific medical societies. Autism spectrum disorders in childhood, adolescence, and adulthood - Part 1: Diagnostics - interdisciplinary S3 guideline of the DGKJP and the DGPPN and the participating professional societies, professional associations, and patient organizations long version]. AWMF online.

Arora, B. (2017). Importance of emotional intelligence in the workplace. *International Journal of Engineering and Applied Sciences*, *4*(4), 257492 ISSN: 2394-3661.

Baldwin, S., Costley, D., & Warren, A. (2014). Employment activities and experiences of adults with high-functioning autism and Asperger's disorder. *Journal of Autism and Developmental Disorders*, *44*(10), 2440–2449. https://doi.org/10.1007/s10803-014-2112-z

Baron-Cohen, S., Wheelwright, S., Skinner, R., Martin, J., & Clubley, E. (2001). The autism-spectrum quotient (AQ): Evidence from asperger syndrome/high-funtioning autism, males and females, scientists and methematicians. *Journal of Autism and Developmental Disorders*, *31*, 5–17.

Bartlett, M. S. (1951). The effect of standardization on a χ^2 approximation in factor analysis. *Biometrika*, *38*(3–4), 337–344. https://doi.org/10.1093/biomet/38.3-4.337

Bender, R., & Lange, S. (2001). Adjusting for multiple testing—When and how? *Journal of Clinical Epidemiology*, *54*(4), 343–349. https://doi.org/10.1016/S0895-4356(00)00314-0

Bloch, C., Burghof, L., Lehnhardt, F. G., Vogeley, K., & Falter-Wagner, C. (2021). Alexithymia traits outweigh autism traits in the explanation of depression in adults with autism. *Scientific Reports*, *11*(1), 1–7. https://doi.org/10.1038/s41598-021-81696-5

Bottema-Beutel, K., Kapp, S. K., Lester, J. N., Sasson, N. J., & Hand, B. N. (2020). Avoiding ableist language: Suggestions for autism researchers. *Autism in Adulthood*, *3*, 18–29. https://doi.org/10.1089/aut.2020.0014

Brunello, G., & Schlotter, M. (2011). Non cognitive skills and personality traits: Labour market relevance and their development in Education & Training Systems. In *IZA discussion papers 5743*. Institute of Labor Economics (IZA).

Bühner, M. (2011). *Einführung in die test- und Fragebogenkonstruktion (3. Aufl.) [introduction to test and questionnaire construction]*. Pearson Studium.

Buijsman, R., Begeer, S., & Scheeren, A. M. (2023). 'Autistic person' or 'person with autism'? Person-first language preference in Dutch adults with autism and parents. *Autism*, *27*(3), 788–795. https://doi.org/10.1177/13623613221117914

Bury, S. M., Jellett, R., Spoor, J. R., & Hedley, D. (2020). "It defines who I am" or "It's something I have": What language do [autistic] Australian adults [on the autism spectrum] prefer? *Journal of Autism and Developmental Disorders*, *53*, 1–11. https://doi.org/10.1007/s10803-020-04425-3

Chen, J. L., Leader, G., Sung, C., & Leahy, M. (2015). Trends in employment for individuals with autism spectrum disorder: A review of the research literature. *Review Journal of Autism and Developmental Disorders*, *2*(2), 115–127.

Croen, L. A., Zerbo, O., Qian, Y., Massolo, M. L., Rich, S., Sidney, S., & Kripke, C. (2015). The health status of adults on the autism spectrum. *Autism*, *19*(7), 814–823. https://doi.org/10.1177/1362361315577517

Demerouti, E., & Bakker, A. B. (2011). The job demands-resources model: Challenges for future research. *SA Journal of Industrial Psychology* 2011, *37*(2), 1–9.

Demerouti, E., Bakker, A. B., Nachreiner, F., & Schaufeli, W. B. (2001). The job demands-resources model of burnout. *Journal of Applied Psychology*, *86*(3), 499–512. https://doi.org/10.1037/0021-9010.86.3.499

Espelöer, J., Proft, J., Falter-Wagner, C. M., & Vogeley, K. (2022). Alarmingly large unemployment gap despite of above-average education in adults with ASD without intellectual disability in Germany: A cross-sectional study. *European Archives of Psychiatry and Clinical Neuroscience*, *273*, 731–738. https://doi.org/10.1007/s00406-022-01424-6

Falter-Wagner, C. M., Bloch, C., Burghof, L., Lehnhardt, F. G., & Vogeley, K. (2022). Autism traits outweigh alexithymia traits in the explanation of mentalising performance in adults with autism but not in adults with rejected autism diagnosis. *Molecular Autism*, *13*(1), 32. https://doi.org/10.1186/s13229-022-00510-9

Fazekas, K. (2020). The growing importance of non-cognitive skills in job search and at work. In K. Fazekas, M. Csillag, Z. Hermann, & A. Scharle (Eds.), *The Hungarian labour market 2019* (pp. 134–136). Institute of Economics, Centre for Economic and Regional Studies.

Fombonne, E., MacFarlane, H., & Salem, A. C. (2021). Epidemiological surveys of ASD: Advances and remaining challenges. *Journal of Autism and Developmental Disorders*, *51*, 4271–4290. https://doi.org/10.1007/s10803-021-05005-9

Frank, F., Jablotschkin, M., Arthen, T., Riedel, A., Fangmeier, T., Hölzel, L. P., & Tebartz van Elst, L. (2018). Education and employment status of adults with autism spectrum disorders in Germany - a cross-sectional-survey. *BMC Psychiatry*, *18*(1), 75. https://doi.org/10.1186/s12888-018-1645-7

Gaskin, C. J., & Happell, B. (2014). On exploratory factor analysis: A review of recent evidence, an assessment of current practice, and recommendations for future use. *International Journal of Nursing Studies*, *51*(3), 511–521. https://doi.org/10.1016/j.ijnurstu.2013.10.005 Epub 2013 Oct 14. PMID: 24183474.

Happé, F. (1999). Autism: Cognitive deficit or cognitive style? *Trends in Cognitive Sciences*, *3*(6), 216–222. https://doi.org/10.1016/S1364-6613(99)01318-2

Herzberg, F. (1972). One more time: How do you motivate employees. In L. E. Davis & J. Taylor (Eds.), *Job design* (pp. 113–115). Penguin.

Howlin, P. (2013). Social disadvantage and exclusion: Adults with autism lag far behind in employment prospects. *Journal of the American Academy of Child and Adolescent Psychiatry*, *52*(9), 897–899.

Howlin, P., & Moss, P. (2012). Adults with autism spectrum disorders. *Canadian Journal of Psychiatry*, *57*(5), 275–283. https://doi.org/10.1177/070674371205700502

Hurlbutt, K., & Chalmers, L. (2004). Employment and adults with Asperger syndrome. *Focus on Autism and Other Developmental Disabilities*, *19*(4), 215–222.

Johnson, T. D., & Joshi, A. (2016). Dark clouds or silver linings? A stigma threat perspective on the implications of an autism diagnosis for workplace well-being. *Journal of Applied Psychology*, *101*(3), 430–449. https://doi.org/10.1037/apl0000058

Kaiser, H. F., & Rice, J. (1974). Little jiffy, mark iv. *Educational and Psychological Measurement*, *34*(1), 111–117. https://doi.org/10.1177/001316447403400115

Kapp, S. K., Gillespie-Lynch, K., Sherman, L. E., & Hutman, T. (2013). Deficit, difference, or both? Autism and neurodiversity. *Developmental Psychology*, *49*(1), 59–71. https://doi.org/10.1037/a0028353

Kirchner, J. C., & Dziobek, I. (2014). Towards successful employment of adults with autism: A first analysis of special interests and factors deemed important for vocational performance. *Scandinavian Journal of Child and Adolescent Psychiatry and Psychology*, *2*(2), 77–85.

Lehnhardt, F. G., Gawronski, A., Volpert, K., Schilbach, L., Tepest, R., & Vogeley, K. (2012). Das psychosoziale Funktionsniveau spätdiagnostizierter Patienten mit Autismus-Spektrum-Störungen-eine retrospektive Untersuchung im Erwachsenenalter [psychosocial functioning of adults with late diagnosed autism spectrum disorders--a retrospective study]. *Fortschritte der Neurologie-Psychiatrie*, *80*(2), 88–97. https://doi.org/10.1055/s-0031-1281642

Little, R. J. A. (1988). A test of missing completely at random for multivariate data with missing values. *Journal of the American Statistical Association*, *83*(404), 1198–1202. https://doi.org/10.1080/01621459.1988.10478722

Maslahati, T., Bachmann, C. J., Höfer, J., Küpper, C., Stroth, S., Wolff, N., Poustka, L., Roessner, V., Kamp-Becker, I., Hoffmann, F., & Roepke, S. (2022). How do adults with autism Spectrum disorder participate in the labor market? A German multi-center survey. *Journal of Autism and Developmental Disorders*, *52*(3), 1066–1076. https://doi.org/10.1007/s10803-021-05008-6

Monk, R., Whitehouse, A. J., & Waddington, H. (2022). The use of language in autism research. *Trends in Neurosciences*, *45*, 791–793. https://doi.org/10.1016/j.tins.2022.08.009

Müller, E., Schuler, A., Burton, B. A., & Yates, G. B. (2003). Meeting the vocational support needs of individuals with Asperger syndrome and other autism spectrum disabilities. *Journal of Vocational Rehabilitation*, *18*(3), 163–175.

Müller, E., Schuler, A., & Yates, G. B. (2008). Social challenges and supports from the perspective of individuals with Asperger syndrome and other autism spectrum disabilities. *Autism*, *12*(2), 173–190. https://doi.org/10.1177/1362361307086664 PMID: 18308766.

Olesen, S. C., Butterworth, P., Leach, L. S., Kelaher, M., & Pirkis, J. (2013). Mental health affects future employment as job loss affects

mental health: Findings from a longitudinal population study. *BMC Psychiatry*, *13*(1), 144. https://doi.org/10.1186/1471-244X-13-144

Proft, J., Gawronski, A., Krämer, K., Schoofs, T., Kockler, H., & Vogeley, K. (2016). Autismus im Beruf. *Zeitschrift für Psychiatrie Psychologie Und Psychotherapie*, *64*(4), 277–285. https://doi.org/10.1024/1661-4747/a000289

Riedel, A., Schröck, C., Ebert, D., Fangmeier, T., Bubl, E., & Tebartz van Elst, L. (2016). Überdurchschnittlich ausgebildete Arbeitslose--Bildung, Beschäftigungsverhältnisse und Komorbiditäten bei Erwachsenen mit hochfunktionalem Autismus in Deutschland [Well Educated Unemployed--On Education, Employment and Comorbidities in Adults with High-Functioning Autism Spectrum Disorders in Germany]. *Psychiatrische Praxis*, *43*(1), 38–44. https://doi.org/10.1055/s-0034-1387494

Ritchie, S. J., & Tucker-Drob, E. M. (2018). How much does education improve intelligence? A meta-analysis. *Psychological Science*, *29*(8), 1358–1369. https://doi.org/10.1177/0956797618774253

Riggio, R. E. (2020). Social skills in the workplace. *The Wiley Encyclopedia of Personality and Individual Differences: Clinical, Applied, and Cross-Cultural Research*, *I*, 527–531. https://doi.org/10.1002/9781119547181.ch352

Roux, A. M., Shattuck, P. T., Cooper, B. P., Anderson, K. A., Wagner, M., & Narendorf, S. C. (2013). Postsecondary employment experiences among young adults with an autism spectrum disorder. *Journal of the American Academy of Child and Adolescent Psychiatry*, *52*(9), 931–939.

Sarrett, J. (2017). Interviews, disclosures, and misperceptions: Autistic adults' perspectives on employment related challenges. *Disability Studies Quarterly*, *37*(2). https://doi.org/10.18061/dsq.v37i2.5524

Schaufeli, W. B. (2017). Applying the job demands-resources model. *Organizational Dynamics*, *2*(46), 120–132.

Shattuck, P. T., Narendorf, S. C., Cooper, B., Sterzing, P. R., Wagner, M., & Taylor, J. L. (2012). Postsecondary education and employment among youth with an autism spectrum disorder. *Pediatrics*, *129*(6), 1042–1049. https://doi.org/10.1542/peds.2011-2864

Federal employment agency (Statistik der Bundesagentur für Arbeit). (2022). Arbeitslosigkeit und Langzeitarbeitslosigkeit im Zeitverlauf. Einzelausgaben - Statistik der Bundesagentur für Arbeit (arbeitsagentur.de). Retrieved June 10.

Taylor, J. L., & Seltzer, M. M. (2011). Employment and post-secondary educational activities for young adults with autism spectrum disorders during the transition to adulthood. *Journal of Autism and Developmental Disorders*, *41*(5), 566–574.

Tepest, R. (2021). The meaning of diagnosis for different designations in talking about autism. *Journal of Autism and Developmental Disorders*, *51*, 760–761. https://doi.org/10.1007/s10803-020-04584-3

Vogeley, K., Kirchner, J. C., Gawronski, A., Tebartz van Elst, L., & Dziobek, I. (2013). Toward the development of a supported employment program for individuals with high-functioning autism in Germany. *European Archives of Psychiatry and Clinical Neuroscience*, *263*(2), 197–203. https://doi.org/10.1007/s00406-013-0455-7

World Health Organization. (1992). *The ICD-10 classification of mental and behavioral disorders: Clinical descriptions and diagnostic guidelines*. World Health Organization.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Espelöer, J., Proft, J., Kemmer, P., Falter-Wagner, C. M., & Vogeley, K. (2023). What is specific about employment status, workplace experiences and requirements in individuals with autism in Germany? *Autism Research*, *16*(7), 1389–1402. https://doi.org/10.1002/aur.2958