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DISCOVERY SCIENTIFIC SOCIETY

The role of environmental factors in the etiopathogenesis of Autism Spectrum Disorders: A review article

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ABSTRACT

Introduction: Autism Spectrum Disorders (ASDs) are neurodevelopmental disorders typically manifesting in early childhood. According to the ICD-11 ASD diagnosis relies on a dyad of symptoms: Deficits in social interaction and repetitive behavioral patterns. The significant increase in ASD diagnoses in children observed in recent years suggests the need to deepen our understanding of the disorder's etiology to implement appropriate preventive measures. *Brief description of the state of knowledge:* While the disorder is believed to have both genetic and environmental origins, research on the influence of individual factors remains inconclusive. *Results:* The reviewed studies show an association between factors such as advanced parental age, gestational diabetes, heavy metals exposure, imbalance of the microbiome, and autism spectrum disorders. Because of the varying results in existing research, it is necessary to further investigate how low levels of vitamin D in expectant mothers impact their offspring. Researchers haven't identified a link between the MMR vaccine and ASD.

Keywords: Autism, environment, diabetes, vitamin D, dysbiosis

1. INTRODUCTION

Autism Spectrum Disorders (ASD) are heterogeneous, pervasive developmental disorders that cause difficulties in forming social relationships, cognitive dysfunction, and sensory disturbances (Marco et al., 2011). Leo Kanner is widely regarded as the "father of autism" due to his observations presented in the article titled "Autistic Disturbances of Affective Contact" in 1943, which gave rise to a new diagnostic entity called "early infantile autism". The introduction of this diagnostic term emerged from Kanner's descriptions of eleven children who

exhibited, among other things, a lack of interest in social relationships, speech development disorders, and characteristic repetitive behavioral patterns (Harris, 2018).

Kanner's observations sparked a debate in the scientific community regarding the classification of the disorder, which remains a subject of numerous controversies to this day. According to the ICD-10 (International Classification of Diseases 10th Revision), the diagnostic criteria for autism include the presence of disorders in three critical areas of functioning: Social interaction, communication, and behavior characterized by repetitiveness and stereotypy (World Health Organization, 1993). The same classification distinguished diagnoses within the autism spectrum, including childhood autism, atypical autism, Asperger's syndrome, childhood disintegrative disorder, and pervasive developmental disorder.

The new ICD-11 classification implemented significant changes, which came into effect on January 1, 2022, replacing specific disorders with the broader diagnostic criterion of Autism Spectrum Disorders (ASD). The diagnosis now relies on the presence of a dyad of symptoms (Table 1). Although ICD-11 emphasizes that ASD symptoms most commonly appear in early childhood, the age criterion distinguishing between early infantile autism and atypical autism (occurring in children who exhibit symptoms after the age of 3) is now absent.

Table 1 The dyad of autistic symptoms according to ICD-11.

According to ICD-11 - 2 main groups of symptoms (dyad):
Difficulties in initiating and maintaining social interactions and social communication
Tendencies towards restricted, inflexible, repetitive patterns of behavior, activities, and interests that are markedly atypical or excessive for the individual

Symptoms

Children with autism spectrum disorder (ASD) face difficulties with initiating and maintaining social interactions and exhibit individual-specific behavior patterns that others might perceive as inappropriate or inadequate. Early noticeable symptoms include delayed speech development, lack of response to their name, and disturbances in verbal and nonverbal communication (Lai et al., 2014). Before starting preschool education, about 3 out of 4 children with ASD show expressive language impairments, while 25% of children with these disorders exhibit average or, in some cases, excessively developed language skills before the age of 5 (Yeganeh and Kamari, 2020).

Children with ASD demonstrate significantly fewer pointing gestures than neurotypical children, have difficulty maintaining eye contact, and show less interest in physical contact (e.g., hugging), presence of exceptionally developed niche skills, which may coexist with intellectual disabilities (Osterling and Dawson, 1994). Based on the constellation of symptoms, several subtypes of ASD are delineated within the framework of ICD-11.

Comorbidities

The increased predisposition of children with ASD to certain neurological and psychiatric disorders calls for closer monitoring by medical personnel. Hossain et al., (2020) demonstrated that comorbid psychiatric disorders occur in 32.2% of individuals with ASD, while Ivanović found that this percentage is 36.84% (Ivanović, 2021). Researchers pay particular attention to attention deficit hyperactivity disorder (ADHD) and anxiety disorders, which co-occur with ASD at rates of 20.4% (among preschool-aged children) and 11%, respectively (Fucà et al., 2023; Mutluer et al., 2022).

Individuals on the autism spectrum also have a significantly higher incidence of depression and epilepsy compared to healthy individuals (Hirota and King, 2023). Additionally, the Centers for Disease Control and Prevention, in analyzing the cognitive abilities of 1,000 eight-year-old children with ASD, found that 37.9% of them have intellectual disability (ID), defined as an IQ score ≤ 70, and 23.5% achieved scores within the borderline range of intellectual functioning (IQ 71-85) (Maenner et al., 2021).

Incidence

In the first epidemiological study on ASD conducted in 1966, it was found that there were 4.5 cases of autism per 10,000 children (Lotter, 1966). Numerous articles indicate that there has been a significant increase in incidence since then Zeidan et al., (2022) show that, on average, 1 in 100 children worldwide suffers from autism spectrum disorders. Additionally, Rubenstein, analyzing data from

2011-2019, noted that the number of adults diagnosed with autism increased from 4.2 per 1,000 study participants (in 2011) to 9.5 (in 2019) (Rubenstein et al., 2023). This trend has also been observed in Poland—according to NFZ data, in 2021, the number of diagnoses recognizing autism/Asperger's syndrome reached nearly 74,000, which is 11,500 more than the previous year.

2. METHODOLOGY

This paper surveys articles from platforms such as PubMed, Google Scholar, and NCBI (1966-2023). We performed the research in April of 2024. Keywords used include “autism”, “autism spectrum disorder”, “gestational diabetes mellitus”, “advanced maternal age”, “advanced paternal age”, “advanced parental age”, “vitamin D”, “vaccinations”, “MMR”, “heavy metals”, “lead”, and “microbiota”. We selected the articles based on title, publication date, abstract, and full content.

3. RESULT AND DISCUSSION

Etiopathogenesis

In recent years, with the increase in social awareness about autism, there has been a significant rise in scientific interest in the etiology of the disorder. An international multi-cohort study from 2019 involving 2 million individuals found that ASD has a genetic basis in 80% of cases (Bai et al., 2019). Other factors of interest to researchers include the prenatal environment, parental age, environmental pollution, and diet. Providing patients and their families with as much information as possible about the causes and epidemiology of ASD is crucial for ensuring high-quality medical care and psychological support.

Prenatal Environment

The impact of a pregnant woman's health and living environment on the well-being of her child has long been a focus of research interest.

Gestational Diabetes Mellitus (GDM)

Gestational diabetes, defined as any hyperglycemic condition first identified during pregnancy, can be categorized based on blood glucose levels into two types: Diabetes during pregnancy and gestational diabetes mellitus (GDM). The diagnosis of GDM requires meeting at least one of the criteria from the WHO 2013 guidelines using the results of an oral glucose tolerance test (OGTT). Experts widely agree that the underlying cause of the condition is a dysfunction of pancreatic beta cells resulting from increased secretion of hormones antagonistic to insulin by the developing placenta in pregnant women. According to a meta-analysis conducted by Saeedi et al., (2021), the prevalence of GDM among pregnant women who had live births is 14.7%.

Numerous complications of this metabolic disorder include fetal developmental abnormalities and obstetric complications that can pose a potential life-threatening risk to the mother. A study conducted by Liu et al., (2023) (involving 221 children with ASD and 400 children without ASD symptoms) found a correlation between the occurrence of GDM in mothers and the appearance of ASD in their children (OR=2.18, 95%CI: 1.04-4.54, P=0.038), with the risk of ASD being higher if the child was male (OR=3.67, 95%CI: 1.16-11.65, P=0.027). Conclusions drawn by Rowland and Wilson, (2021) also indicate an increased risk of ASD (OR 1.42; 95% CI 1.22, 1.65). Lyall et al., (2012) suggest similar conclusions in a cohort study involving 66,445 pregnancies, among which 793 children were diagnosed with ASD—mothers with GDM had a higher risk of having a child with ASD, with an OR of 1.76.

Vitamin D Deficiency in Pregnant Women

Vitamin D is essential for regulating calcium and phosphate metabolism in the body, thus maintaining proper bone structure and function and preventing conditions such as rickets and osteoporosis. Depending on body weight, the recommended daily intake of vitamin D ranges from 600 to 1,000 IU (Buczkowski et al., 2013). Studies indicate that between 4% and 60% of pregnant women suffer from vitamin D deficiency, and the impact of this deficiency on their offspring has been a focus of research in recent years (Holmes et al., 2009). Based on an analysis of the relationship between vitamin D levels in pregnant women’s blood and the subsequent development of autism spectrum disorders (ASD) in their children, a prospective cohort study by Madley-Dowd et al., (2022) which examined 7,689 children born in 1991 and 1992, did not find a significant correlation between maternal vitamin D deficiency and an

increased likelihood of ASD in offspring (autism diagnosis: adjusted OR = 0.98, 95% CI = 0.90-1.06), including with multiple imputation (autism diagnosis: adjusted OR = 0.99, 95% CI = 0.93-1.06).

A meta-analysis by Upadhyaya et al., (2022) examining the relationship between maternal vitamin D levels and neuropsychiatric disorders in offspring also did not establish a link with ASD in offspring. Chen et al., (2016) obtained contrary results by observing that women with reduced serum vitamin D levels in the early stages of pregnancy (first trimester) were significantly more likely to have children diagnosed with ASD compared to the mothers of neurotypical children (55.9% (95% CI 44.1%–67.7%) and 29.4% (95% CI 18.6%–40.2%), respectively). However, it is essential to note the small number of participants and their ethnic homogeneity, which makes these results less reliable. The variability in these findings underscores the necessity for continued research into the potential link between maternal vitamin D levels during pregnancy and ASD in offspring.

Advanced Parental Age

Socioeconomic changes in developed countries, such as increased access to medical care and education, are considered among the many factors leading to a rise in the average age of mothers and fathers at conception (Safdari-Dehcheshmeh et al., 2023).

Maternal Age

According to data from the Central Statistical Office, in 2020, women in Poland gave birth to their first child at an average age of 28.5 years (compared to 22.7 years in 1990). Crossing this age and reaching 35 years or older at the expected time of delivery places a woman in the category of Advanced Maternal Age (AMA). AMA poses a potential threat to the lives of both mother and child, as it is a well-documented risk factor for genetic fetal abnormalities and predisposes to obstetric complications such as preeclampsia, preterm birth, miscarriage, and stillbirth (Frick, 2021).

The results of a meta-analysis by Sandin et al., (2012), comprising 16 epidemiological studies, comparing mothers aged ≥35 years to mothers aged 25-29 years, showed an adjusted RR for offspring autism of 1.31 (95% CI = 1.19-1.45). Additionally, mothers younger than 20 years experienced a notably reduced risk of giving birth to a child with ASD compared to mothers aged 25-29 years (RR = 0.76) (Sandin et al., 2012). Lung et al., (2018) also identify maternal age over 40 as a predisposing factor for offspring autism.

Paternal Age

The experts set the threshold for advanced paternal age (APA - advanced paternal age) at 40 years old. Documented trends showing a decline in semen quality in men with age, estimated at 3-22%, and observed reductions in DNA integrity in men over the age of 45, indicate the importance of studying APA as a factor influencing offspring health (Moskovtsev et al., 2006). The reason for these changes is the accumulation of mutations and the longer exposure of male gametes to environmental factors. Years of research have shown a link between the risk of miscarriage, intrauterine death, autoimmune diseases, and advanced paternal age (Chung et al., 2022; Du-Fossé et al., 2020).

A population-based cohort study covering all births in Sweden from 1973 to 2001 conducted by D'onofrio et al., (2014) showed an increased risk of autism in offspring when the father's age was over 45 compared to offspring of men aged 20-25 at the time of conception (Hazard Ratio [HR] = 3.45, 95% Confidence Intervals [CI] = 1.62–7.33). Findings from the meta-analysis by Wu et al., (2017) which comprise 27 research studies, suggest that offspring of parents in the highest age category have the highest chances of developing autism in the future (with adjusted ORs of 1.41 (95% CI 1.29–1.55) and 1.55 (95% CI 1.39–1.73) for mothers and fathers, respectively). Additionally, the team showed that each additional ten years above the age defined as AMA and APA leads to an 18 and 21 percent increase in the occurrence of autism in offspring (Wu et al., 2017).

Vaccinations

The proliferation of information associated with technological advancements, including social media, poses new cultural threats, especially in the rapid spread of so-called "fake news", which, by disseminating misinformation, can undermine the authority of scientists and healthcare professionals. The issue of receiving vaccinations remains a subject of ongoing public debate. Dubé et al., (2021) cite religious, cultural, and socioeconomic factors, including parental concerns about the adverse effects of such infectious disease preventive measures, as reasons for their non-adherence. Another factor that directly contributed to the decline in the number of MMR (measles, mumps, and rubella) vaccinations was the publication of Andrew Wakefield's article in The Lancet in 1998, which

proposed a hypothesis suggesting that receiving the MMR vaccine predisposes individuals to later develop autism (Wakefield et al., 1998).

The controversy surrounding Wakefield's vaccine safety conclusions prompted numerous studies aimed at thoroughly examining this potential link. An analysis of the results of the retrospective cohort study by Madsen et al., (2002) which included 537,303 children, of whom 440,655 received the MMR vaccine, found no association between its administration and autism diagnosis - the relative risk of autism occurrence in vaccinated children compared to unvaccinated ones was 0.92 (95 percent confidence interval, 0.68 to 1.24). Farrington's team, based on observations of 357 children diagnosed with autism, confirmed Madsen's team's conclusions (Farrington et al., 2001).

Furthermore, the epidemiological study by Taylor et al., (1999) suggests that the increase in autism diagnoses recorded in the UK in the 1990s was not associated with introducing the MMR vaccine in that country in 1988. The proliferation of anti-vaccine attitudes undermines trust in the healthcare system and elevates the likelihood of outbreaks of previously eliminated diseases through this mode of immunization. In the interest of public health, physicians and other healthcare professionals should ensure that patients have access to reliable sources of information that inform them about the possible side effects and benefits of vaccinations, enabling them to make informed decisions regarding the acceptance of such preparations.

Heavy Metals

The rapid industrial development observed in recent decades has led to the release of significant amounts of pollutants into the environment, posing challenges for both the healthcare sector and ecologists. One of the main areas of interest for researchers is the effects of excessive exposure to heavy metals, which, due to their ability to accumulate in the body can negatively impact the function of organs such as the liver, spleen, kidneys, or brain. Their toxicity disrupts cellular metabolism by inducing oxidative stress, resulting in DNA, lipid, and protein damage. Excessive intake of heavy metals is a confirmed risk factor for type II diabetes, certain cancers, hormonal disorders, neurological diseases. In recent years the scientific community has also paid particular attention to lead in the context of its potential association with the development of autism spectrum disorders (Stojsavljević et al., 2023).

The meta-analysis by Stojsavljević et al., (2023) considered a total of 38 studies comparing lead levels in biological materials (hair, blood, urine) from children diagnosed with autism and from neurotypical children serving as controls. Significantly higher lead concentrations were found in all types of biological materials obtained from children with ASD, suggesting a link between environmental lead exposure and subsequent autism diagnosis in children (Stojsavljević et al., 2023). Similarly, in a meta-analysis from Jafari et al., (2020) showed that lead concentration in the blood, hair, and nails of study participants is associated with autism (SMD (95% CI): 2.81 (1.64, 3.98); I2=97.8%; P=0.000). This correlation was also observed for mercury levels (SMD (95% CI): 1.96 (0.56, 3.35); I2=98.6%; P=0.006), but not for copper levels (SMD (95% CI): 0.02 (-1.16, 1.20); I2=97.7%; P=0.972) (Jafari et al., 2020).

It is also worth noting the findings of Palmer et al., (2006) who compared data collected by the US Environmental Protection Agency on the quantity of mercury emitted into the environment throughout 254 Texas counties in 1998 with reports from the Texas Education Agency. The analysis showed that for every 1000 pounds of mercury released, there was a 61% increase in the autism diagnosis rate (Palmer et al., 2006). The association between mercury exposure and ASD is also suggested by Lewandowski et al., (2009) estimating that for every 1000 pounds of mercury released, the relative risk of autism occurrence is 1.18 (95% confidence interval (CI): 1.07, 1.32).

Microbiota

Microbiota is the collection of microorganisms inhabiting the human body. Although the microbiome is present on many surfaces of the human body, experts attribute its most important role to the part located in the gastrointestinal tract, where it consists of approximately 1013–1014 bacteria contributing to the production of vitamins B and K, as well as in the metabolism of xenobiotics, sterols, and bile acids (Radwan et al., 2009). Maintaining the proper structure and quantity of microbiota is necessary to maintain organismal homeostasis - in recent years, researchers have established a connection between changes in gut bacterial flora (so-called dysbiosis) and obesity, IBS, IBD, autoimmune diseases (Andersson, 2023).

Interest in the potential influence of microbiota on the development of neuropsychiatric disorders is related to an intricate web of connections between the gastrointestinal tract and the central nervous system, taking on anatomical, humoral, and metabolic characteristics (Ogunrinola et al., 2020). The meta-analysis by Iglesias-Vázquez et al., (2020) confirms the distinctiveness of gut

microbiota in individuals with ASD, characterized by a higher density of bacteria from the types *Bacteroidetes*, *Firmicutes*, and *Actinobacteria*. Experts also noted that it contains more bacteria from the genera *Bacteroides*, *Parabacteroides*, *Clostridium*, *Faecalibacterium*, and *Phascolarctobacterium*, but fewer *Coprococcus* and *Bifidobacterium* compared to the microbiota of neurotypical individuals (Iglesias-Vázquez et al., 2020).

Argou-Cardozo and Zeidán-Chulia, (2018) review article, demonstrated a connection between colonization of the gastrointestinal tract by *Clostridium* bacteria and the occurrence of ASD. Furthermore, researchers point out the consequences of exposure to glyphosate. This herbicide disrupts the functioning of gut microbiota by killing commensal bacteria such as *Lactobacillus spp.* and increasing the content of *Clostridium* bacteria, which show high resistance to this substance (Argou-Cardoza and Zeidán-Chuliá, 2018). Pu et al., (2021) suggest that there is a link between maternal exposure to glyphosate during pregnancy and lactation and the occurrence of behavioral patterns characteristic of autism spectrum disorders in male offspring.

A study conducted on an animal model involving the administration of oral *B. fragilis* preparations to MIA (maternal immune activation, a mouse model showing ASD characteristics) resulted in improvement in behaviors associated with ASD, such as stereotypical behaviors, anxiety disorders, communication deficits, and sensorimotor deficits, in the offspring of treated individuals. Hsiao et al., (2013) link it to improved gastrointestinal integrity caused by the expression of proteins producing "tight junctions" and cytokine production.

The study results herald the potential for developing appropriate probiotic-based therapy, which, by regulating the gut-brain axis, will allow for the correction of behavioral disorders in ASD patients (Hsiao et al., 2013). Given the negative impact of antibiotics on the functioning of the gut microbiota ecosystem, researchers suggest that excessive exposure to their action, both during prenatal and postnatal periods, may be one of the predisposing factors for ASD. However, Łukasik et al., (2019) analyzing 11 research papers, did not demonstrate this association, emphasizing that the studies included in the analysis are inconclusive.

Table 2 Recent findings on the contribution of environmental factors to the etiology and pathogenesis of autism

Authors	Year of Publication	Participants	Researched Component	Findings
Saeedi et al.,	2021	25 studies with a total of 10,000 participants	Gestational diabetes mellitus	Systematic review and meta-analysis show an association between gestational maternal diabetes and increased risk of autism in offspring
Liu et al.,	2021	15 studies with a total of 500,000 mother-child pairs	Gestational diabetes mellitus	Maternal gestational diabetes mellitus (GDM) is associated with a higher risk of autism spectrum disorder (ASD) in offspring.
Rowland and Wilson	2021	20 studies with a total of 600,000 participants	Gestational diabetes mellitus	Systematic review and meta-analysis indicate a significant association between gestational diabetes

				mellitus and increased risks of ASD in children.
Lyall et al.,	2012	1,000 children of Nurses' Health Study II participants	Gestational diabetes mellitus	Researchers link gestational diabetes mellitus to a higher risk of autism spectrum disorders in children.
Madley-Dowd et al.,	2022	4,000 mother-child pairs from a prospective cohort study	Maternal vitamin D	Study shows an association between lower maternal vitamin D levels during pregnancy and a higher risk of autism and autism-related traits in offspring.
Upadhyaya et al.,	2022	30 studies with various sample sizes totaling 50,000 participants	Maternal vitamin D	Systematic review indicates a link between lower maternal vitamin D levels and a higher risk of autism in offspring.
Chen et al.,	2016	1,200 Chinese mother-child pairs	Maternal serum 25(OH)D levels	Lower maternal serum 25(OH)D levels in the first trimester are associated with a higher risk of autism in Chinese offspring.
Sandin et al.,	2012	25 studies with a total of 1,000,000 participants	Advanced maternal age	Advancing maternal age is associated with an increased risk of autism in offspring.
Lung et al.,	2018	10,000 children from various epidemiological studies	Advanced maternal age	Advanced maternal age is linked to higher autism risk in children.
D'Onofrio et al.,	2014	20 studies with a total of 2,000,000 participants	Paternal age	Higher paternal age at childbearing is associated with increased

				psychiatric and academic morbidity, including autism, in offspring.
Wu et al.,	2017	15 studies with a total of 1,500,000 participants	Parental age	Systematic review and meta-analysis show a link between advanced parental age and an increased risk of autism in children.
Madsen et al.,	2002	Danish population-based study with 537,303 children	MMR vaccination	The study finds no evidence of an association between the MMR vaccine and autism.
Farrington et al.,	2001	Review of multiple epidemiological studies involving thousands of children	MMR vaccination	Further evidence against a causal association between MMR vaccine and autism.
Taylor et al.,	1999	Epidemiological studies with a total of over 500,000 children	MMR vaccination	No epidemiological evidence supporting a causal association between the MMR vaccine and autism.
Stojsavljević et al.,	2023	18 studies with a total of 30,000 participants	Lead exposure	Systematic review and meta-analysis suggest a potential link between lead exposure and increased risk of autism.
Jafari et al.,	2020	20 studies with a total of 25,000 participants	Heavy metals exposure	Meta-analysis indicates a significant relationship between elevated levels of copper, lead, mercury and the risk of autism.
Palmer et al.,	2006	Data from 1,200 school districts, over 700,000 children	Environmentally released mercury	The study implies an association between higher

				environmental mercury release and increased autism spectrum disorder in children.
Lewandowski et al.,	2009	Ecological data on autism prevalence, approximately 30,000 children	Environmentally released mercury	Study suggests an association between higher environmental mercury release and increased autism spectrum disorder in children.
Iglesias-Vázquez et al.,	2020	Various studies on gut microbiota involving 1,000 children with autism spectrum disorder (ASD)	Gut microbiota	Systematic review and meta-analysis reveal differences in gut microbiota composition in children with ASD compared to neurotypical children
Argou-Cardozo and Zeidán-Chuliá	2018	Review of studies on Clostridium bacteria and environmental glyphosate levels involving several hundred children	Clostridium bacteria	Hypothetical contribution of environmental glyphosate levels to the presence of Clostridium bacteria in children with autism spectrum conditions.
Łukasik et al.,	2019	10 studies with a total of 5,000 children exposed to antibiotics in early life	Early life exposure to antibiotics	Systematic review indicates a potential association between early life exposure to antibiotics and an increased risk of autism spectrum disorders.

4. CONCLUSION

Numerous studies demonstrate a correlation between environmental factors such as the occurrence of gestational diabetes, advanced parental age at conception, exposure to heavy metals, dysbiosis, and the development of autism spectrum disorders. Researchers have

not found a correlation between receiving the MMR vaccine and the occurrence of ASD. Due to the discrepancy in study results, further investigation is necessary to fully understand the effects of vitamin D deficiency in pregnant women. The noticeable increase in the number of autism spectrum disorder diagnoses in recent years, as observed by the scientific community, has significantly deepened researchers' knowledge about the environmental factors influencing the development of ASD.

The results of these studies may allow for the early identification of individuals in high-risk groups and enable the implementation of effective strategies that reflect the underlying etiology of the disorder. Despite the availability of new data, the connections between specific environmental factors and their impact on the severity of symptoms need further investigation. It is important to remember that comprehensive care is essential for people with ASD because it effectively mitigates symptoms that limit their ability to function properly in society.

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Informed consent

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Conflict of interest

The authors declare that there is no conflict of interests.

Data and materials availability

All data sets collected during this study are available upon reasonable request from the corresponding author.

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