frontiers | Research Topics

Physical and medical conditions associated with autism

Edited by
Martina Micai, Mila Vulchanova, Valentina Riva and David Saldaña

Published in
Frontiers in Psychiatry

ISSN 1664-8714
ISBN 978-2-8325-5106-6
DOI 10.3389/978-2-8325-5106-6

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Physical and medical conditions associated with autism

Topic editors

Martina Micai — National Institute of Health (ISS), Italy

Mila Vulchanova — Norwegian University of Science and Technology, Norway

Valentina Riva — Eugenio Medea (IRCCS), Italy

David Saldaña — Sevilla University, Spain

Citation

Micai, M., Vulchanova, M., Riva, V., Saldaña, D., eds. (2024). *Physical and medical conditions associated with autism*. Lausanne: Frontiers Media SA.

doi: 10.3389/978-2-8325-5106-6

Table of contents

frontiers | Frontiers in Psychiatry

TYPE Editorial
PUBLISHED 21 June 2024
DOI 10.3389/fpsyt.2024.1447188

OPEN ACCESS

EDITED AND REVIEWED BY
Antonio M. Persico,
University of Modena and Reggio Emilia, Italy

*CORRESPONDENCE
Martina Micai
martina.micai@iss.it

RECEIVED 11 June 2024
ACCEPTED 17 June 2024
PUBLISHED 21 June 2024

CITATION
Micai M, Saldaña D, Vulchanova M and Riva V (2024) Editorial: Physical and medical conditions associated with autism.
Front. Psychiatry 15:1447188.
doi: 10.3389/fpsyt.2024.1447188

Editorial: Physical and medical conditions associated with autism

Martina Micai[1]*, David Saldaña[2], Mila Vulchanova[3] and Valentina Riva[4]

[1]Coordinamento e Promozione della Ricerca, Istituto Superiore di Sanità, Rome, Italy, [2]Laboratorio de Diversidad Cognición y Lenguaje, Departamento de Psicología Evolutiva y de la Educación, Universidad de Sevilla, Seville, Spain, [3]Language Acquisition and Language Processing Lab, Department of Language and Literature, Norwegian University of Science and Technology (NTNU), Trondheim, Norway, [4]Scientific Institute, IRCCS E. Medea, Child Psychopathology Unit, Lecco, Bosisio Parini, Italy

KEYWORDS

autism, physical health, mental health, comorbidity, dual diagnosis

Editorial on the Research Topic
Physical and medical conditions associated with autism

This Research Topic, entitled "Physical and Medical Conditions Associated with Autism," aims to provide evidence and data to enhance our understanding of the prevalence, causes, interventions, and long-term impacts of physical and medical conditions in autistic individuals. The aim is to address these and other related questions, while raising awareness of how associated physical and medical conditions affect the lives of autistic individuals.

Certain physical and medical conditions, such as sleep-wake disorders, epilepsy, and sensory impairments, are well-recognized as being more common among autistic individuals compared to the general population. However, the prevalence and impact of other conditions, including cardiovascular issues, immune dysregulation and inflammation, genetic conditions, and constipation remain to be thoroughly established. The presence of these co-occurring conditions can hinder the success of treatments and worsen the quality of life for both autistic individuals and their careers. However, there are substantial gaps in the literature regarding these co-occurring conditions in autistic individuals.

The Research Topic is now published and includes nine different papers written by colleagues/samples from Europe (Spain, Italy, Netherlands, Romania), Asia (China and Taiwan), America, and Australia. This Research Topic consists of five original articles, three review articles, and one brief research report, published in 2022, 2023, and 2024.

1 Genetic and molecular mechanisms

This study by Zhou and Gao (2022) investigates the cuproptosis signaling pathway, a novel form of regulated cell death, in the context of ASD. The researchers collected gene expression profiles from brain samples of ASD model mice and blood samples from

humans with ASD. They identified crucial genes in the cuproptosis pathway, such as FDX1, DLAT, LIAS, and ATP7B, using machine-learning models. The study found that the artificial neural network (ANN) model had the highest accuracy, sensitivity, and specificity, suggesting its potential for early ASD identification.

2 Immune dysregulation and inflammation

The reviews by Erbescu et al. (2022) and Jyonouchi (2024) explore the role of immune system dysregulation in autism spectrum disorder (ASD) and the potential of anti-inflammatory therapies. Erbescu et al. (2022) highlight the complex interaction between the central nervous system and the immune system, emphasizing the disruption of cytokine levels that contribute to neuroinflammation in ASD. They discuss various immune molecules involved in antigen presentation and inflammatory responses, maternal immune activation, brain-reactive antibodies, and autoimmunity as prenatal and postnatal factors in ASD pathophysiology. Oxidative stress, mitochondrial dysfunction, and gastrointestinal inflammation are also analyzed as contributing factors. The importance of genetic and epigenetic factors linked to immune dysregulation is highlighted, suggesting their potential as biomarkers for ASD. Jyonouchi (2024) examines the potential of anti-inflammatory therapies for ASD, noting that many ASD subjects do not respond to first-line behavioral and pharmacological interventions. The analysis focuses on the role of neuroinflammation in ASD pathogenesis, influenced by genetic, epigenetic, and environmental factors. Promising results from anti-inflammatory therapies targeting metabolic changes and oxidative stress are discussed. The study highlights the potential of repurposing existing anti-inflammatory medications and the need for a deep understanding of emerging agents, such as biologic and gatekeeper blockers, tailored to specific inflammatory pathways in ASD patients.

3 Epidemiological and health profiles

The study by Vidriales-Fernández et al. (2023) provides an epidemiological analysis of the health 2,629 registries of autistic individuals in Spain. It highlights a higher prevalence of nervous system disorders, mental health diagnoses, and other co-occurring conditions among autistic individuals. The study identifies increased health risks for women, older individuals, and those with intellectual disabilities, noting a high use of psychopharmacological treatments from early childhood.

Lee's study examines the link between early childhood constipation and the risk of developing ASD, using a nationwide population-based cohort in Taiwan. The research found that constipated 3 year-old children had a significantly higher incidence of ASD compared to non-constipated children. The study highlights the potential role of gut microbiota alterations in ASD pathogenesis and emphasizes the importance of monitoring gastrointestinal health in early childhood.

Distefano's et al. (2023) study investigates the prevalence and impact of sleep disorders in 163 preschool autistic children. Children with poor sleep had higher scores in all areas assessed by the Children's Sleep Habits Questionnaire (CSHQ) and on the Child Behavior Checklist (CBCL) across all domains. The analysis showed that severe sleep disorders were linked to higher scores in internalizing, externalizing, and total problems on the CBCL syndromic scales, as well as on all DSM-oriented CBCL subscales. Additionally, the study found that the connection between sleep disorders and restricted and repetitive behaviors (RRBs) is explained by anxiety-related symptoms. The findings highlight the need for screening and early intervention for sleep problems.

4 Treatment and strategies

Liu's et al. (2023) systematic review and meta-analysis evaluates the efficacy of non-invasive brain stimulation (NIBS) in ASD. The analysis of 22 randomized controlled trials shows positive effects of NIBS on repetitive behaviors, cognitive function, and executive function in autistic individuals. The findings call for more rigorous, large-scale studies to validate its efficacy and establish standardized treatment protocols.

Warreman et al. (2024)'s study explores the prevalence of metabolic syndrome (MetS) in 17,705 adults with autistic traits. The research finds that MetS is more common in females with high autistic traits compared to those with low traits, while no significant difference is observed in males. The study identifies associations between MetS and poorer self-reported health, reduced physical activity, and altered leukocyte counts. The findings underscore the importance of cardiovascular prevention strategies tailored to individuals with autistic traits, particularly females.

Yau et al. (2024)'s research investigates the barriers to effective use of augmentative alternative communication (AAC) devices for minimally verbal or nonspeaking Australian autistic individuals. Through semi-structured interviews and focus groups with 30 parents, educators, and clinicians, the study identifies common themes such as stakeholder knowledge, attitudes, stigma, resource availability, AAC-user engagement, and device fit. The research highlights contrasting perspectives among stakeholders, particularly regarding stigma, resource struggles, and communication processes.

5 Conclusions

In summary, increased awareness of specific physical and medical conditions associated with autism enables targeted interventions and better outcomes. Comprehensive medical assessments of autistic individuals are crucial to clarify their co-

occurring conditions, identify differential diagnoses, and decide on tailored, individualized interventions.

Author contributions

MM: Conceptualization, Data curation, Writing – original draft, Writing – review & editing. DS: Writing – review & editing. MV: Writing – review & editing. VR: Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. DS was funded by Grant PID2021-122658NB-I00 funded by MICIU/AEI/10.13039/501100011033 and by "ERDF A way of making Europe". VR was funded by the Italian Ministry of Health (Ricerca corrente) for biomedical research.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

frontiers | Frontiers in Psychiatry

TYPE Review
PUBLISHED 19 October 2022
DOI 10.3389/fpsyt.2022.1006612

OPEN ACCESS

EDITED BY
Valentina Riva,
Eugenio Medea (IRCCS), Italy

REVIEWED BY
Sarah Hohmann,
Heidelberg University, Germany
Ourania Tsitsilonis,
National and Kapodistrian University of Athens, Greece
Marco Vinceti,
University of Modena and Reggio Emilia, Italy
Viviana Roman,
Institute of Virology-Center of Immunology, Romania

*CORRESPONDENCE
Aurora Arghir
aurora.arghir@ivb.ro

SPECIALTY SECTION
This article was submitted to Autism, a section of the journal Frontiers in Psychiatry

RECEIVED 29 July 2022
ACCEPTED 23 September 2022
PUBLISHED 19 October 2022

CITATION
Erbescu A, Papuc SM, Budisteanu M, Arghir A and Neagu M (2022) Re-emerging concepts of immune dysregulation in autism spectrum disorders.
Front. Psychiatry 13:1006612.
doi: 10.3389/fpsyt.2022.1006612

Re-emerging concepts of immune dysregulation in autism spectrum disorders

Alina Erbescu[1,2], Sorina Mihaela Papuc[1], Magdalena Budisteanu[1,3,4], Aurora Arghir[1]* and Monica Neagu[1,2,5]

[1]Victor Babes National Institute of Pathology, Bucharest, Romania, [2]Faculty of Biology, Doctoral School, University of Bucharest, Bucharest, Romania, [3]Prof. Dr. Alex. Obregia Clinical Hospital of Psychiatry, Bucharest, Romania, [4]Faculty of Medicine, Titu Maiorescu University, Bucharest, Romania, [5]Colentina Clinical Hospital, Bucharest, Romania

Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by communication and social interaction deficits, and by restricted interests and stereotyped, repetitive behavior patterns. ASD has a strong genetic component and a complex architecture characterized by the interplay of rare and common genetic variants. Recently, increasing evidence suggest a significant contribution of immune system dysregulation in ASD. The present paper reviews the latest updates regarding the altered immune landscape of this complex disorder highlighting areas with potential for biomarkers discovery as well as personalization of therapeutic approaches. Cross-talk between the central nervous system and immune system has long been envisaged and recent evidence brings insights into the pathways connecting the brain to the immune system. Disturbance of cytokine levels plays an important role in the establishment of a neuroinflammatory milieu in ASD. Several other immune molecules involved in antigen presentation and inflammatory cellular phenotypes are also at play in ASD. Maternal immune activation, the presence of brain-reactive antibodies and autoimmunity are other potential prenatal and postnatal contributors to ASD pathophysiology. The molecular players involved in oxidative-stress response and mitochondrial system function, are discussed as contributors to the pro-inflammatory pattern. The gastrointestinal inflammation pathways proposed to play a role in ASD are also discussed. Moreover, the body of evidence regarding some of the genetic factors linked to the immune system dysregulation is reviewed and discussed. Last, but not least, the epigenetic traits and their interactions with the immune system are reviewed as an expanding field in ASD research. Understanding the immune-mediated pathways that influence brain development and function, metabolism, and intestinal homeostasis, may lead to the identification of robust diagnostic or predictive biomarkers for ASD individuals. Thus, novel therapeutic approaches could be developed, ultimately aiming to improve their quality of life.

KEYWORDS

autism spectrum disorder, immune-related genes, cytokine, neuroinflammation, epigenetic factors

Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental condition with multifactorial etiology. The clinical picture is characterized by social interaction and communication deficits, as well as by restricted interests and stereotyped and repetitive behavior patterns (1). ASD prevalence has increased in recent decades, with current estimates of at least 1% of all children (2, 3). ASD is primarily a clinical diagnosis based on Diagnostic And Statistical Manual Of Mental Disorders, Fifth Edition (DSM 5) criteria. Depending on the degree of dependence on the entourage in the daily life, there are three levels of ASD severity: level 1 (requiring support), level 2 (requiring substantial support), and level 3 (requiring very substantial support) (1). ASD is characterized by an important clinical heterogeneity, related primarily to the severity of its specific features. Secondly, the heterogeneity lies in the association of other neurologic and psychiatric conditions such as epilepsy, motor abnormalities, intellectual disability, attention deficit hyperactivity disorder, sleep disorders, anxiety, and depression (4–7). In addition, a vulnerability to diverse medical conditions, such as gastrointestinal diseases, allergies, infectious disorders, immune dysfunctions, autoimmune conditions, and less common, congenital cardiac anomalies and hearing impairment, was observed in ASD populations (7, 8). Autism is currently considered a spectrum of deficits, across which a quantitative variation of behavioral and cognitive impairments is observed (9). In addition to the neurobehavioral phenotype, multiple systems and organs are involved, recent research suggesting that alteration of pleiotropic genes (i.e., a gene that independently influence several distinct phenotypic traits) and disruption of essential molecular mechanisms might underlie these various comorbid conditions (8).

ASD has a strong genetic component and a complex architecture characterized by an interplay of rare and common genetic variation (9–13). Various rare genetic variants with major individual phenotypic effect were discovered and proved to have a substantial contribution to ASD individual liability. However, each of these anomalies are extremely rare, accounting for <1% of ASD individuals (14–17). Common genetic variation, on the contrary, is estimated to collectively account as a major contributor to ASD liability, while individually has a small effect size (10, 12). The sustained efforts to decipher ASD genetics led to identification of many genes linked to ASD and neurodevelopmental disorders (NDDs). Autism resources such as SFARI Genes (https://gene.sfari.org/database/human-gene/, accessed August 5th 2022), includes 1,075 genes to date. One hundred and two genes were found to be strongly associated with ASD and NDDs risk in one of the largest whole exome sequencing studies (18). Immune response featured as one of the main biological pathways in which these genes were involved, beside neuronal communication, gene expression regulation, and cytoskeleton organization (18).

The extreme genetic heterogeneity of ASD is considered explanatory for the high variability of clinical presentations. However, there is a growing appreciation of the fact that the wide spectrum of genetic defects seems to disrupt common molecular functions and pathways (9, 19, 20).

Besides genetic factors and early lesions or immaturity of the brain, environmental factors may play a role in ASD, by themselves or in combination with the other risk factors. Air pollution during pregnancy, or in the first year of life (21, 22), as well as exposure during pregnancy to heavy metals, such as arsenic, cadmium, lead (23, 24), organic toxicants (25), or pesticides (26) were proposed as risk factors for ASD in offspring.

Nutritional deficiencies during pregnancy, such as low levels of Vitamin D (27) further discussed in this paper, were reported in various studies as factors that may contribute to an increase risk of ASD in offspring (27). In addition, gestational diabetes mellitus and hypertensive disorder of pregnancy may be associated with a risk of ASD in offspring, however further research is warranted to validate these findings (28, 29).

Genetic alterations in interaction with environmental risk factors converge toward the immune system (30). The genetic, epidemiologic, and immunologic studies bring new insights that can facilitate patient stratification, clinical management, and discovery of new therapeutic approaches.

The present paper reviews the updates regarding immune dysregulation, immune-related risk genes/pathways in ASD, highlighting some promising areas for potential biomarkers and therapeutic targets discovery.

Immune system involvement in ASD

As the immune system is intricately linked to all systems and organs, it plays an important role in maintaining the entire body homeostasis. Crosstalk between the central nervous system and immune system has been hypothesized previously; recent evidence emerging from various studies of neuroanatomy, neuroendocrinology and cell biology began to elucidate the pathways connecting the brain to immune system (31). In this regard, maternal immune activation appearing in the first 3 months of fetal development has been suggested to be involved in the disruption of normal neurodevelopment (32). Numerous ASD gene expression studies pointed toward an immune system deregulation (33–35). Among the significantly upregulated genes in postmortem ASD brain tissues, many were identified to be involved in immune and inflammatory response and other immune regulatory processes (33, 34). Epigenomic players, such as microRNAs (miRNAs), were also shown to be dysregulated in ASD individuals, in blood and brain tissue (36, 37). These miRNAs target ASD genes and regulate, in correlation with transcription factors, complex metabolic and immune components (37). Additionally, genome-wide

association studies brought new data on autistic-like traits or ASD that were associated with common variation affecting immune pathways (38, 39).

Dysregulation of both the innate and adaptive immune responses appear to be involved in ASD (40–42). The innate immune cells (e.g., monocytes, macrophages, and microglia) as well as immune molecules associated with innate immune responses were found to be altered in ASD individuals (40, 43–46). Elevated levels of cytokines, such as interleukin (IL)-1 β, IL-6, and tumor necrosis factor alpha (TNF- α), were detected in the plasma or postmortem brain samples of individuals with ASDs (40, 47–49). Besides monocytes, microglia have been the focus of intense investigation in ASD research. Microglia acts as an essential player in neural circuits formation; microglia activation, by up-regulated IL-1β, IL-6, IL-17, IL-18, IL-33, and TNF-α, has been proposed as a potential contributor to ASD pathogenesis (30, 34, 48, 50, 51). Adaptive immune responses are mediated by B and T lymphocytes (B-cells and T-cells) as a response to antigen exposure. B-cells contribute to the immune dysregulation in ASD, recent studies revealing high levels of pro-inflammatory cytokines (IL-6 and TNF-α) and low levels of anti-inflammatory cytokine - IL-10 in peripheral blood lymphocytes of ASD children (52). The analysis of T helper cells (Th cells) compartment and NK cell signaling pathways, placed at the interface between innate and adaptive immune responses, provided further evidence supporting the pro-inflammatory ASD environment (40, 42, 53, 54).

Systemic and central nervous system (CNS) inflammatory processes, through various immune cell compartments and specific cytokine networks, lead to a marked neuroinflammation suggested to contribute to ASD pathophysiology (50, 55–57).

Cytokine dysregulation in ASD

Cytokines are intercellular signaling molecules, responsible for immune regulation and inflammatory responses; selected cytokines panels proved useful biomarkers when studying inflammation patterns (58–61). Cytokines are strictly linked to the immune response, although they can influence a wide range of different processes. Common and rare variants in cytokine genes, other regulatory genes or enhancer elements have been described in association with variation of cytokine levels among healthy individuals (62). Moreover, genetic variation regulating cytokine gene expression was associated with susceptibility to various immune-mediated and complex disorders (63, 64). The role of immune-related genes in ASD has been the focus of many recent studies. Among all known cytokines, IL-1 stands out as a molecule involved in many neuronal physiological pathways. IL1-β modulates neural plasticity and, historical examples show that IL1-β is necessary for long-term potentiation maintenance of hippocampal CA1 region (65). Animal model studies using specific antagonists, such as IL-1RA, showed that low levels of IL1-β are essential for normal synaptic plasticity (66), while abnormally elevated levels or depleted IL-1 lead to memory impairments (67, 68). Proinflammatory cytokines, such as IL-1, IL-6, TNF-α, and many others, have been reported to be dysregulated in various neuropsychiatric disorders (69, 70). Various members of IL-1 family, as main inflammatory cytokines, were investigated in different cohorts of ASD children. IL-1β triggers an inflammatory response through lymphocyte and macrophage activation. A chronic inflammatory state is sustained by increased tissue infiltration of inflammatory cells mediated by IL-1β (71). Another pro-inflammatory cytokine, IL-6 is an important immune factor involved in brain development; IL-6 impairs neurons' cellular adhesion and migration, as well as synapse development (72, 73). TNF-α is essential to inflammation regulation, especially inflammatory cytokine production (74); the impairment of TNF-α synthesis has long been suggested as relevant for immune disorders and complex diseases pathogenesis (75).

Analysis of peripheral cytokine messenger RNA (mRNA) expression has shown that *TNF-α* and *IL-6* were found statistically significant up-regulated in the blood (76) and in B cells of ASD subjects as compared to controls (52). Another study confirming the cytokine inflammatory status in ASD has shown that mRNA expression for *IL-1β, IL-4, IFN-γ, IL-9,* Janus Kinase 1 *(JAK1),* and Signal transducer and activator of transcription 5 *(STAT5)*, in peripheral blood mononucleate cells (PBMC), was found significantly elevated in ASD individuals compared to controls (77). *IL-31*, coding another cytokine involved in chronic inflammation (78) was found to be increased in ASD. Pro-inflammatory cytokines production in stimulated monocytic cells from peripheral blood was investigated by Enstrom et al. in ASD (45). After stimulation with toll-like receptors ligands, the monocytes had a significant increase in IL-1 β, IL-6, and TNF-α secretion in ASD children compared to typically developing age-matched controls. Ashwood et al. found increased IL-1β, IL-6, IL-8, IL-12p40 plasma levels in children with ASD; the increased cytokine levels seemed to be associated with more severe symptoms of core ASD domains (40). Another study has shown that ASD children had increased levels of TNF-α protein and decreased expression of TNF and HNRNPL-related immunoregulatory long non-coding RNA (*THRIL*) gene. *THRIL* was shown to negatively regulate TNF-α expression in macrophages, thus this regulatory pathway could be disturbed in ASD individuals (79). The elevated peripheral proinflammatory cytokines landscape showed a potential correlation with ASD comorbidities, namely epilepsy; higher levels of IL-12p40 were detected in ASD individuals having a positive seizure history compared with ASD and no epilepsy (54).

IL-16, a chemoattractant that modulates T cell activation was studied in ASD's PBMCs and several dysregulations were found. Thus CD4+IL-16+, CD8+IL-16+, CD14+IL-16+, CCR3+IL-16+, and CXCR7+IL-16+ cells were found increased in ASD with a concomitantly increased expression

of IL-1β+IL-16+, IL-6+IL-16+, and TNF-α+IL-16+. All these results qualify the chemoattractant IL-16 as another driver of immune alteration (80).

In addition, several meta-analyses found significantly increased peripheral pro-inflammatory cytokine levels, such as IL-1β, IL-6, and IFN-γ and decreased levels of transforming growth factor (TGF)-1β as an anti-inflammatory cytokine, in ASD individuals compared to controls (69, 81, 82).

Cytokine studies performed on blood spots from neonatal cohorts allowed the assessment of circulating levels prior to an ASD diagnosis. Krakowiak et al. showed that IL-1β and IL-4 circulatory levels detected at birth are independently associated with ASD, the clinical diagnosis being established from 2 to 5 years of age. A correlation with ASD severity was also observed, IL-4 being associated with severe forms of ASD (according to Autism Diagnostic Observation Schedule score) and IL-1β with mild ASD. The increased expression of IL-1β and IL-4 indicate prenatal immune abnormalities, and thus support a potential contribution to ASD pathogenesis (83). In another study, higher concentrations of IL-6 and IL-8 were found in neonatal blood spots from individuals who later developed ASD compared to the general population. In addition, significantly increased eotaxin-1, IFN-γ, and IL-12p70 levels were found when comparing ASD with children with developmental delay (84).

Concordant results were obtained for adult psychiatric disorders where circulatory cytokine levels were significantly increased (IL1-RA, IL-18, TNF, IL-6, and C-Reactive Protein). Over-expression of genes coding inflammatory molecules was also observed to be positively correlated with disease severity (85).

Adaptive immune responses are triggered by exposure to antigens and are generally mediated by Th cells. Th cells can be categorized, depending on the cytokines produced and their functional consequences, into pro-inflammatory Th1 or anti-inflammatory Th2. Although various cytokine studies in ASD children reported increased levels of either Th1 (40, 45, 86, 87) or Th2 cytokines (88), the evidence converge toward a pro-inflammatory ASD environment (40, 53, 54).

The immune dysfunction observed in ASD individuals is also supported by post-mortem brain tissue and cerebrospinal fluid (CSF) studies. Vargas et al. showed evidence for active inflammatory processes in the cerebral cortex and cerebellum of ASD patients, supported by elevated levels of cytokines such as chemoattractant protein (MCP)−1 and IL-6 (48). Another study showed the presence of elevated levels of IL-6, IL-8, TNF-α, granulocyte-macrophage colony-stimulating factor (GM-CSF), and IFN-γ, with no significant differences for IL-4, IL-5, and IL-10 between port-mortem brain samples from ASD and control groups, thus pointing toward Th1 pathway activation and subsequently increased adaptive response (49). Increased levels of IL-6 were found by Wei et al. in the cerebellum of post-mortem tissue samples from ASD individuals and were interpreted as potential contributors to the alteration of the balance between excitatory and inhibitory circuits (89).

CSF also proved valuable for immune-related detection of biomarkers in ASD. Smedler et al. analyzed over 200 proteins from CSF and serum collected from twins with various neurodevelopmental conditions including ASD, in a study aimed to detect ASD markers. The study showed that autistic behavior and ASD were associated with serum B-cell activating factor (BAFF) and Cystatin B (CSTB) (90). BAFF is a cytokine belonging to the TNF ligand family, known to be involved in autoimmune disorders (91), as well as in psychiatric disorders, such as schizophrenia and bipolar disorder (92). In an animal model of ASD, it was shown that TNF superfamily member 13b gene (*Tnfsf13b*), encoding BAFF, is up-regulated in the prefrontal cortex; moreover, the study proposed *Tnfsf13b* among the immune genes that may play a role in social behavior regulation (93).

Although there are many studies supporting a proinflammatory component of ASD and immune dysregulation, some limitations of these studies must be taken into account. As cytokine concentrations and ratio can fluctuate, standardization is required with regard to the analysis type, sample source and the molecular player examined (gene, mRNA, protein and so on) (94).

An overview of the main immune cells and their secreted cytokines involved in neuro-inflammation in ASD are presented in Figure 1.

HLA and immunoglobulins dysregulation in ASD

The major histocompatibility complex (MHC) region is a complex genomic system localized on chromosome 6p21.3p22.1. It encompasses the human leukocyte antigen (HLA) gene cluster, that has important biological roles in immune system activity, as well as in neurodevelopment and neuroplasticity (41, 95–98). HLA cluster is one of the most polymorphic regions in the human genome and includes three distinct functional classes annotated from I to III. Considering the variety of functions of HLA genes in immune regulation and nervous system development and homeostasis, numerous studies suggested a connection between HLA alleles/haplotypes and psychiatric disorders (35, 99, 100). Several association studies revealed specific HLA alleles related to ASD, such as *HLA-DRB1* and *HLA-A2* (101–103). However, the analysis of the complex MHC region in large autism genome-wide association studies (GWAS) was hindered by the complexity and haplotype diversity of this region, thus precluding further association of HLA alleles and autism (12, 100).

Tissue expression levels of the HLA-DR alpha (HLA-DRA), MHC class II cell surface receptor, were significantly

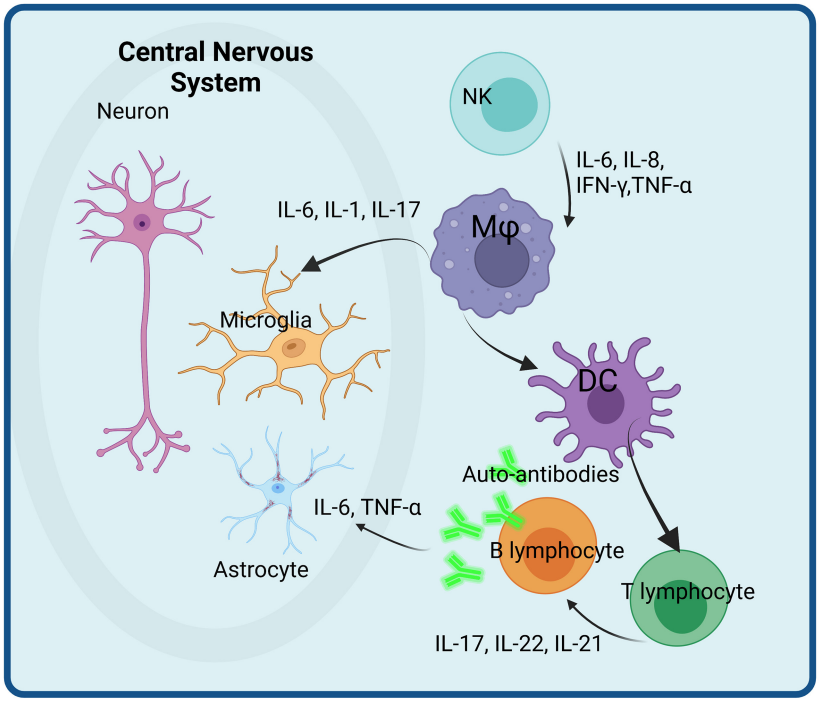


FIGURE 1
Immune cells and their secreted immune-related molecules with potential roles in neuro-inflammation in ASD. Activated NK cells secrete pro-inflammatory cytokines (IL-6, IL-8, IFN, and TNF) that activate macrophages (Mφ) with enhanced secretion of IL-6, IL-1, and IL-17 that impacts directly the microglial populations. Activated dendritic cells (DC) play a role in T and B lymphocytes activation, that enhance the pro-inflammatory cytokine secretion as well as the differentiation of B cells in plasma cells. The auto-antibodies thus produced further attack astrocytes. Created with BioRender.com.

reduced in the gray matter of post-mortem ASD brain samples compared to control samples. In addition, the expression of the Mannose Receptor C-Type 1 (*MRC1*), an anti-inflammatory gene, was found significantly increased in the white matter of ASD individuals. MRC1+ cells are involved in the neuroinflammatory cellular processes, namely in the removal of apoptotic/necrotic cells (47).

The pro-inflammatory microglia phenotype is characterized by HLA-DR and CD68 expression. HLA-DR is mainly involved in antigen presentation required for T-cell function, while CD68 is mainly associated with phagocytosis functions (47). The alteration of their expression in the post mortem ASD brain samples differs with regard to the studied cerebral area (47, 48). MRC1 activation induces IL-10 secretion, while IL-10 induces MRC1 expression in cultured macrophages (104). The inflammatory alterations reported by Sciara et al. within the brain tissue reside in the increased vasculature areas and may lead to altered myelination and, ultimately, may contribute to the complex ASD phenotype (47).

In another study evaluating post-mortem brain tissue in ASD, DiStasio et al. showed that the perivascular lymphocytic cuffs display increased numbers of lymphocytes in over 60% of the investigated ASD samples. Total T lymphocytes predominate over B lymphocytes and cytotoxic T CD8+ over helper CD4+ T lymphocytes. These perivascular lymphocytic infiltrates are associated with astrocyte blebs irrespective of the diagnostic age. The authors suggest that the astrocytic blebs are the result of cytotoxic T-lymphocytes. Taking into account the association of ASD with specific HLA alleles and MHC molecules, the authors suggest that cytotoxic T CD8+ lymphocytes in ASD target MHC-expressing astrocytes (105). The damaged astrocyte, damage performed directly or cytokine-mediated, cannot offer metabolic support to axons, potentially impairing axon function (106). This assertion sustains firstly the direct immune involvement in the neuronal physiology and secondly depicts the involvement of other related pathways, like the metabolic ones. An interesting parallel with other autoimmune diseases, has shown that in Type 1 Diabetes, specific HLA alleles are associated with CD8+ T-lymphocyte autoreactivity and islet autoimmunity promoting pancreatic islet cell destruction (107, 108). Hence, MHC alleles specific to ASD were reported several years ago (103). Another hypothesis proposes that astrocyte/immune cells related markers

detected in CSF or serum can represent future biomarkers in ASD (105).

Immunoglobulins (Ig) represent another category of immune-related molecules that have been recently gaining interest in ASD research. A review study published in 2012 focused on the maternal-fetal transfer of brain-reactive antibodies and its impact on the risk of developing ASD suggesting that these antibodies may play a role in the behavioral outcome (109). Also, cerebellar-specific autoantibodies were found in children with ASD, correlated with lower adaptive and cognitive function; however, the study could not determine a clear pathogenic significance of this phenomenon (110). These antibodies cross the placenta, recognize self-proteins and can hinder fetal development, as blood brain barrier is still not completely formed (111). Therefore, maternal autoantibodies may be proposed as markers for ASD diagnosis (35, 112). Autoantibodies toward folate receptor alpha (FRα) were also found in ASD children, autoantibodies that are involved in autoimmune diseases development and oxidative stress (113). These autoantibodies hinder folate passage across the blood-brain barrier to the brain. The mothers of ASD children can have these types of autoantibodies leading to an impairment of folate passage across the placenta. Another interesting area is the rather complex folate-dependent one-carbon metabolism including the methylation cycle, the trans-sulfuration pathway and the folate cycle (114). These metabolic pathways are linked to the DNA synthesis/repair, DNA, RNA and proteins methylation, oxidative stress, cellular proliferation/apoptosis, and many other processes (114) linking other complex pathways that can relate to the pathophysiology of ASD (115). Moreover, FRα autoantibodies that can impair folate transport and oxidative biomarkers can lead to the discovery of new therapeutic strategies (113).

Another interesting Ig molecule was recently associated with ASD. IgA concentration and specificities are associated with multiple factors (e.g., subject age, gut microbiota composition, T cell abundance) (116). Hence, IgA was reported as significantly elevated in the gut of ASD children (117). Virulence factor-related gut microbiota (VFGM) genes were found positively correlated to the IgA levels of ASD children; a specific VFGM gene configuration was associated with ASD. VFGM genes detected in ASD were found to be more diverse as compared to typically developing children (118). Group B streptococcus (GBS) genes represented the most prominent VFGM group in ASD. In ASD animal models it was shown that maternal GBS can induce autistic-like litter (119). Wang et al. have shown that three bacterial lipopolysaccharide (LPS) genes (e.g., *kfiC, Cj1137c, wlaN*) were found significantly enriched in gut microbiota of ASD children, positively correlating with gut IgA and VFGM gene diversity (118). This study confirmed the findings of an earlier experimental animal study (120). Therefore, recent studies pinpoint that there are clear links within the immune-gut-brain axis in ASD (121, 122), as further elaborated in the paper. Moreover, the regulation of intestinal microbiota of pregnant female mice was shown to prevent ASD-like behaviors in their offspring corroborated with a normalization of pro-inflammatory cytokines IL-6 and IL-17a (123). Therefore, dysregulated intestinal microbiota and its potential role in sustaining the inflammation in ASD is a topic to be developed in the near future (118).

Immune-related genes and pathways

In immune systems cells, cystatins are involved in antigen processing and presentation, in phagocytosis, cytokines modulation, and nitric oxide expression (124). Concomitantly, cystatins are involved in neurogenesis processes and potentially in synaptic plasticity, as showed in human studies and animal models (125, 126). Cystatin B (CSTB) serum level was strongly associated with autistic features in a recent twin study on ASD and other neurodevelopmental disorders (90). Moreover, Unverricht-Lundborg disease is a rare recessive myoclonic epilepsy caused by pathogenic variants in *CSTB* gene (omim.org/entry/254800, accessed on April 20, 2022). Although the typical phenotype does not include autistic features, a recent report of three patients with Unverricht-Lundborg disease brought into attention ASD as part of the clinical picture (127). Taking into account the potential roles of *CSTB* in the nervous system, future studies are needed to establish if ASD is associated with certain genetic defects in the *CSTB* gene.

Several other immune-related genes involved in immunologic disorders have also been investigated in association with ASD. One example is the adenosine deaminase (*ADA*) gene encoding the adenosine deaminase which plays an important role in purine metabolism. Adenosine deaminase has an important role in immune system function. When mutated it induces a severe combined human immune deficiency (SCID), profound lymphopenia on all classes and a dysfunctional purine metabolism (128). Certain *ADA* alleles were reported as associated with ASD in earlier studies. A significantly increased frequency of *ADA* Asp8Asn polymorphism (*ADA2* allele) was reported by two independent case-control studies of individuals with autism of Italian descent (129, 130). This variant has been associated with a low-activity of the ADA enzyme (129). However, no significant increase in the frequency of the ADA2 allele was observed in a case-control study of North America ASD families, the authors suggesting the existence of a potential risk haplotype in the Italian ASD population (131). As other studies reported neurological and behavioral problems in patients with ADA deficiency and SCID, but not autism (132, 133), further studies are warranted in order to dissect the *ADA* gene role in ASD.

Fibroblast growth factor (FGF) family is involved in complex regeneration processes and initiates the inflammatory events (134), having roles in brain development, regulating cortical

size and connectivity as well (135). By using whole exome sequencing to investigate Saudi families with ASD members, Al-Mubarak et al. detected a rare variant of *FGF5* among other risk genes with roles in brain development and function (136). Several lines of evidence suggested that dysregulation of FGF signaling may contribute to ASD pathophysiology (137–139). Specifically, the macrocephaly observed in early childhood in ASD which is attributed to increased head growth mainly in the first 2 years of life, is hypothesized to be, at least in some children, caused by alterations in *FGF* genes expression (135). Similarly, the lipid and protein phosphatase and tensin homolog (*PTEN*) regulates the physiology of many immune cells as well as embryonic stem cells proliferation, including the neurogenic ones (135, 140). The signaling downstream from cytokine and T- and B-cell receptors, integrins, and growth factor receptors depends on PTEN activity. Therefore, mutations in *PTEN* gene have tremendous effect, like dysfunction of the immune system, autoimmunity, and lymphoid hyperplasia (141). Germline mutations in *PTEN* were initially reported in individuals with autosomal dominant forms of familial tumor predisposition syndromes (142, 143), currently termed as PTEN hamartoma tumor syndrome; some of these presented neurodevelopmental problems, including ASD (144). Several studies reported heterozygous germline *PTEN* mutations in individuals with macrocephaly, developmental delay and ASD, leading to delineation of a new syndrome, Macrocephaly/autism syndrome (MIM605309) (145, 146). Studies on animal models showed that *PTEN* selective deletion in cerebral cortex and hippocampus neurons leads to macrocephaly and behavioral abnormalities (147). The individuals with *PTEN* variants display a various spectrum of immune dysfunction. This varies from asymptomatic lymphopenia to different forms of lymphoid hyperplasia, such as hyperplasia of the adenoids and/or tonsils leading to recurrent upper respiratory tract infections, gastrointestinal polyps with follicular lymphoid hyperplasia, adenoid lymphoid hyperplasia, and thymus hyperplasia (148–150).

In the last years, numerous studies revealed an overlap between autism and cancer genes with many common genes involved in major cell-signaling pathways and metabolic processes dysregulation (151). Among these, the genes involved in PI3K-Akt-mTOR signaling axis, such as *PTEN, NF1* (neurofibromin 1), *TSC1*(TSC complex subunit 1), *TSC2* (TSC complex subunit 2), were associated with inherited risk for both cancer and ASD (152, 153).

Alternative splicing and co-expression analyses of total RNA from PBMC isolated from ASD twins and their parents has shown that zinc finger protein 322 (*ZNF322)* and nuclear receptor subfamily 4 group A member 1 (*NR4A1)* display differentially alternative splicing (154). ZNF322 is a member of the zinc-finger transcription factor family with a putative role in regulation of the ubiquitous MAPK signaling pathways (155) while NR4A1 is a key general regulator in the induction of T cell dysfunction (156). Since the genes coding these molecules seem to play crucial roles in their networks, further studies for their testing and validation as biomarkers for ASD are needed.

Another molecule linked to the immune system function is vitamin D. Besides the roles played in calcium homeostasis, vitamin D has important immune functions (157). Low levels of vitamin D were reported in association with increased levels of proinflammatory cytokines in various disorders such as cancer and psychiatric conditions (158–162). Specifically, vitamin D metabolites have a regulatory repressive effect on IL-8 promoter activation, through vitamin D receptor (VDR) stimulation (163). Animal model studies and human epidemiological studies also suggested that vitamin D deficiency has an important impact on nervous system development (164–166). The assessment of vitamin D deficiency during pregnancy in two population-based cohorts from the Netherlands (165) and Sweden (166) showed association with a greater risk of ASD occurrence in offspring. As vitamin D acts upon binding to vitamin D receptor (VDR), common variants in *VDR* gene (e.g., FokI, BsmI, ApaI, and TaqI polymorphisms) were investigated for their functional consequences on the Vitamin D-VDR complex. These polymorphisms were also investigated in association with various disorders such as immune disorders, cancer, and neuropsychiatric disorders, including autism (167–170). Although some positive associations were found, conflicting results were also generated drawing the attention toward factors such as methodological and cohort differences between the associations studies, as well as environmental effect upon genes and gene-to-gene interactions. A more recent genotyping study of the above-mentioned polymorphism in ASD children, their parents, healthy siblings and controls showed that FokI polymorphism was robustly correlated with ASD; in addition, the frequency of FokI polymorphism in mothers of ASD children revealed an increased risk of having a child with ASD. Authors point out that FokI (T) minor allele codes for a less active protein in comparison to the FokI (C) allele. Therefore, a reduced biological activity of VDR-vitamin D complex is envisaged which may explain the sustained inflammation in pregnant mothers and ASD children with FokI (TT) genotype (171).

Mitochondrial biology dysfunctions and oxidative stress in ASD

The imbalance between production and removal of reactive oxygen species (ROS) generating oxidative stress has recently gained attention in autism research (172–175). ROS are physiologically produced to kill pathogens or as metabolic intermediates. ROS are generated from electron transport chains, drug metabolism, from exposure to chemicals, pollutants, and/or upon radiation. Nicotinamide adenine dinucleotide phosphate oxidase (NOX) isoforms generate endogenous ROS, and are localized to various cellular

membranes being involved in acute and chronic brain diseases (176). Monocyte—macrophage lineage and neutrophils are the main immune cells that generate ROS. ROS constitute the first line of defense against pathogens, but these species also regulate the activity of innate and adaptive immunity. As a primary or secondary signal molecule, ROS are involved in various pro-inflammatory mechanisms (177) including neuro-inflammation (178). B lymphocytes from ASD individuals showed increased gene expression of toll like receptor 4 (*TLR4*) and nicotinamide adenine dinucleotide phosphate oxidase 2 (NADPH oxidase2—*NOX2*) (179). At the same time, T cells displaying the similar feature and contributing to the oxidative stress governed by innate cells (180) (Table 1).

Another enzyme, myeloperoxidase (MPO) is primarily located in immune cells and plays an important role in the immune system, which produces some ROS, particularly hypochlorous acid (HOCl) having the purpose to kill invading pathogens (181). A recent study showed that serum MPO was found significantly higher in ASD compared to control subjects (182). Another important species, nitric oxide (NO) is generated by NO synthases (NOSs) involved in physiological and pathological conditions (183, 184). Interestingly, low concentrations of NO generated by neuronal NOS or endothelial NOS have a physiological neuroprotective function and are involved in the signaling pathway, while higher concentrations of NO synthesized by inducible NOS (iNOS) are neurotoxic (183, 184). iNOS was found in various cell types of the immune system, such as macrophages, dendritic cells, neutrophils, to non-immune cells like epithelial cells from the gut and lung mucosa, smooth muscle cells, and stromal cells of secondary lymphoid organs (178). This was linked to ASD (185) and may be involved in damaging the DNA as well as the enzymatic apparatus that regulates the neurotransmitters (174, 180). Multiple dysregulated molecular players were identified by various studies focused on the oxidative stress in ASD children.

Increased gene expression of *IL-6* and of *HSP70i* (a stress protein) and increased plasma levels of peroxiredoxin (2 and 5) were found by Abruzzo et al. in ASD children compared to controls (186). Peroxiredoxin is a peroxide scavenger, molecular chaperone and contributor to modulation of the cytokine storm Assuming the role of stress proteins involvement in neuroinflammation, this opens avenues for potential ASD's new therapeutic approaches and for plasma peroxiredoxin as a possible biomarker/indicator of disease severity (186).

Significant increase of oxidative DNA damage was found in peripheral lymphocytes from ASD individuals, along with increased plasma ceruloplasmin and copper concentrations, thiol proteins, and superoxide dismutase (SOD) levels, while vitamin C and A levels had lower values in comparison to controls (113). SOD enzymes play critical roles in cell protection against ROS by converting the superoxide to hydrogen peroxide (H2O2) (187). Sequence polymorphisms in the genes encoding for SOD enzymes, namely *SOD1*, *SOD2*, and *SOD3* were investigated in several human disorders (187–189). *SOD1* is associated with familial amyotrophic lateral sclerosis (MIM # 105400), mutations in this gene being reported in ∼20% of patients (https://www.omim.org/entry/105400, accessed on April 22, 2022). Two single nucleotide polymorphisms in *SOD1* (rs2234694 and rs36233090) were reported in correlation with an increased ASD risk. These variants are localized in non-coding regions of the gene and are predicted to have regulatory effects (187). It was recently shown that within harvested neutrophils and monocytes from ASD patients there is an increased SOD expression. However, this is associated with upregulated expression of nitrotyrosine (a marker of oxidant damage), proving the dysregulated antioxidant network (190). Similar results were obtained in T cells from ASD patients, namely an increased antioxidant potential coupled with an inflammatory pattern (191).

Another recent study explored the genetic polymorphisms of glutathione transferases (GSTs) in ASD (192), enzymes with multiple functions, such as inactivation of epoxides and hydroperoxides, molecules generated during oxidative stress. Moreover, GSTs are involved in the synthesis of important biomolecules such as prostaglandins, leukotrienes, and hormones (testosterone and progesterone) (193). Six GSTs gene subfamilies have been described (194), with several highly polymorphic members. Several polymorphisms which influence gene transcription and cause functional alterations at protein level were identified, such as homozygous deletions in glutathione S-transferase mu 1 *(GSTM1),* glutathione S-transferase theta 1 *(GSTT1)* and single nucleotide polymorphism in glutathione S-transferase pi 1 (*GSTP1),* glutathione S-transferase alpha 1 *(GSTA1)* genes (192). *GSTM1* null allele was identified as a potential risk factor of ASD in offspring of mothers receiving medication during pregnancy. This highlights the fact that oxidative stress-related genetic factors added to the environmental factors may contribute to ASD development (192).

Mitochondria play a myriad of physiological functions in metabolism (e.g., glucose oxidation, biosynthesis of fatty acid, amino acid and hormones), ROS signaling, cellular survival regulation including apoptosis and innate immunity (195). Recently reviewed by Chen et al. mitochondrial dysfunction and oxidative stress activate the innate immune system with deleterious roles in inflammatory-related (196) and various autoimmune diseases (197), cancer (198), neurological disorders (199), and diabetes (200). Bennuri et al. studied the consequences of prolonged ROS exposure in a lymphoblastoid cell line (LCL) model of ASD, mainly the adaptive changes that occur in mitochondria function. Gene expression changes were detected, with increased expression for *SOD2,* uncoupling protein 2 *(UCP2)*, and mammalian target of rapamycin kinase (*MTOR)* genes. Also, an increased expression of protein kinase AMP-activated catalytic subunit alpha 2 (*PRKAA2)* gene was observed with potential functional consequences on mTORC1

(201). All these genes are highly involved in mitochondrial respiration. Prolonged exposure to ROS induced changes in mitochondrial respiration. It was also suggested that mTORC1 pathway regulates mitochondrial activity in ASD in relation to S6 kinase beta-1 (S6K1) regulation. S6K1 pathway activated by mTORC1, can sustain mitochondrial response to stress. Therefore, in the context of chronic oxidative stress, normalizing mitochondrial functionality in ASD individuals can gain new therapeutic value (201). In a Chilean cohort of children with ASD a significant increase of the mitochondrial DNA levels was reported along with an increase in the protein oxidation (189). The study also proposed a screening of gene expression in a panel of genes relevant for mitochondria function, consisting of HIG1 Hypoxia Inducible Domain Family Member 2A *(HIGD2A)*, superoxide dismutase 2 (*SOD2)*, mitofusin 1 (*MFN1)*, mitofusin 2 (*MFN2),* dynamin 1 like (*DRP1)*, fission mitochondrial 1 (*FIS1),* and OPA1 mitochondrial dynamin like GTPase (*OPA1).* However, the only gene expression change that reached statistical significance was the increased expression of *MFN2* (189). Mitochondria are organelles characterized by structural and molecular dynamics undergoing fusion and division (202). All the above-mentioned genes are involved in the fusion/division processes in mitochondria. Probably the increase in mitochondrial DNA levels identified in ASD children is a compensatory process aimed at maintaining the mitochondrial functions (189).

Oxidative stress, caused by ROS or reactive nitrogen species, may disrupt the homeostasis of many cells and tissues, leading to mitochondrial and metabolic dysfunctions, altered immune responses, and central and systemic inflammation (177). The full understanding of the role of oxidative stress in ASD is still a matter of intense research and may open future perspectives for a better understanding of ASDs.

Table 1 depicts oxidative stress and antioxidants in different immune cells (B cells, T cells, monocytes, neutrophils) relevant for ASD individuals.

The complex network of genes that encode immune systems elements and immune-related ones in ASD is briefly outlined in Figure 2 and summarized in Table 2.

Immune-gut axis in ASD

Microbiome composition differs in ASD individuals compared with typically developing ones (203), and numerous reports describe gut dysbiosis associated with metabolic imbalance and immune dysregulation in ASD. The question to be answered is if the gut dysbiosis is a consequence or a cause of ASD? Recent studies tried to answer this question and to decipher the genetic factors that intermingle in ASD with the triad microbiota-metabolism-immune system (122, 204, 205). The gastrointestinal system (GI) is intricately connected with the immune system, and chronic inflammatory processes are suggested to contribute to the GI symptoms which frequently occur in individuals with ASD (206–208). Intestinal barrier dysregulations trigger pro-inflammatory processes and release pro-inflammatory cytokines and activated monocytes. These can reach the blood-brain barrier through the bloodstream, thus contributing to neuroinflammation (94, 209).

A whole-exome sequencing study in a group of ASD children with GI symptoms performed by Liu et al. revealed significantly increased single nucleotide variants (SNVs) distribution in genes involved in innate immune response, glycosylation and retrograde axonal transport. The identified SNVs correlated with the microbiome composition and the obtained data emphasized that the interaction of host genetics and gut microbiome may induce immune deregulation and metabolism inference (204). In another recent study, Wang et al. showed that VFGM gene imbalance may reflect dysregulation of the gut's immune function in ASD, and suggests common mechanisms such as gut inflammation and gut microbiota influencing neuroinflammation in ASD (118).

RNA sequencing (RNAseq) performed on GI tissue from ASD children detected differentially expressed transcripts (DETs) regulating immune and inflammatory response (210). T cell receptor (TCR) activation was mainly depicted in Th1 and Th2 arms including multiple signaling pathways (e.g., iCOS-iCOSL TREM1, NF-kappaB, Toll-like receptor signaling). Most of the identified DETs include metabolic pathways (tryptophan, serotonin and melatonin degradation, ketogenesis, ketolysis, oxidative phosphorylation), endocrine pathways (activation of pregnane X receptor, retinoid X receptor, and farnesoid X receptor) and mitochondrial dysregulation (210). Tryptophan degradation was signaled as deficient in ASD, being pointed out as a possible biomarker (211). A substantial number of transcripts in the mitochondrial pathways were found upregulated in GI-ASD, namely nicotinamide adenine dinucleotide (NADH) dehydrogenase, NADH:ubiquinone oxidoreductase core subunit 4L *(ND4L)*, cytochrome B (*CYB)*, ubiquinol-cytochrome c reductase core protein 1 (*UQCRC1)*, and cyclooxygenases (*COX*)—*COX1*, *COX2, COX5B, COX6A1, COX6B2* (210). TNF-α transcript and other additional TNF-related transcripts were found up-regulated in both GI and peripheral lymphocytes in ASD children (210, 212) contributing to the mitochondrial dysfunction in the context of gut inflammation. Therefore, with regard to the immune-gut axis in ASD, some important points should be underlined. Taking into account that immune dysregulation, immune and autoimmune diseases were described in ASD individuals or families (213), and that gut microbiota imbalance has an important impact on both immune and nervous systems, the interactions of brain, gut and immune system may contribute to ASD pathophysiology (118, 122, 205).

Gut microbiota, has an intense communication with many other systems so that the "Immune-Gut-Brain

TABLE 1 Reactive oxygen species and anti-oxidants generated by immune cells in ASD.

Oxidative stress					
Oxygen species	**Enzyme**	**Innate immune cells**	**Adaptive immune cell**	**ASD**	**References**
ROS: superoxide (O2–), hydroxyl (OH), hydrogen peroxide (H2O2), singlet oxygen (1O2)	Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase	Monocyte, macrophage, neutrophils	B lymphocytes increased NADPH oxidase	Increased generation	(176–178)
			T lymphocytes increased NADPH oxidase		(180)
Hypochlorous acid (HOCl)	Myeloperoxidase (MPO)	Granulocytes	–	Increased generation	(182)
Nitric oxide (NO)	Inducible nitric oxide synthase (iNOS)	Monocyte, macrophage, dendritic cells, neutrophils	–	NO is neurotoxic	(174, 180)
Antioxidant mechanisms					
Enzyme	**Action**	**Cell source**		**ASD**	**References**
Superoxide dismutase (SOD)	Conversion of superoxide to H2O2	All cells		Increased levels are found in neutrophils and monocytes	(113, 187, 190)
Glutathione peroxidase (GSHPx)	Inactivation of free peroxides in cells	All cells		Decreased activity in neutrophils and monocytes	(190)
Glutathione reductase (GR)	Provides reduced glutathione to control ROS	All cells		Decreased activity in neutrophils; unchanged activity in monocytes	(190)

axis" seems to have an important impact in neurodevelopmental disorders.

A diagram of the putative interplay of the immune system-gut microbiota and CNS dysregulation is presented in Figure 3.

Immune-related epigenetics of ASD

Epigenetics has followed the research foot-steps of genomics and proteomics and, in recent years, it expanded in various fields, from cancer (214) to autoimmune diseases (215), aiming to explain complex biological processes. The epigenetic machinery consists of a multitude of molecules and processes that are interlinked. DNA methylation/demethylation, histone modification, non-coding RNAs [e.g., long non-coding RNAs (lncRNA), circular RNA, miRNA] are just a few molecular players that can induce heritable phenotypic changes not affecting *per se* the DNA sequence. If epigenetics disrupts gene expression it can lead to various major human pathologies and epigenetics molecules can be markers for patient's diagnostic, stratification, and/or therapy (216).

Epigenetic changes are proposed ASD contributors, as mediators at the crossroads between environmental factors and genome during development (217, 218). DNA methylation is essential for brain development, being one of the most frequently studied epigenetic regulation mechanism (219, 220). Studies of genome-wide DNA methylation patterns were performed on various tissues from ASD individuals, such as blood (221), buccal epithelium (220, 222, 223), brain (218, 224–226), cord blood and placenta (227–229). Each of the above-mentioned tissues has advantages, such as accessibility for peripheral tissues or a more homogeneous cellular composition for buccal epithelium, as well as limitations, such as extremely reduced sample sizes for post-mortem brain

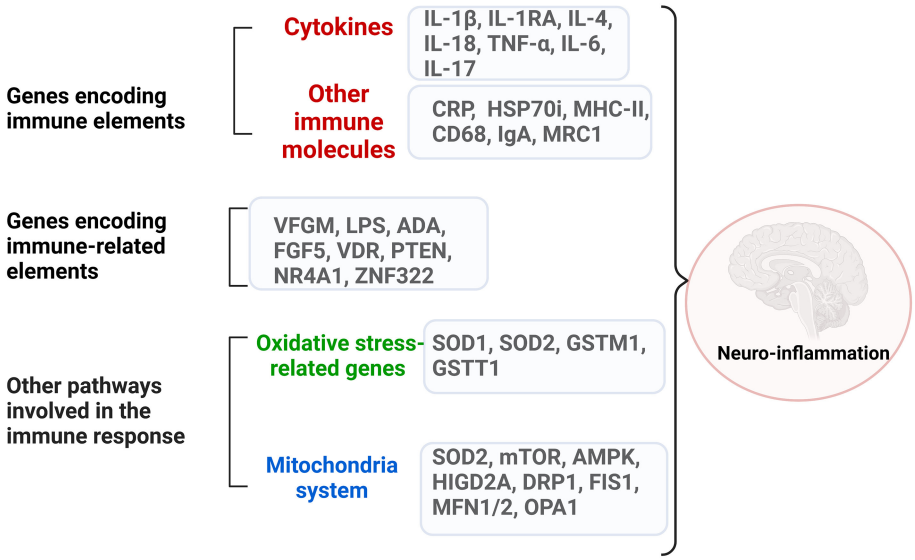


FIGURE 2
The complex network of dysregulated genes encoding immune system elements and immune-related ones in ASD. Neuroinflammation can be generated through dysregulated genes from three categories: genes that encode directly immune molecules, like cytokines and surface molecules on the immune cells; genes that encode elements involved in major immune pathways like growth factors, proliferation processes; genes that encode molecules involved in oxidative stress and mitochondria pathways. Created with BioRender.com.

tissue or cell heterogeneity for peripheral blood (220, 222). Jangjoo et al. compared the genome-wide DNA methylation (DNAm) profiles in blood samples from children with ASD and typically controls and found no significant differences between the two groups (221). However, a subset of ASD children had a DNAm pattern distinct from the rest of ASD children and from controls. These methylation differences were mainly associated with deregulations of immune cell type circulating proportions, although ASD risk genes were also differentially methylated (221). Several studies reported brain DNA methylation alterations, in areas such as prefrontal cortex, temporal cortex, cingulate gyrus, subventricular zone, and cerebellum, in ASD individuals compared to controls (218, 224–226). These data indicate the existence of common differentially methylated regions in ASD thus bringing new evidence that support the role of epigenetic changes in ASD pathophysiology and contribute to the discovery of new candidate genes (218, 224–226). The perinatal tissues, such as placenta and cord blood revealed interesting data regarding epigenetic changes in early developmental. Due to its distinct pattern of DNA methylation, similar to oocytes and preimplantation embryos (230), placenta proved to be a promising tissue for studying DNA methylation changes in ASD (217, 228, 231). The detection of differentially methylated regions in ASD, as epigenetic markers, highlight the value of DNA methylation investigation prior to symptom onset and bring novel insights for early recognition and therapeutic approaches.

Non-coding RNAs are other epigenetic players recently identified in ASD. Changes in the expression level of lncRNAs were reported in ASD as summarized in Table 3. Among epigenetic regulators altered in ASD, several miRNAs involved in the development of immune system and immune responses were reported, along with those regulating major pathways like PI3K/Akt/mTOR and epidermal growth factor receptor (EGFR) intracellular signaling (232).

Several studies reported the involvement of small RNA both in central nervous and the immune system regulation. Down-regulation of hsa_can_1002-m was observed in the cerebral cortex of ASD individuals. This miRNA is predicted to modulate activity of EGFR and FGF receptor (FGFR) signaling pathways which are involved in brain development and inflammatory/immune processes (36).

A recent study using small RNAseq analysis on lymphoblastoid cell lines derived from ASD children identified a series of miRNAs with dysregulated expression. The predicted targeted genes of these miRNAs are involved in important pathways, such as MAPK signaling, cytokine–cytokine receptor interaction, spliceosome, calcium signaling, and WNT signaling. Further expression analysis of genes targeted by two selected miRNAs, miR-181a-5p and miR-320a, which were down-regulated in ASD individuals compared to controls, showed dysregulation of genes involved both in the central nervous and immune system, namely AKT serine/threonine kinase 2 (*AKT2*), AKT serine/threonine kinase 3 (*AKT3*), *TNF-α*, calcium/calmodulin dependent protein kinase II alpha *(CAMK2A)*, and beta *(CAMK2B)* (242).

Overall, the epigenetic changes in ASD can contribute to altered gene expression of targeted genes. The

TABLE 2 Summary of immune-related genes with dysregulated expression in various ASD studies.

Gene name	Gene alteration detected in ASD studies (ref)	Protein function (https://www.uniprot.org/; https://www.ncbi.nlm.nih.gov/protein/)
NADPH oxidase2—*NOX2*	Increased gene expression (179)	Major subunit of the phagocytic NADPH oxidase which generates superoxide
HLA-DRB1	Human Leukocyte Antigen (HLA)-DR4 polymorphic allele (103)	Part of HLA class II beta chain molecules. Presents antigens on antigen presenting cells (APC), guiding antigen-specific T-helper effector functions
HLA-A	HLA-A2 polymorphic allele (103)	Belongs to HLA class I molecules. Presents antigens on APC cells, guiding functions of antigen-specific cytotoxic CD8-positive T cells
Heat Shock 70 KDa Protein 1A—*HSP70I*	Increased gene expression (186)	Chaperone molecule involved in various cellular processes, with an essential role in the protein quality control system
Interferon-gamma IFN-γ—*IFNG*	Increased gene expression (52)	Belongs to type II interferon class molecules. Soluble cytokine which is produced by innate and adaptive immune system cells
Interleukin 1 Receptor Antagonist—*IL1R1*	Increased gene expression (85)	Belongs to interleukin 1 cytokine family. Inhibits the activities of IL-1A, IL-1B and modulates a variety of immune and inflammatory responses related with IL-1
Interleukin 6—*IL-6*	Increased gene expression (52, 76, 186)	Cytokine involved in various biological functions, with important role in immunity, tissue regeneration, and metabolism
Interleukin 9—*IL-9*	Increased gene expression (77)	Pleiotropic cytokine involved in immune response, regulates the function of various hematopoietic cells, stimulates cell proliferation, and prevents apoptosis. IL-9 stimulates a receptor complex (IL-9R/IL2RG) which leads to activation the JAK-STAT pathway
Interleukin 10—*IL-10*	Decreased gene expression (52)	Pleiotropic cytokine with immunosuppressive activity. Receptor complex ligation activates JAK/STAT signaling
Interleukin 16—*IL-16*	Increased gene expression (80)	Pleiotropic cytokine with chemoattractant function for a variety of CD4+ immune cells
Interleukin 17A—*IL-17A*	Increased gene expression (52)	Member of the IL-17 receptor family. Proinflammatory cytokine produced by activated T cells
Mannose receptor C-type 1—*MRC1*	Increased gene expression (47)	Type I membrane receptor involved in mediation of glycoproteins endocytosis by macrophages
Mammalian target of rapamycin—*MTOR*	Increased gene expression (201)	Serine/threonine protein kinase with central role in regulation of many fundamental cell processes: cellular metabolism, growth and survival in response to hormones, growth factors, nutrients, energy, and stress signals. mTOR constitute the catalytic subunit of two distinct protein complexes: mTORC1 which promotes translation initiation and controls protein synthesis, and mTORC2 which is a regulator of the actin cytoskeleton, and promotes cell survival and cell cycle progression
Neurofibromin 1—*NF1*	Rare sequence variants (https://gene.sfari.org/database/human-gene/, accessed August 5th 2022)	Multifunctional protein involved in several cell signaling pathways (Ras/MAPK, Akt/mTOR, cAMP/PKA) and regulates fundamental cellular processes, such as proliferation and migration, cytoskeletal dynamics, neurite outgrowth, dendritic-spine density, and dopamine levels
Protein kinase AMP-activated catalytic subunit alpha 2—*PRKAA2*	Increased gene expression (201)	Catalytic subunit of AMP-activated protein kinase (AMPK), an important energy-sensing enzyme with critical role in cellular energy status
Phosphatase and tensin homolog—*PTEN*	Rare sequence variants (https://gene.sfari.org/database/human-gene/, accessed August 5th 2022)	Enzyme with tumor suppressor function. Presents dual specificity for protein and lipid phosphatase. Regulates important cellular processes, such as proliferation, differentiation, growth, migration, death, apoptosis of the cells through the PI3K/AKT/mTOR signaling pathway

(Continued)

TABLE 2 (Continued)

Gene name	Gene alteration detected in ASD studies (ref)	Protein function (https://www.uniprot.org/; https://www.ncbi.nlm.nih.gov/protein/)
Superoxide dismutase 1—*SOD1*	Single nucleotide polymorphisms (187)	An antioxidant enzyme that metabolizes the free superoxide radicals in the body to molecular oxygen and hydrogen peroxide
Superoxide dismutase 2—*SOD2*	Increased gene expression (201)	Member of the iron/manganese superoxide dismutase family with important role in oxidative stress management
Toll like receptor 4—*TLR4*	Increased gene expression (179)	Member of the TLR family which plays an essential role in regulation of immune responses to infection
Tumor necrosis factor alpha—TNF *(TNF-α)*	Increased gene expression (52, 76)	Multifunctional proinflammatory cytokine that belongs to the TNF superfamily involved in the regulation of a wide spectrum of biological processes including cell survival, proliferation, differentiation, and cell death
Tuberous sclerosis 1—*TSC1*	Rare sequence variants (https://gene.sfari.org/database/human-gene/, accessed August 5th 2022)	Tumor suppressor. Interacts with TSC2 generating a protein complex which negatively regulates mTORC1 signaling, the main regulator of anabolic cell growth. Co-chaperone function inhibiting the ATPase activity of Hsp90
Tuberous sclerosis 2—*TSC2*	Rare sequence variants (https://gene.sfari.org/database/human-gene/, accessed August 5th 2022)	Tumor suppressor. Interacts with TSC1 and form the TSC protein complex which controls cellular growth
Uncoupling protein 2—*UCP2*	Increased gene expression (201)	Member of mitochondrial anion carrier protein family with important role in prevention of oxidative stress

epigenetic alterations and the subsequent transcriptional changes converge functionally to known ASD pathways (243). Moreover, epigenetic modification in response to environmental factors open new avenues toward understanding this complex entity and possibly to therapeutic interventions (244).

Discussion

ASD is a neurodevelopmental disorder characterized by impairment of social interaction and communication, as well as restricted interests and stereotyped and repetitive behavior patterns (1).

ASD has a multifactorial etiology. The genetic and environmental risk factors interplay has detrimental consequences primarily on the central nervous systems; other systems such as the immune and digestive system are also dysregulated. Sequence variants have been reported in numerous genes, however there are still no "autism genes" but "brain-genes" (https://staging.spectrumnews.org, Accessed September 14th 2022). The epigenetic changes observed in ASD can contribute to the altered gene expression of many targeted genes. Moreover, the epigenetic changes in response to environmental factors open new avenues toward understanding this complex entity and possibly to therapeutic interventions (243, 244).

Among the environmental factors that increase ASD risk, those acting in the prenatal period and in the maternal environment in which the fetus is developing, are regarded as the most harmful. Maternal immune activation, brain-reactive antibodies and autoimmunity are proposed contributors to ASD pathophysiology (32, 35, 111–113).

Both genetic and epigenetic factors can contribute to the immune dysregulations in ASD. Early studies have shown that PBMCs from ASD children are characterized by an excess of pro-inflammatory cytokines, their function being dysregulated by the lack of anti-inflammatory mechanisms (245). Other studies have also provided evidence supporting the role of IL-17A in neuro-inflammatory processes; in addition, monocytes, B lymphocytes, and neutrophils produce oxidative and inflammatory mediators (43, 44, 52). Recently NK cells joined the immune arsenal proposed to contribute to neuro-inflammation in ASD (246). Among the mediators of immune dysregulation, cytokines are considered to have a major role. IL-1 is involved in various neuronal physiological pathways, e.g., modulation of neural plasticity, synaptic plasticity, neuronal calcium signaling, thus has been in the spot light of ASD research (66–68, 247). Other immune molecules, such as IL-6, TNF-α, IL-8, IL-31, IL-16, and IL-12p40, also contribute to the inflammatory milieu in ASD (45, 78, 80). Therefore, the entire immune system, with its complex network of molecules and immune cells, is involved in the dysregulation of innate and adaptive immune responses in ASD. Oxidative stress is a direct

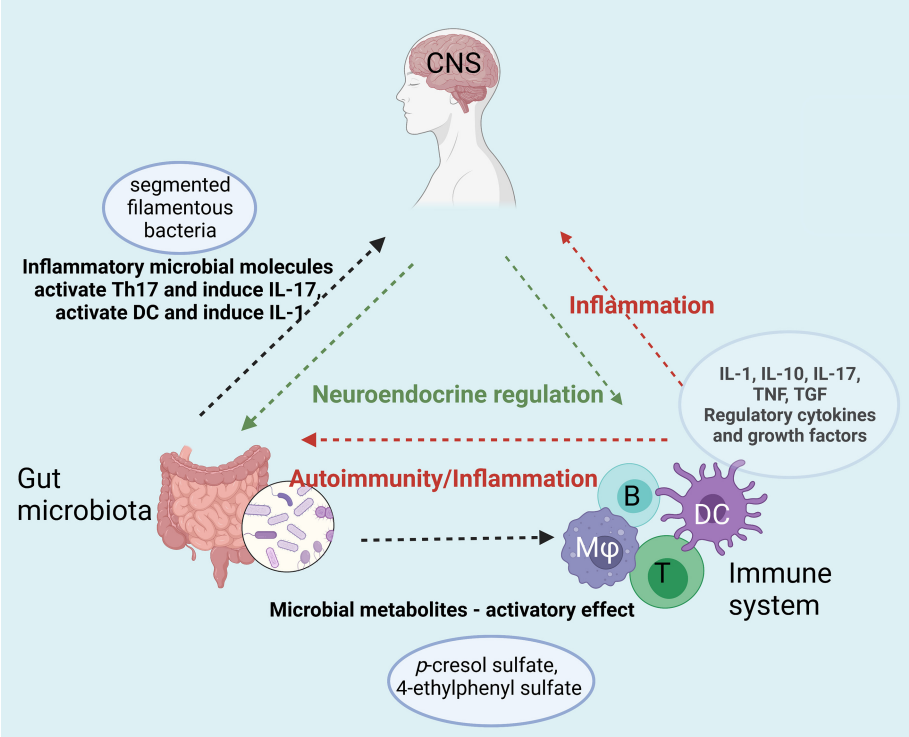


FIGURE 3
Immune-Gut-Brain network that converge toward sustaining neuroinflammation in ASD. Gut microbiota enhances the inflammatory status by specific microbial metabolites (e.g., p-cresol sulfate, 4-ethyphenyl sulfate) that activate both innate (dendritic cells, macrophages) and adaptive immune cells (T and B lymphocytes). The enhanced pro-inflammatory cytokine secretion and the auto-antibodies generated upon this hyperactivation act on both digestive system and on the central nervous system sustaining the neuroinflammation. Moreover, gut microbiota enhances the inflammatory status by specific microbial molecules that append mainly to the segmented filamentous bacteria that activate Th17 and induce IL-17 secretion [B-B, lymphocyte; T-T, lymphocyte; DC, dendritic cell; (Mφ), monocyte/macrophage]. Created with BioRender.com.

consequence of immune dysregulation; this phenomenon has been reported in many acute and chronic brain disorders (176, 186). ROS are involved in neuroinflammation, and although the innate immune cells are the main source of ROS, adaptive immune cells T and B cells also contribute to the oxidative stress observed in ASD (178–180). The imbalance generated by the increased ROS production and decrease of anti-oxidant regulatory mechanisms leads to oxidative stress. Oxidative stress, further dysregulates mitochondrial physiology, metabolic pathways, gut homeostasis, and other immune responses, contributing to neuroinflammation (177).

Further research on the proinflammatory molecular arsenal, with its main players, the cytokines, may contribute to establishing ASD immune endophenotypes, and consequently driving current and future ASD therapeutic interventions to more personalized approaches (248, 249). Moreover, neurobehavioral symptoms severity appears to correlate with the extent of systemic immune alteration (41). This opens new perspectives for prediction of clinical evolution and therapeutic guidance. The field of biological biomarkers in ASD is promising, with current markers awaiting further validation and new markers being continuously discovered. Panels of immune molecules have the potential to become robust ASD-related biomarkers that may aid the diagnosis, patient stratification, and monitoring (50).

Conclusions

Significant discoveries regarding the molecular and immunological features of individuals with ASD have been reported during the past years. Multiple studies have been focused on the genetic architecture of ASD, known to be complex and highly heterogeneous. Gathering prenatal and early post-natal comprehensive information, individualized profiling can be done in order to advance the clinical care toward precision medicine approaches in ASD. Identification of immune-related genes and their interactions on several levels has shown that the main immune dysregulation resides in the inflammatory area impacting early neuronal development and function. Gene encoding immune molecules, such as cytokines

TABLE 3 Non-coding RNAs found altered in ASD.

Symbol (Name)	Expression sites (https://gtexportal.org/home/)	Expression level	References
RP11-466P24.2 (ENST00000502589)	Various tissues, including white blood cells and brain	Decreased	(233)
SYP Antisense RNA 1–SYP-AS1 (ENST00000527880)	Brain and adrenal tissue	Decreased	(233)
Syntaxin Binding Protein 5 Antisense RNA 1–STXBP5-AS1 (ENST00000433499)	Various tissues, including immune cells and nervous system; found in breast cancer	Decreased	(233)
Interferon Gamma Antisense RNA 1–IFNG-AS1 (ENSG00000255733)	Various tissues, including immune cells and nervous system; found in autoimmune-diseases	Decreased	(234)
AK128569 (uc001mff.1)	Various tissues, including immune cells, EBV transformed lymphocytes	Increased	(233)
Synaptotagmin 9 Antisense RNA 1–SYT9AS, CTD-2516F10.2 (ENST00000504206)	Various tissues, including basal ganglia	Increased	(233)
Moesin Pseudogene 1 Antisense RNA 1–MSNP1AS (ENSG00000251593)	Various tissues, including immune cells, nervous system, endocrine system, and various internal organs	Increased	(235, 236)
Ribosomal Protein S10 Pseudogene 2 Antisense RNA 1–RPS10P2-AS1	Fetal temporal cortex and adult peripheral blood	Increased	(237)
Long Intergenic Non-Protein Coding RNA 693–LINC00693	Various tissues, including immune cells and nervous system; found in autoimmune-diseases	Increased	(238)
Long Intergenic Non-Protein Coding RNA 689–LINC00689	Various tissues, including lymph nodes, placenta, testis	Increased	(238)
Maternally Expressed 3–MEG3 (ENSG00000258663)	Various tissues, including endocrine system, nervous system	Increased	(239)
Nuclear Paraspeckle Assembly Transcript 1–NEAT1 (ENSG00000245532)	Various tissues, including immune cells, nervous system, endocrine system, and various internal organs	Increased	(240)
Taurine Up-Regulated 1–TUG1 (ENSG00000253352)	Various tissues, including immune cells, lymph nodes and nervous system	Increased	(240)
SH3 And Multiple Ankyrin Repeat Domains 2 Antisense RNA–SHANK2 AS (ENSG00000226627)	Low expression in various tissues	Increased	(241)

and several other immune-related elements involved in antigen processing, oxidative stress and mitochondrion function converge toward the overall pro-inflammatory status of ASD.

A better understanding of the immune-mediated pathways and their impact on many other biological processes and systems, such as metabolism, the endocrine and gastrointestinal systems, may contribute to the discovery of new therapeutic targets in ASD, ultimately aiming to improve the quality of life of ASD individuals.

Author contributions

MN and AE conceived, designed the structure, and contributed to the writing of the paper. AA, SP, and MB contributed to the writing of the paper and the preparation of figures. MN, AE, and AA edited the paper. All authors approved the manuscript.

Funding

The research leading to these results has received funding from the EEA Grant 2014-2021, under the project contract no 6/2019. Article processing charge (APC) was funded from the same grant.

Acknowledgments

AE develops the Ph.D., thesis: Genomic structural variation in autism spectrum disorders.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer MV declared a past co-authorship with one of the authors MN to the handling editor.

Publisher's note

References

1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 5th edition. Arlington, VA: American Psychiatric Association (2013). doi: 10.1176/appi.books.9780890425596

2. Maenner MJ, Shaw KA, Baio J, Washington A, Patrick M, DiRienzo M 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) 69:1–12. doi: 10.15585/mmwr.ss6904a1

3. Fuentes J, Hervás A, Howlin P, ESCAP ASD Working Party. ESCAP practice guidance for autism: a summary of evidence-based recommendations for diagnosis and treatment. *Eur Child Adolesc Psychiatry.* (2021) 30:961–84. doi: 10.1007/s00787-020-01587-4

4. Lai MC, Lombardo MV, Baron-Cohen S. Autism. *Lancet.* (2014) 383:896–910. doi: 10.1016/S0140-6736(13)61539-1

5. Diaz-Beltran L, Esteban FJ, Varma M, Ortuzk A, David M, Wall DP. Cross-disorder comparative analysis of comorbid conditions reveals novel autism candidate genes. *BMC Genomics.* (2017) 18:315. doi: 10.1186/s12864-017-3667-9

6. 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:e20193447. doi: 10.1542/9781610024716-part01-ch002

7. Dizitzer Y, Meiri G, Flusser H, Michaelovski A, Dinstein I, Menashe I. Comorbidity and health services' usage in children with autism spectrum disorder: a nested case-control study. *Epidemiol Psychiatr Sci.* (2020) 29:e95. doi: 10.1017/S2045796020000050

8. 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:e0153329. doi: 10.1371/journal.pone.0153329

9. Geschwind DH. Genetics of autism spectrum disorders. *Trends Cogn Sci.* (2011) 15:409–16. doi: 10.1016/j.tics.2011.07.003

10. Gaugler T, Klei L, Sanders SJ, Bodea CA, Goldberg AP, Lee AB, et al. Most genetic risk for autism resides with common variation. *Nat Genet.* (2014) 46:881–5. doi: 10.1038/ng.3039

11. Autism Spectrum Disorders Working Group of the Psychiatric Genomics Consortium. Meta-analysis of GWAS of over 16,000 individuals with autism spectrum disorder highlights a novel locus at 10q24.32 and a significant overlap with schizophrenia. *Mol Autism*. (2017) 8:21. doi: 10.1186/s13229-017-0137-9

12. Grove J, Ripke S, Als TD, Mattheisen M, Walters RK, Won H, et al. Identification of common genetic risk variants for autism spectrum disorder. *Nat Genet.* (2019) 51:431–44. doi: 10.1038/s41588-019-0344-8

13. Fakhro KA. Genomics of autism. *Adv Neurobiol.* (2020) 24:83–96. doi: 10.1007/978-3-030-30402-7_3

14. De Rubeis S, He X, Goldberg AP, Poultney CS, Samocha K, Cicek AE, et al. Synaptic, transcriptional and chromatin genes disrupted in autism. *Nature.* (2014) 515:209–15. doi: 10.1038/nature13772

15. Sanders SJ, He X, Willsey AJ, Ercan-Sencicek AG, Samocha KE, Cicek AE, et al. Insights into autism spectrum disorder genomic architecture and biology from 71 risk loci. *Neuron.* (2015) 87:1215–33. doi: 10.1016/j.neuron.2015.09.016

16. Bourgeron T. From the genetic architecture to synaptic plasticity in autism spectrum disorder. *Nat Rev Neurosci.* (2015) 16:551–63. doi: 10.1038/nrn3992

17. 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. doi: 10.1038/nm.4071

18. Satterstrom FK, Kosmicki JA, Wang J, Breen MS, De Rubeis S, An JY, et al. Large-scale exome sequencing study implicates both developmental and functional changes in the neurobiology of autism. *Cell.* (2020) 180:568–84.e23. doi: 10.1016/j.cell.2019.12.036

19. Iakoucheva LM, Muotri AR, Sebat J. Getting to the cores of autism. *Cell.* (2019) 178:1287–98. doi: 10.1016/j.cell.2019.07.037

20. Cheroni C, Caporale N, Testa G. Autism spectrum disorder at the crossroad between genes and environment: contributions, convergences, and interactions in ASD developmental pathophysiology. *Mol Autism.* (2020) 11:69. doi: 10.1186/s13229-020-00370-1

21. Volk HE, Hertz-Picciotto I, Delwiche L, Lurmann F, McConnell R. Residential proximity to freeways and autism in the CHARGE study. *Environ Health Perspect.* (2011) 119:873–7. doi: 10.1289/ehp.1002835

22. Jung CR, Lin YT, Hwang BF. Air pollution and newly diagnostic autism spectrum disorders: a population-based cohort study in Taiwan. *PLoS ONE.* (2013) 8:e75510. doi: 10.1371/journal.pone.0075510

23. Grandjean P, Landrigan PJ. Developmental neurotoxicity of industrial chemicals. *Lancet.* (2006) 368:2167–78. doi: 10.1016/S0140-6736(06)69665-7

24. Rossignol DA, Genuis SJ, Frye RE. Environmental toxicants and autism spectrum disorders: a systematic review. *Transl Psychiatry.* (2014) 4:e360. doi: 10.1038/tp.2014.4

25. Pino-López M, Romero-Ayuso DM. Trastornos del espectro autista y exposiciones ocupacionales de los progenitores [parental occupational exposures and autism spectrum disorder in children]. *Rev Esp Salud Publica.* (2013) 87:73–85. doi: 10.4321/S1135-57272013000100008

26. Roberts EM, English PB. Bayesian modeling of time-dependent vulnerability to environmental hazards: an example using autism and pesticide data. *Stat Med.* (2013) 32:2308–19. doi: 10.1002/sim.5600

27. Siniscalco D, Cirillo A, Bradstreet JJ, Antonucci N. Epigenetic findings in autism: new perspectives for therapy. *Int J Environ Res Public Health.* (2013) 10:4261–73. doi: 10.3390/ijerph10094261

28. Rowland J, Wilson CA. The association between gestational diabetes and ASD and ADHD: a systematic review and meta-analysis. *Sci Rep.* (2021) 11:5136. doi: 10.1038/s41598-021-84573-3

29. Brand JS, Lawlor DA, Larsson H, Montgomery S. Association between hypertensive disorders of pregnancy and neurodevelopmental outcomes among offspring. *JAMA Pediatr.* (2021) 175:577–85. doi: 10.1001/jamapediatrics.2020.6856

30. Estes ML, McAllister AK. Immune mediators in the brain and peripheral tissues in autism spectrum disorder. *Nat Rev Neurosci.* (2015) 16:469–86. doi: 10.1038/nrn3978

31. Dantzer R. Neuroimmune interactions: from the brain to the immune system and vice versa. *Physiol Rev.* (2018) 98:477–504. doi: 10.1152/physrev.00039.2016

32. Courchesne E, Pramparo T, Gazestani VH, Lombardo MV, Pierce K, Lewis NE. The ASD living biology: from cell proliferation to clinical phenotype. *Mol Psychiatry.* (2019) 24:88–107. doi: 10.1038/s41380-018-0056-y

33. Voineagu I, Wang X, Johnston P, Lowe JK, Tian Y, Horvath S, et al. Transcriptomic analysis of autistic brain reveals convergent molecular pathology. *Nature.* (2011) 474:380–4. doi: 10.1038/nature10110

34. Gupta S, Ellis SE, Ashar FN, Moes A, Bader JS, Zhan J, et al. Transcriptome analysis reveals dysregulation of innate immune response genes and neuronal activity-dependent genes in autism. *Nat Commun.* (2014) 5:5748. doi: 10.1038/ncomms6748

35. Meltzer A, Van de Water J. The role of the immune system in autism spectrum disorder. *Neuropsychopharmacology.* (2017) 42:284–98. doi: 10.1038/npp.2016.158

36. Wu YE, Parikshak NN, Belgard TG, Geschwind DH. Genome-wide, integrative analysis implicates microRNA dysregulation in autism spectrum disorder. *Nat Neurosci.* (2016) 19:1463–76. doi: 10.1038/nn.4373

37. Gao H, Zhong J, Huang Q, Wu X, Mo X, Lu L, et al. Integrated systems analysis explores dysfunctional molecular modules and regulatory factors in

children with autism spectrum disorder. *J Mol Neurosci.* (2021) 71:358–68. doi: 10.1007/s12031-020-01658-w

38. Golovina E, Fadason T, Lints TJ, Walker C, Vickers MH, O'Sullivan JM. Understanding the impact of SNPs associated with autism spectrum disorder on biological pathways in the human fetal and adult cortex. *Sci Rep.* (2021) 11:15867. doi: 10.1038/s41598-021-95447-z

39. Arenella M, Cadby G, De Witte W, Jones RM, Whitehouse AJ, Moses EK. Potential role for immune-related genes in autism spectrum disorders: evidence from genome-wide association meta-analysis of autistic traits. *Autism.* (2022) 26:361–72. doi: 10.1177/13623613211019547

40. 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) 25:40–5. doi: 10.1016/j.bbi.2010.08.003

41. Michel M, Schmidt MJ, Mirnics K. Immune system gene dysregulation in autism and schizophrenia. *Dev Neurobiol.* (2012) 72:1277–87. doi: 10.1002/dneu.22044

42. Horiuchi F, Yoshino Y, Kumon H, Hosokawa R, Nakachi K, Kawabe K, et al. Identification of aberrant innate and adaptive immunity based on changes in global gene expression in the blood of adults with autism spectrum disorder. *J Neuroinflammation.* (2021) 18:102. doi: 10.1186/s12974-021-02154-7

43. Nadeem A, Ahmad SF, Attia SM, Bakheet SA, Al-Harbi NO, Al-Ayadhi LY. Activation of IL-17 receptor leads to increased oxidative inflammation in peripheral monocytes of autistic children. *Brain Behav Immun.* (2018) 67:335–44. doi: 10.1016/j.bbi.2017.09.010

44. Nadeem A, Ahmad SF, Attia SM, Al-Ayadhi LY, Bakheet SA, Al-Harbi NO. Oxidative and inflammatory mediators are upregulated in neutrophils of autistic children: role of IL-17A receptor signaling. *Prog Neuropsychopharmacol Biol Psychiatry.* (2019) 90:204–11. doi: 10.1016/j.pnpbp.2018.12.002

45. Enstrom AM, Onore CE, Van de Water JA, Ashwood P. Differential monocyte responses to TLR ligands in children with autism spectrum disorders. *Brain Behav Immun.* (2010) 24:64–71. doi: 10.1016/j.bbi.2009.08.001

46. Hughes HK, Rowland ME, Onore CE, Rogers S, Ciernia AV, Ashwood P. Dysregulated gene expression associated with inflammatory and translation pathways in activated monocytes from children with autism spectrum disorder. *Transl Psychiatry.* (2022) 12:39. doi: 10.1038/s41398-021-01766-0

47. Sciara AN, Beasley B, Crawford JD, Anderson EP, Carrasco T, Zheng S, et al. Neuroinflammatory gene expression alterations in anterior cingulate cortical white and gray matter of males with autism spectrum disorder. *Autism Res.* (2020) 13:870–84. doi: 10.1002/aur.2284

48. 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. doi: 10.1002/ana.20315

49. Li X, Chauhan A, Sheikh AM, Patil S, Chauhan V, Li XM, et al. Elevated immune response in the brain of autistic patients. *J Neuroimmunol.* (2009) 207:111–6. doi: 10.1016/j.jneuroim.2008.12.002

50. Prata J, Santos SG, Almeida MI, Coelho R, Barbosa MA. Bridging autism spectrum disorders and schizophrenia through inflammation and biomarkers - pre-clinical and clinical investigations. *J Neuroinflammation.* (2017) 14:179. doi: 10.1186/s12974-017-0938-y

51. Kim HJ, Cho MH, Shim WH, Kim JK, Jeon EY, Kim DH, et al. Deficient autophagy in microglia impairs synaptic pruning and causes social behavioral de-fects. *Mol Psychiatry.* (2017) 22:1576–84. doi: 10.1038/mp.2016.103

52. Nadeem A, Ahmad SF, Al-Harbi NO, Al-Ayadhi LY, Sarawi W, Attia SM et al. Imbalance in pro-inflammatory and anti-inflammatory cytokines milieu in B cells of children with autism. *Mol Immunol.* (2022) 141:297–304. doi: 10.1016/j.molimm.2021.12.009

53. Alabdali A, Al-Ayadhi L, El-Ansary A. Association of social and cognitive impairment and biomarkers in autism spectrum disorders. *J Neuroinflammation.* (2014) 11:4. doi: 10.1186/1742-2094-11-4

54. Inga Jácome MC, Morales Chacòn LM, Vera Cuesta H, Maragoto Rizo C, Whilby Santiesteban M, Ramos Hernandez L, et al. Peripheral inflammatory markers contributing to comorbidities in autism. *Behav Sci.* (2016) 6:E29 doi: 10.3390/bs6040029

55. Jiang NM, Cowan M, Moonah SN, Petri WA Jr. The impact of systemic inflammation on neurodevelopment. *Trends Mol Med.* (2018) 24:794–804. doi: 10.1016/j.molmed.2018.06.008

56. Freitas BC, Mei A, Mendes APD, Beltrão-Braga PCB, Marchetto MC. Modeling inflammation in autism spectrum disorders using stem cells. *Front Pediatr.* (2018) 6:394. doi: 10.3389/fped.2018.00394

57. Young AM, Chakrabarti B, Roberts D, Lai MC, Suckling J, Baron-Cohen S. From molecules to neural morphology: understanding neuroinflammation in autism spectrum condition. *Mol Autism.* (2016) 7:9. doi: 10.1186/s13229-016-0068-x

58. Brocker C, Thompson D, Matsumoto A, Nebert DW, Vasiliou V. Evolutionary divergence and functions of the human interleukin (IL) gene family. *Hum Genomics.* (2010) 5:30–55. doi: 10.1186/1479-7364-5-1-30

59. Kordulewska NK, Kostyra E, Piskorz-Ogórek K, Moszyńska M, Cieślińska A, Fiedorowicz E, et al. Serum cytokine levels in children with spectrum autism disorder: differences in pro- and anti-inflammatory balance. *J Neuroimmunol.* (2019) 337:577066. doi: 10.1016/j.jneuroim.2019.577066

60. Tortelli R, Zecca C, Piccininni M, Benmahamed S, Dell'Abate MT, Barulli MR, et al. Plasma inflammatory cytokines are elevated in ALS. *Front Neurol.* (2020) 11:552295. doi: 10.3389/fneur.2020.552295

61. Diesch T, Filippi C, Fritschi N, Filippi A, Ritz N. Cytokines in saliva as biomarkers of oral and systemic oncological or infectious diseases: a systematic review. *Cytokine.* (2021) 143:155506. doi: 10.1016/j.cyto.2021.155506

62. van Deuren RC, Arts P, Cavalli G, Jaeger M, Steehouwer M, van de Vorst M, et al. Impact of rare and common genetic variation in the interleukin-1 pathway on human cytokine responses. *Genome Med.* (2021) 13:94. doi: 10.1186/s13073-021-00907-w

63. Duffin KC, Krueger GG. Genetic variations in cytokines and cytokine receptors associated with psoriasis found by genome-wide association. *J Invest Dermatol.* (2009) 129:827–33. doi: 10.1038/jid.2008.308

64. Richard AC, Peters JE, Lee JC, Vahedi G, Schäffer AA, Siegel RM, et al. Targeted genomic analysis reveals widespread autoimmune disease association with regulatory variants in the TNF superfamily cytokine signalling network. *Genome Med.* (2016) 8:76. doi: 10.1186/s13073-016-0329-5

65. Avital A, Goshen I, Kamsler A, Segal M, Iverfeldt K, Richter-Levin G, et al. Impaired interleukin-1 signaling is associated with deficits in hippocampal memory processes and neural plasticity. *Hippocampus.* (2003) 13:826–34. doi: 10.1002/hipo.10135

66. Schmid AW, Lynch MA, Herron CE. The effects of IL-1 receptor antagonist on beta amyloid mediated depression of LTP in the rat CA1 *in vivo*. *Hippocampus.* (2009) 19:670–6. doi: 10.1002/hipo.20542

67. Goshen I, Kreisel T, Ounallah-Saad H, Renbaum P, Zalzstein Y, Ben-Hur T, et al. A dual role for interleukin-1 in hippocampal-dependent memory processes. *Psychoneuroendocrinology.* (2007) 32:1106–15. doi: 10.1016/j.psyneuen.2007.09.004

68. Bourgognon JM, Cavanagh J. The role of cytokines in modulating learning and memory and brain plasticity. *Brain Neurosci Adv.* (2020) 4:2398212820979802. doi: 10.1177/2398212820979802

69. Yarlagadda A, Alfson E, Clayton AH. The blood brain barrier and the role of cytokines in neuropsychiatry. *Psychiatry.* (2009) 6:18–22.

70. 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. doi: 10.1016/j.jpsychires.2019.05.019

71. Turner MD, Nedjai B, Hurst T, Pennington DJ. Cytokines and chemokines: at the crossroads of cell signalling and inflammatory disease. *Biochim Biophys Acta.* (2014) 1843:2563–82. doi: 10.1016/j.bbamcr.2014.05.014

72. Masi A, Glozier N, Dale R, Guastella AJ. The immune system, cytokines, and biomarkers in sutism spectrum disorder. *Neurosci Bull.* (2017) 33:194–204. doi: 10.1007/s12264-017-0103-8

73. Xu N, Li X, Zhong Y. Inflammatory cytokines: potential biomarkers of immunologic dysfunction in autism spectrum disorders. *Mediators Inflamm.* (2015) 2015:531518. doi: 10.1155/2015/531518

74. Ricci S, Businaro R, Ippoliti F, Lo Vasco VR, Massoni F, Onofri E, et al. Altered cytokine and BDNF levels in autism spectrum disorder. *Neurotox Res.* (2013) 24:491–501. doi: 10.1007/s12640-013-9393-4

75. Parameswaran N, Patial S. Tumor necrosis factor-α signaling in macrophages. *Crit Rev Eukaryot Gene Expr.* (2010) 20:87–103. doi: 10.1615/CritRevEukarGeneExpr.v20.i2.10

76. Eftekharian MM, Ghafouri-Fard S, Noroozi R, Omrani MD, Arsang-Jang S, Ganji M, et al. Cytokine profile in autistic patients. *Cytokine.* (2018) 108:120–6. doi: 10.1016/j.cyto.2018.03.034

77. Ahmad SF, Nadeem A, Ansari MA, Bakheet SA, Al-Ayadhi LY, Attia SM. Upregulation of IL-9 and JAK-STAT signaling pathway in children with autism. *Prog Neuropsychopharmacol Biol Psychiatry.* (2017) 79 (Pt B):472–80. doi: 10.1016/j.pnpbp.2017.08.002

78. Borgia F, Custurone P, Li Pomi F, Cordiano R, Alessandrello C, Gangemi S. IL-31: state of the art for an inflammation-oriented interleukin. *Int J Mol Sci.* (2022) 23:6507. doi: 10.3390/ijms23126507

79. Xie J, Huang L, Li X, Li H, Zhou Y, Zhu H, et al. Immunological cytokine profiling identifies TNF-α as a key molecule dysregulated in autistic children. *Oncotarget.* (2017) 8:82390–8. doi: 10.18632/oncotarget.19326

80. Ahmad SF, Ansari MA, Nadeem A, Bakheet SA, Al-Ayadhi LY, Attia SM. Elevated IL-16 expression is associated with development of immune dysfunction in children with autism. *Psychopharmacology.* (2019) 236:831–8. doi: 10.1007/s00213-018-5120-4

81. 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) 20:440–6. doi: 10.1038/mp.2014.59

82. 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. doi: 10.3389/fpsyt.2021.670200

83. Krakowiak P, Goines PE, Tancredi DJ, Ashwood P, Hansen RL, Hertz-Picciotto I, et al. Neonatal cytokine profiles associated with autism spectrum disorder. *Biol Psychiatry.* (2017) 81:442–51. doi: 10.1016/j.biopsych.2015.08.007

84. Heuer LS, Croen LA, Jones KL, Yoshida CK, Hansen RL, Yolken R, et al. An exploratory examination of neonatal cytokines and chemokines as predictors of autism risk: the early markers for autism study. *Biol Psychiatry.* (2019) 86:255–64. doi: 10.1016/j.biopsych.2019.04.037

85. Hylén U, Eklund D, Humble M, Bartoszek J, Särndahl E, Bejerot S. Increased inflammasome activity in markedly ill psychiatric patients: an explorative study. *J Neuroimmunol.* (2020) 339:577119. doi: 10.1016/j.jneuroim.2019.577119

86. Saad K, Abdallah AEM, Abdel-Rahman AA, Al-Atram AA, Abdel-Raheem YF, Gad EF, et al. Polymorphism of interleukin-1β and interleukin-1 receptor antagonist genes in children with autism spectrum disorders. *Prog Neuropsychopharmacol Biol Psychiatry.* (2020) 103:109999. doi: 10.1016/j.pnpbp.2020.109999

87. Jyonouchi H, Sun S, Le H. Proinflammatory and regulatory cytokine production associated with innate and adaptive immune responses in children with autism spectrum disorders and developmental regression. *J Neuroimmunol.* (2001) 120:170–9. doi: 10.1016/S0165-5728(01)00421-0

88. Molloy CA, Morrow AL, Meinzen-Derr J, Schleifer K, Dienger K, Manning-Courtney P, et al. Elevated cytokine levels in children with autism spectrum disorder. *J Neuroimmunol.* (2006) 172:198–205. doi: 10.1016/j.jneuroim.2005.11.007

89. Wei H, Zou H, Sheikh AM, Malik M, Dobkin C. Brown WT, Li X. IL-6 is increased in the cerebellum of autistic brain and alters neural cell adhesion, migration and synaptic formation. *J Neuroinflamm.* (2011) 8:52. doi: 10.1186/1742-2094-8-52

90. Smedler E, Kleppe J, Neufeld J, Lundin K, Bölte S, Landén M. Cerebrospinal fluid and serum protein markers in autism: a co-twin study. *J Neurochem.* (2021) 158:798–806. doi: 10.1111/jnc.15338

91. Steri M, Orrù V, Idda ML, Pitzalis M, Pala M, Zara I, et al. Overexpression of the cytokine BAFF and autoimmunity risk. *N Engl J Med.* (2017) 376:1615–26. doi: 10.1056/NEJMoa1610528

92. Engh JA, Ueland T, Agartz I, Andreou D, Aukrust P, Boye B, et al. Plasma levels of the cytokines B cell-activating factor (BAFF) and a proliferation-inducing ligand (APRIL) in schizophrenia, bipolar, and major depressive disorder: a cross sectional, multisite study. *Schizophr Bull.* (2022) 48:37–46. doi: 10.1093/schbul/sbab106

93. Ma L, Piirainen S, Kulesskaya N, Rauvala H, Tian L. Association of brain immune genes with social behavior of inbred mouse strains. *J Neuroinflammation.* (2015) 12:75. doi: 10.1186/s12974-015-0297-5

94. Siniscalco D, Schultz S, Brigida AL, Antonucci N. Inflammation and neuro-immune dysregulations in autism spectrum disorders. *Pharmaceuticals.* (2018) 11:56. doi: 10.3390/ph11020056

95. Shiina T, Hosomichi K, Inoko H, Kulski JK. The HLA genomic loci map: expression, interaction, diversity and disease. *J Hum Genet.* (2009) 54:15–39. doi: 10.1038/jhg.2008.5

96. Goddard CA, Butts DA, Shatz CJ. Regulation of CNS synapses by neuronal MHC class I. *Proc Natl Acad Sci USA.* (2007) 104:6828–33. doi: 10.1073/pnas.0702023104

97. Cohly HH, Panja A. Immunological findings in autism. *Int Rev Neurobiol.* (2005) 71:317–41. doi: 10.1016/S0074-7742(05)71013-8

98. Bilbo SD, Schwarz JM. The immune system and developmental programming of brain and behavior. *Front Neuroendocrinol.* (2012) 33:267–86. doi: 10.1016/j.yfrne.2012.08.006

99. Bennabi M, Gaman A, Delorme R, Boukouaci W, Manier C, Scheid I, et al. HLA-class II haplotypes and autism spectrum disorders. *Sci Rep.* (2018) 8:7639. doi: 10.1038/s41598-018-25974-9

100. Tamouza R, Krishnamoorthy R, Leboyer M. Understanding the genetic contribution of the human leukocyte antigen system to common major psychiatric disorders in a world pandemic context. *Brain Behav Immun.* (2021) 91:731–9. doi: 10.1016/j.bbi.2020.09.033

101. Lee LC, Zachary AA, Leffell MS, Newschaffer CJ, Matteson KJ, Tyler JD, et al. HLA-DR4 in families with autism. *Pediatr Neurol.* (2006) 35:303–7. doi: 10.1016/j.pediatrneurol.2006.06.006

102. Chien YL, Wu YY, Chen CH, Gau SS, Huang YS, Chien WH, et al. Association of HLA-DRB1 alleles and neuropsychological function in autism. *Psychiatr Genet.* (2012) 22:46–9. doi: 10.1097/YPG.0b013e32834915ae

103. Torres AR, Sweeten TL, Johnson RC, Odell D, Westover JB, Bray-Ward P, et al. Common genetic variants found in HLA and KIR immune genes in autism spectrum disorder. *Front Neurosci.* (2016) 10:463. doi: 10.3389/fnins.2016.00463

104. Makita N, Hizukuri Y, Yamashiro K, Murakawa M, Hayashi Y. IL-10 enhances the phenotype of M2 macrophages induced by IL-4 and confers the ability to increase eosinophil migration. *Int Immunol.* (2015) 27:131–41. doi: 10.1093/intimm/dxu090

105. DiStasio MM, Nagakura I, Nadler MJ, Anderson MP. T lymphocytes and cytotoxic astrocyte blebs correlate across autism brains. *Ann Neurol.* (2019) 86:885–98. doi: 10.1002/ana.25610

106. Wender R, Brown AM, Fern R, Swanson RA, Farrell K, Ransom BR. Astrocytic glycogen influences axon function and survival during glucose deprivation in central white matter. *J Neurosci.* (2000) 20:6804–10. doi: 10.1523/JNEUROSCI.20-18-06804.2000

107. Pinkse GG, Tysma OH, Bergen CA, Kester MG, Ossendorp F, van Veelen PA, et al. Autoreactive CD8 T cells associated with beta cell destruction in type 1 diabetes. *Proc Natl Acad Sci USA.* (2005) 102:18425–30. doi: 10.1073/pnas.0508621102

108. Redondo MJ, Steck AK, Pugliese A. Genetics of type 1 diabetes. *Pediatr Diabetes.* (2018) 19:346–53. doi: 10.1111/pedi.12597

109. Braunschweig D, Van de Water J. Maternal autoantibodies in autism. *Arch Neurol.* (2012) 69:693–9. doi: 10.1001/archneurol.2011.2506

110. Goines P, Haapanen L, Boyce R, Duncanson P, Braunschweig D, Delwiche L, et al. Autoantibodies to cerebellum in children with autism associate with behavior. *Brain Behav Immun.* (2011) 25:514–23. doi: 10.1016/j.bbi.2010.11.017

111. Braunschweig D, Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Croen LA, et al. Autism: maternally derived antibodies specific for fetal brain proteins. *Neurotoxicology.* (2008) 29:226–31. doi: 10.1016/j.neuro.2007.10.010

112. Braunschweig D, Krakowiak P, Duncanson P, Boyce R, Hansen RL, Ashwood P, et al. Autism-specific maternal autoantibodies recognize critical proteins in developing brain. *Transl Psychiatry.* (2013) 3:e277. doi: 10.1038/tp.2013.50

113. Ramaekers VT, Sequeira JM, Thöny B, Quadros EV. Oxidative stress, folate receptor autoimmunity, and CSF findings in severe infantile autism. *Autism Res Treat.* (2020) 2020:9095284. doi: 10.1155/2020/9095284

114. Melnyk S, Fuchs GJ, Schulz E, Lopez M, Kahler SG, Fussell JJ, et al. Metabolic imbalance associated with methylation dysregulation and oxidative damage in children with autism. *J Autism Dev Disord.* (2012) 42:367–77. doi: 10.1007/s10803-011-1260-7

115. Thorsen M. Oxidative stress, metabolic and mitochondrial abnormalities associated with autism spectrum disorder. *Prog Mol Biol Transl Sci.* (2020) 173:331–54. doi: 10.1016/bs.pmbts.2020.04.018

116. Bessman NJ, Sonnenberg GF. Emerging roles for antigen presentation in establishing host-microbiome symbiosis. *Immunol Rev.* (2016) 272:139–50. doi: 10.1111/imr.12425

117. Zhou J, He F, Yang F, Yang Z, Xie Y, Zhou S, et al. Increased stool immunoglobulin A level in children with autism spectrum disorders. *Res Dev Disabil.* (2018) 82:90–4. doi: 10.1016/j.ridd.2017.10.009

118. Wang M, Doenyas C, Wan J, Zeng S, Cai C, Zhou J, et al. Virulence factor-related gut microbiota genes and immunoglobulin A levels as novel markers for machine learning-based classification of autism spectrum disorder. *Comput Struct Biotechnol J.* (2020) 19:545–54. doi: 10.1016/j.csbj.2020.12.012

119. Allard MJ, Bergeron JD, Baharnoori M, Srivastava LK, Fortier LC, Poyart C, et al. A sexually dichotomous, autistic-like phenotype is induced by group B *Streptococcus maternofetal* immune activation. *Autism Res.* (2017) 10:233–45. doi: 10.1002/aur.1647

120. Fernández de Cossío L, Guzmán A, van der Veldt S, Luheshi GN. Prenatal infection leads to ASD-like behavior and altered synaptic pruning in the mouse offspring. *Brain Behav Immun.* (2017) 63:88–98. doi: 10.1016/j.bbi.2016.09.028

121. Vuong HE, Hsiao EY. Emerging roles for the gut microbiome in autisms disorder. *Biol Psychiatry.* (2017) 81:411–23. doi: 10.1016/j.biopsych.2016.08.024

122. Doenyas C. Gut microbiota, inflammation, and probiotics on neural development in autism spectrum disorder. *Neuroscience.* (2018) 374:271–86. doi: 10.1016/j.neuroscience.2018.01.060

123. Wang X, Yang J, Zhang H, Yu J, Yao Z. Oral probiotic administration during pregnancy prevents autism-related behaviors in offspring induced by maternal immune activation via anti-inflammation in mice. *Autism Res.* (2019) 12:576–88. doi: 10.1002/aur.2079

124. Zavasnik-Bergant T. Cystatin protease inhibitors and immune functions. *Front Biosci.* (2008) 13:4625–37 doi: 10.2741/3028

125. Di Matteo F, Pipicelli F, Kyrousi C, Tovecci I, Penna E, Crispino M, et al. Cystatin B is essential for proliferation and interneuron migration in individuals with EPM1 epilepsy. *EMBO Mol Med.* (2020) 12:e11419. doi: 10.15252/emmm.201911419

126. Penna E, Cerciello A, Chambery A, Russo R, Cernilogar FM, Pedone EM, et al. Cystatin B involvement in synapse physiology of rodent brains and human cerebral organoids. *Front Mol Neurosci.* (2019) 12:195. doi: 10.3389/fnmol.2019.00195

127. Tandon R, Pradhan S. Autistic features in Unverricht-Lundborg disease. *Epilepsy Behav Rep.* (2019) 12:100323. doi: 10.1016/j.ebr.2019.100323

128. Bradford KL, Moretti FA, Carbonaro-Sarracino DA, Gaspar HB, Kohn DB. Adenosine deaminase (ADA)-deficient severe combined immune deficiency (SCID): molecular pathogenesis and clinical manifestations. *J Clin Immunol.* (2017) 37:626–37. doi: 10.1007/s10875-017-0433-3

129. Bottini N, De Luca D, Saccucci P, Fiumara A, Elia M, Porfirio MC, et al. Autism: evidence of association with adenosine deaminase genetic polymorphism. *Neurogenetics.* (2001) 3:111–3. doi: 10.1007/s100480000104

130. Persico AM, Militerni R, Bravaccio C, Schneider C, Melmed R, Trillo S, et al. Adenosine deaminase alleles and autistic disorder: case-control and family-based association studies. *Am J Med Genet.* (2000) 96:784–90. doi: 10.1002/1096-8628(20001204)96:6<784::AID-AJMG18>3.0.CO;2-7

131. Hettinger JA, Liu X, Holden JJ. The G22A polymorphism of the ADA gene and susceptibility to autism spectrum disorders. *J Autism Dev Disord.* (2008) 38:14–9. doi: 10.1007/s10803-006-0354-0

132. Rogers MH, Lwin R, Fairbanks L, Gerritsen B, Gaspar HB. Cognitive and behavioral abnormalities in adenosine deaminase deficient severe combined immunodeficiency. *J Pediatr.* (2001) 139:44–50. doi: 10.1067/mpd.2001.115023

133. Camici M, Micheli V, Ipata PL, Tozzi MG. Pediatric neurological syndromes and inborn errors of purine metabolism. *Neurochem Int.* (2010) 56:367–78. doi: 10.1016/j.neuint.2009.12.003

134. Fitzpatrick EA, Han X, Xiao Z, Quarles LD. Role of fibroblast growth factor-23 in innate immune responses. *Front Endocrinol.* (2018) 9:320. doi: 10.3389/fendo.2018.00320

135. Vaccarino FM, Grigorenko EL, Smith KM, Stevens HE. Regulation of cerebral cortical size and neuron number by fibroblast growth factors: implications for autism. *J Autism Dev Disord.* (2009) 39:511–20. doi: 10.1007/s10803-008-0653-8

136. Al-Mubarak B, Abouelhoda M, Omar A, AlDhalaan H, Aldosari M, Nester M, et al. Whole exome sequencing reveals inherited and de novo variants in autism spectrum disorder: a trio study from Saudi families. *Sci Rep.* (2017) 7:5679. doi: 10.1038/s41598-017-06033-1

137. Iwata T, Hevner RF. Fibroblast growth factor signaling in development of the cerebral cortex. *Dev Growth Differ.* (2009) 51:299–323. doi: 10.1111/j.1440-169X.2009.01104.x

138. Turner CA, Eren-Koçak E, Inui EG, Watson SJ, Akil H. Dysregulated fibroblast growth factor (FGF) signaling in neurological and psychiatric disorders. *Semin Cell Dev Biol.* (2016) 53:136–43. doi: 10.1016/j.semcdb.2015.10.003

139. Kumar S, Reynolds K, Ji Y, Gu R, Rai S, Zhou CJ. Impaired neurodevelopmental pathways in autism spectrum disorder: a review of signaling mechanisms and crosstalk. *J Neurodev Disord.* (2019) 11:10. doi: 10.1186/s11689-019-9268-y

140. Eng C. PTEN: one gene, many syndromes. *Hum Mutat.* (2003) 22:183–98. doi: 10.1002/humu.10257

141. Taylor H, Laurence ADJ, Uhlig HH. The role of PTEN in innate and adaptive immunity. *Cold Spring Harb Perspect Med.* (2019) 9:a036996. doi: 10.1101/cshperspect.a036996

142. Liaw D, Marsh DJ, Li J, Dahia PL, Wang SI, Zheng Z, et al. Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. *Nat Genet.* (1997) 16:64–7. doi: 10.1038/ng0597-64

143. Marsh DJ, Dahia PL, Zheng Z, Liaw D, Parsons R, Gorlin RJ, et al. Germline mutations in PTEN are present in Bannayan-Zonana syndrome. *Nat Genet.* (1997) 16:333–4. doi: 10.1038/ng0897-333

144. Mester J, Charis E. PTEN hamartoma tumor syndrome. *Handb Clin Neurol.* (2015) 132:129–37. doi: 10.1016/B978-0-444-62702-5.00009-3

145. Butler MG, Dasouki MJ, Zhou XP, Talebizadeh Z, Brown M, Takahashi TN, et al. Subset of individuals with autism spectrum disorders and extreme macrocephaly associated with germline PTEN tumour suppressor gene mutations. *J Med Genet.* (2005) 42:318–21. doi: 10.1136/jmg.2004.024646

146. Varga EA, Pastore M, Prior T, Herman GE, McBride KL. The prevalence of PTEN mutations in a clinical pediatric cohort with autism spectrum disorders, developmental delay, and macrocephaly. *Genet Med.* (2009) 11:111–7. doi: 10.1097/GIM.0b013e31818fd762

147. Kwon CH, Luikart BW, Powell CM, Zhou J, Matheny SA, Zhang W, et al. Pten regulates neuronal arborization and social interaction in mice. *Neuron.* (2006) 50:377–88. doi: 10.1016/j.neuron.2006.03.023

148. Heindl M, Händel N, Ngeow J, Kionke J, Wittekind C, Kamprad M, et al. Autoimmunity, intestinal lymphoid hyperplasia, and defects in mucosal B-cell homeostasis in patients with PTEN hamartoma tumor syndrome. *Gastroenterology.* (2012) 142:1093–6.e6. doi: 10.1053/j.gastro.2012.01.011

149. Browning MJ, Chandra A, Carbonaro V, Okkenhaug K, Barwell J. Cowden's syndrome with immunodeficiency. *J Med Genet.* (2015) 52:856–9. doi: 10.1136/jmedgenet-2015-103266

150. Tsujita Y, Mitsui-Sekinaka K, Imai K, Yeh TW, Mitsuiki N, Asano T, et al. Phosphatase and tensin homolog (PTEN) mutation can cause activated phosphatidylinositol 3-kinase δ syndrome-like immunodeficiency. *J Allergy Clin Immunol.* (2016) 138:1672–80.e10. doi: 10.1016/j.jaci.2016.03.055

151. Gabrielli AP, Manzardo AM, Butler MG. GeneAnalytics pathways and profiling of shared autism and cancer genes. *Int J Mol Sci.* (2019) 20:1166. doi: 10.3390/ijms20051166

152. Yeung KS, Tso WWY, Ip JJK, Mak CCY, Leung GKC, Tsang MHY, 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. doi: 10.1186/s13229-017-0182-4

153. Wen Y, Herbert MR. Connecting the dots: overlaps between autism and cancer suggest possible common mechanisms regarding signaling pathways related to metabolic alterations. *Med Hypotheses.* (2017) 103:118–23. doi: 10.1016/j.mehy.2017.05.004

154. Okay K, Variş PÜ, Miral S, Ekinci B, Yaraş T, Karakülah G, et al. Alternative splicing and gene co-expression network-based analysis of dizygotic twins with autism-spectrum disorder and their parents. *Genomics.* (2021) 113:2561–71. doi: 10.1016/j.ygeno.2021.05.038

155. Li Y, Wang Y, Zhang C, Yuan W, Wang J, Zhu C, et al. ZNF322, a novel human C2H2 Kruppel-like zinc-finger protein, regulates transcriptional activation in MAPK signaling pathways. *Biochem Biophys Res Commun.* (2004) 325:1383–92. doi: 10.1016/j.bbrc.2004.10.183

156. Liu X, Wang Y, Lu H, Li J, Yan X, Xiao M, et al. Genome-wide analysis identifies NR4A1 as a key mediator of T cell dysfunction. *Nature.* (2019) 567:525–9. doi: 10.1038/s41586-019-0979-8

157. DeLuca HF. Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr.* (2004) 80 (6 Suppl):1689S−96S. doi: 10.1093/ajcn/80.6.1689S

158. Wesselink E, Balvers M, Bours MJL, de Wilt JHW, Witkamp RF, van Baar H, et al. The association between circulating levels of vitamin D and inflammatory markers in the first 2 years after colorectal cancer diagnosis. *Therap Adv Gastroenterol.* (2020) 13:1756284820923922. doi: 10.1177/1756284820923922

159. Liu W, Zhang L, Xu HJ, Li Y, Hu CM, Yang JY, et al. The anti-inflammatory effects of vitamin D in tumorigenesis. *Int J Mol Sci.* (2018) 19:2736. doi: 10.3390/ijms19092736

160. Ene CD, Anghel AE, Neagu M, Nicolae I. 25-OH Vitamin D and Interleukin-8: Emerging biomarkers in cutaneous melanoma development and progression. *Mediators Inflamm.* (2015) 2015:904876. doi: 10.1155/2015/904876

161. Grudet C, Malm J, Westrin A, Brundin L. Suicidal patients are deficient in vitamin D, associated with a pro-inflammatory status in the blood. *Psychoneuroendocrinology.* (2014) 50:210–9. doi: 10.1016/j.psyneuen.2014.08.016

162. Faivre S, Roche N, Lacerre F, Dealberto MJ. Vitamin D deficiency in a psychiatric population and correlation between vitamin D and CRP. *Encephale.* (2019) 45:376–83. doi: 10.1016/j.encep.2019.02.005

163. Harant H, Andrew PJ, Reddy GS, Foglar E, Lindley IJ. 1alpha,25-dihydroxyvitamin D3 and a variety of its natural metabolites transcriptionally repress nuclear-factor-kappaB-mediated interleukin-8 gene expression. *Eur J Biochem.* (1997) 250:63–71. doi: 10.1111/j.1432-1033.1997.00063.x

164. Eyles D, Brown J, Mackay-Sim A, McGrath J, Feron F. Vitamin D3 and brain development. *Neuroscience.* (2003) 118:641–53. doi: 10.1016/S0306-4522(03)00040-X

165. Vinkhuyzen AAE, Eyles DW, Burne THJ, Blanken LME, Kruithof CJ, Verhulst F, et al. Gestational vitamin D deficiency and autism-related traits: the generation R study. *Mol Psychiatry.* (2018) 23:240–6. doi: 10.1038/mp.2016.213

166. Lee BK, Eyles DW, Magnusson C, Newschaffer CJ, McGrath JJ, Kvaskoff D, et al. Developmental vitamin D and autism spectrum disorders: findings from the Stockholm youth cohort. *Mol Psychiatry.* (2021) 26:1578–88. doi: 10.1038/s41380-019-0578-y

167. Zmuda JM, Cauley JA, Ferrell RE. Molecular epidemiology of vitamin D receptor gene variants. *Epidemiol Rev.* (2000) 22:203–17. doi: 10.1093/oxfordjournals.epirev.a018033

168. Imani D, Razi B, Motallebnezhad M, Rezaei R. Association between vitamin D receptor (VDR) polymorphisms and the risk of multiple sclerosis (MS): an updated meta-analysis. *BMC Neurol.* (2019) 19:339. doi: 10.1186/s12883-019-1577-y

169. Yan J, Feng J, Craddock N, Jones IR, Cook EH Jr, Goldman D, et al. Vitamin D receptor variants in 192 patients with schizophrenia and other psychiatric diseases. *Neurosci Lett.* (2005) 380:37–41. doi: 10.1016/j.neulet.2005.01.018

170. Biswas S, Kanwal B, Jeet C, Seminara RS. Fok-I, Bsm-I, and Taq-I variants of vitamin D receptor polymorphism in the development of autism spectrum disorder: a literature review. *Cureus.* (2018) 10:e3228. doi: 10.7759/cureus.3228

171. Guerini FR, Bolognesi E, Chiappedi M, Mensi MM, Fumagalli O, Rogantini C, et al. Vitamin D receptor polymorphisms associated with autism spectrum disorder. *Autism Res.* (2020) 13:680–90. doi: 10.1002/aur.2279

172. Koufaris C, Sismani C. Modulation of the genome and epigenome of individuals susceptible to autism by environmental risk factors. *Int J Mol Sci.* (2015) 16:8699–718. doi: 10.3390/ijms16048699

173. Rossignol DA, Frye RE. Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis. *Mol Psychiatry.* (2012) 17:290–314. doi: 10.1038/mp.2010.136

174. James SJ, Melnyk S, Jernigan S, Cleves MA, Halsted CH, Wong DH, et al. Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism. *Am J Med Genet B Neuropsychiatr Genet.* (2006) 141B:947–56. doi: 10.1002/ajmg.b.30366

175. Fang J, Sheng R, Qin ZH. NADPH oxidases in the central nervous system: regional and cellular localization and the possible link to brain diseases. *Antioxid Redox Signal.* (2021) 35:951–73. doi: 10.1089/ars.2021.0040

176. Yang Z, Min Z, Yu B. Reactive oxygen species and immune regulation. *Int Rev Immunol.* (2020) 39:292–8. doi: 10.1080/08830185.2020.1768251

177. Bjørklund G, Meguid NA, El-Bana MA, Tinkov AA, Saad K, Dadar M, et al. Oxidative stress in autism spectrum disorder. *Mol Neurobiol.* (2020) 57:2314–32. doi: 10.1007/s12035-019-01742-2

178. Sonar SA, Lal G. The iNOS activity during an immune response controls the CNS pathology in experimental autoimmune encephalomyelitis. *Front Immunol.* (2019) 10:710. doi: 10.3389/fimmu.2019.00710

179. Al-Harbi NO, Nadeem A, Ahmad SF, Al-Ayadhi LY, Al-Harbi MM, As Sobeai HM, et al. Elevated expression of toll-like receptor 4 is associated with NADPH oxidase-induced oxidative stress in B cells of children with autism. *Int Immunopharmacol.* (2020) 84:106555. doi: 10.1016/j.intimp.2020.106555

180. Nadeem A, Ahmad SF, Bakheet SA, Al-Harbi NO, Al-Ayadhi LY, Attia SM, et al. Toll-like receptor 4 signaling is associated with upregulated NADPH oxidase expression in peripheral T cells of children with autism. *Brain Behav Immun.* (2017) 61:146–154. doi: 10.1016/j.bbi.2016.12.024

181. Arnhold J. The dual role of myeloperoxidase in immune response. *Int J Mol Sci*. (2020) 21:8057 doi: 10.3390/ijms21218057

182. Ceylan MF, Tural Hesapcioglu S, Yavas CP, Senat A, Erel O. Serum ischemia-modified albumin levels, myeloperoxidase activity and peripheral blood mononuclear cells in autism spectrum disorder (ASD). *J Autism Dev Disord.* (2021) 51:2511–7. doi: 10.1007/s10803-020-04740-9

183. Garry PS, Ezra M, Rowland MJ, Westbrook J, Pattinson KTS. The role of the nitric oxide pathway in brain injury and its treatment - from bench to bedside. *Exp Neurol.* (2015) 263:235–43. doi: 10.1016/j.expneurol.2014.10.017

184. Arami KM, Jameie B, Akbar Moosavi S. Neuronal nitric oxide synthase. In: Saravi SSS, editor. *Nitric Oxide Synthase—Simple Enzyme-Complex Roles*. London: IntechOpen (2017). doi: 10.5772/67494

185. Nadeem A, Ahmad SF, Al-Ayadhi LY, Attia SM, Al-Harbi NO, Alzahrani KS, et al. Differential regulation of Nrf2 is linked to elevated inflammation and nitrative stress in monocytes of children with autism. *Psychoneuroendocrinology.* (2020) 113:104554. doi: 10.1016/j.psyneuen.2019.104554

186. Abruzzo PM, Matté A, Bolotta A, Federti E, Ghezzo A, Guarnieri T, et al. Plasma peroxiredoxin changes and inflammatory cytokines support the involvement of neuro-inflammation and oxidative stress in autism spectrum disorder. *J Transl Med.* (2019) 17:332. doi: 10.1186/s12967-019-2076-z

187. Kovač J, Macedoni Lukšič M, Trebušak Podkrajšek K, Klančar G, et al. Rare single nucleotide polymorphisms in the regulatory regions of the superoxide dismutase genes in autism spectrum disorder. *Autism Res.* (2014) 7:138–44. doi: 10.1002/aur.1345

188. Hovnik T, Dolzan V, Bratina NU, Podkrajsek KT, Battelino T. Genetic polymorphisms in genes encoding antioxidant enzymes are associated with diabetic retinopathy in type 1 diabetes. *Diabetes Care.* (2009) 32:2258–62. doi: 10.2337/dc09-0852

189. Carrasco M, Salazar C, Tiznado W, Ruiz LM. Alterations of mitochondrial biology in the oral mucosa of Chilean children with autism spectrum disorder (ASD). *Cells.* (2019) 8:367. doi: 10.3390/cells8040367

190. Nadeem A, Ahmad SF, Attia SM, Al-Ayadhi LY, Al-Harbi NO, Bakheet SA. Dysregulated enzymatic antioxidant network in peripheral neutrophils and monocytes in children with autism. *Prog Neuropsychopharmacol Biol Psychiatry.* (2019) 88:352–59. doi: 10.1016/j.pnpbp.2018.08.020

191. Nadeem A, Ahmad SF, Al-Harbi NO, Alasmari AF, Al-Ayadhi LY, Alasmari F, et al. Upregulation of enzymatic antioxidants in CD4+ T cells of autistic children. *Biochimie.* (2020) 171–2:205–12. doi: 10.1016/j.biochi.2020.03.009

192. Mandic-Maravic V, Mitkovic-Voncina M, Pljesa-Ercegovac M, Savic-Radojevic A, Djordjevic M, Pekmezovic T, et al. Autism spectrum disorders and perinatal complications—is oxidative stress the connection? *Front Psychiatry.* (2019) 10:675. doi: 10.3389/fpsyt.2019.00675

193. Nebert DW, Vasiliou V. Analysis of the glutathione S-transferase (GST) gene family. *Hum Genomics.* (2004) 1:460–4. doi: 10.1186/1479-7364-1-6-460

194. Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. *Annu Rev Pharmacol Toxicol.* (2005) 45:51–88. doi: 10.1146/annurev.pharmtox.45.120403.095857

195. Shadel GS, Horvath TL. Mitochondrial ROS signaling in organismal homeostasis. *Cell.* (2015) 163:560–9. doi: 10.1016/j.cell.2015.10.001

196. Chen Y, Zhou Z, Min W. Mitochondria, oxidative stress and innate immunity. *Front Physiol.* (2018) 9:1487. doi: 10.3389/fphys.2018.01487

197. Barrera MJ, Aguilera S, Castro I, Carvajal P, Jara D, Molina C, et al. Dysfunctional mitochondria as critical players in the inflammation of autoimmune diseases: potential role in Sjögren's syndrome. *Autoimmun Rev.* (2021) 20:102867. doi: 10.1016/j.autrev.2021.102867

198. Neagu M, Constantin C, Popescu ID, Zipeto D, Tzanakakis G, Nikitovic D, et al. Inflammation and metabolism in cancer cell-mitochondria key player. *Front Oncol.* (2019) 9:348. doi: 10.3389/fonc.2019.00348

199. Zhou Z, Austin GL, Young LEA, Johnson LA, Sun R. Mitochondrial metabolism in major neurological diseases. *Cells.* (2018) 7:229. doi: 10.3390/cells7120229

200. Rovira-Llopis S, Bañuls C, Diaz-Morales N, Hernandez-Mijares A, Rocha M, Victor VM. Mitochondrial dynamics in type 2 diabetes: pathophysiological implications. *Redox Biol.* (2017) 11:637–45. doi: 10.1016/j.redox.2017.01.013

201. Bennuri SC, Rose S, Frye RE. Mitochondrial dysfunction is inducible in lymphoblastoid cell lines from children with autism and may involve the TORC1 pathway. *Front Psychiatry.* (2019) 10:269. doi: 10.3389/fpsyt.2019.00269

202. Chan DC. Fusion and fission: interlinked processes critical for mitochondrial health. *Annu Rev Genet.* (2012) 46:265–87. doi: 10.1146/annurev-genet-110410-132529

203. 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) 9:43. doi: 10.1038/s41398-019-0389-6

204. Liu Z, Mao X, Dan Z, Pei Y, Xu R, Guo M, et al. Gene variations in autism spectrum disorder are associated with alteration of gut microbiota, metabolites and cytokines. *Gut Microbes.* (2021) 13:1–16. doi: 10.1080/19490976.2020.1854967

205. Sharon G, Cruz NJ, Kang DW, Gandal MJ, Wang B, Kim YM, et al. Human gut microbiota from autism spectrum disorder promote behavioral symptoms in mice. *Cell.* (2019) 177:1600–18.e17. doi: 10.1016/j.cell.2019.05.004

206. Ansel A, Rosenzweig JP, Zisman PD, Melamed M, Gesundheit B. Variation in gene expression in autism spectrum disorders: an extensive review of transcriptomic studies. *Front Neurosci.* (2017) 10:601. doi: 10.3389/fnins.2016.00601

207. Navarro F, Liu Y, Rhoads JM. Can probiotics benefit children with autism spectrum disorders? *World J Gastroenterol.* (2016) 22:10093–102. doi: 10.3748/wjg.v22.i46.10093

208. Lee M, Krishnamurthy J, Susi A, Sullivan C, Gorman GH, Hisle-Gorman E, et al. Association of autism spectrum disorders and inflammatory bowel disease. *J Autism Dev Disord.* (2018) 48:1523–9. doi: 10.1007/s10803-017-3409-5

209. Fiorentino M, Sapone A, Senger S, Camhi SS, Kadzielski SM, Buie TM, et al. Blood-brain barrier and intestinal epithelial barrier alterations in autism spectrum disorders. *Mol Autism.* (2016) 7:49. doi: 10.1186/s13229-016-0110-z

210. Walker SJ, Langefeld CD, Zimmerman K, Schwartz MZ, Krigsman A. A molecular biomarker for prediction of clinical outcome in children with ASD, constipation, and intestinal inflammation. *Sci Rep.* (2019) 9:5987. doi: 10.1038/s41598-019-42568-1

211. Adams JB, Audhya T, McDonough-Means S, Rubin RA, Quig D, Geis E et al. Nutritional and metabolic status of children with autism vs. neurotypical children, and the association with autism severity. *Nutr Metab.* (2011) 8:34. doi: 10.1186/1743-7075-8-34

212. Walker SJ, Beavers DP, Fortunato J, Krigsman A. A putative blood-based biomarker for autism spectrum disorder-associated ileocolitis. *Sci Rep.* (2016) 6:35820. doi: 10.1038/srep35820

213. Edmiston E, Ashwood P, Van de Water J. Autoimmunity, autoantibodies, and autism spectrum disorder. *Biol Psychiatry.* (2017) 81:383–90. doi: 10.1016/j.biopsych.2016.08.031

214. Dobre EG, Constantin C, Costache M, Neagu M. Interrogating epigenome toward personalized approach in cutaneous melanoma. *J Pers Med.* (2021) 11:901. doi: 10.3390/jpm11090901

215. Pacini G, Paolino S, Andreoli L, Tincani A, Gerosa M, Caporali R, et al. Epigenetics, pregnancy and autoimmune rheumatic diseases. *Autoimmun Rev.* (2020) 19:102685. doi: 10.1016/j.autrev.2020.102685

216. Zhang L, Lu Q, Chang C. Epigenetics in health and disease. *Adv Exp Med Biol.* (2020) 1253:3–55. doi: 10.1007/978-981-15-3449-2_1

217. Vogel Ciernia A, LaSalle J. The landscape of DNA methylation amid a perfect storm of autism aetiologies. *Nat Rev Neurosci.* (2016) 17:411–23. doi: 10.1038/nrn.2016.41

218. Nardone S, Sams DS, Zito A, Reuveni E, Elliott E. Dysregulation of cortical neuron DNA methylation profile in autism spectrum disorder. *Cereb Cortex.* (2017) 27:5739–54. doi: 10.1093/cercor/bhx250

219. Spiers H, Hannon E, Schalkwyk LC, Smith R, Wong CC, O'Donovan MC, et al. Methylomic trajectories across human fetal brain development. *Genome Res.* (2015) 25:338–52. doi: 10.1101/gr.180273.114

220. Gui A, Jones EJH, Wong CCY, Meaburn E, Xia B, Pasco G, et al. Leveraging epigenetics to examine differences in developmental trajectories of social attention: a proof-of-principle study of DNA methylation in infants with older siblings with autism. *Infant Behav Dev.* (2020) 60:101409. doi: 10.1016/j.infbeh.2019.101409

221. Jangjoo M, Goodman SJ, Choufani S, Trost B, Scherer SW, Kelley E, et al. An epigenetically distinct subset of children with autism spectrum disorder resulting from differences in blood cell composition. *Front Neurol.* (2021) 12:612817. doi: 10.3389/fneur.2021.612817

222. Berko ER, Suzuki M, Beren F, Lemetre C, Alaimo CM, Calder RB, et al. Mosaic epigenetic dysregulation of ectodermal cells in autism spectrum disorder. *PLoS Genet.* (2014) 10:e1004402. doi: 10.1371/journal.pgen.1004402

223. Aspra Q, Cabrera-Mendoza B, Morales-Marín ME, Márquez C, Chicalote C, Ballesteros A, et al. Epigenome-wide analysis reveals DNA methylation alteration in *ZFP57* and its target *RASGFR2* in a Mexican population cohort with autism. *Children.* (2022) 9:462. doi: 10.3390/children9040462

224. Ladd-Acosta C, Hansen KD, Briem E, Fallin MD, Kaufmann WE, Feinberg AP. Common DNA methylation alterations in multiple brain regions in autism. *Mol Psychiatry.* (2014) 19:862–71. doi: 10.1038/mp.2013.114

225. Nardone S, Sams DS, Reuveni E, Getselter D, Oron O, Karpuj M, et al. DNA methylation analysis of the autistic brain reveals multiple dysregulated biological pathways. *Transl Psychiatry.* (2014) 4:e433. doi: 10.1038/tp.2014.70

226. Corley MJ, Vargas-Maya N, Pang APS, Lum-Jones A, Li D, Khadka V, et al. Epigenetic delay in the neurodevelopmental trajectory of DNA methylation states in autism spectrum disorders. *Front Genet.* (2019) 10:907. doi: 10.3389/fgene.2019.00907

227. Bahado-Singh RO, Vishweswaraiah S, Aydas B, Radhakrishna U. Placental DNmethylation changes and the early prediction of autism in full-term newborns. *PLoS ONE.* (2021) 16:e0253340. doi: 10.1371/journal.pone.0253340

228. Zhu Y, Gomez JA, Laufer BI, Mordaunt CE, Mouat JS, Soto DC, et al. Placental methylome reveals a 22q13.33 brain regulatory gene locus associated with autism. *Genome Biol.* (2022) 23:46. doi: 10.1186/s13059-022-02613-1

229. Mordaunt CE, Jianu JM, Laufer BI, Zhu Y, Hwang H, Dunaway KW, et al. Cord blood DNA methylome in newborns later diagnosed with autism spectrum disorder reflects early dysregulation of neurodevelopmental and X-linked genes. *Genome Med.* (2020) 12:88. doi: 10.1186/s13073-020-00785-8

230. Schroeder DI, Jayashankar K, Douglas KC, Thirkill TL, York D, Dickinson PJ, et al. Early developmental and evolutionary origins of gene body DNA methylation patterns in mammalian placentas. *PLoS Genet.* (2015) 11:e1005442. doi: 10.1371/journal.pgen.1005442

231. Schroeder DI, Schmidt RJ, Crary-Dooley FK, Walker CK, Ozonoff S, Tancredi DJ, et al. Placental methylome analysis from a prospective autism study. *Mol Autism.* (2016) 7:51. doi: 10.1186/s13229-016-0114-8

232. Ghafouri-Fard S, Noroozi R, Brand S, Hussen BM, Eghtedarian R, Taheri M, et al. Emerging role of non-coding RNAs in autism spectrum disorder. *J Mol Neurosci.* (2022) 72:201–16. doi: 10.1007/s12031-021-01934-3

233. Wang Y, Zhao X, Ju W, Flory M, Zhong J, Jiang S, et al. Genome-wide differential expression of synaptic long noncoding RNAs in autism spectrum disorder. *Transl Psychiatry.* (2015) 5:e660. doi: 10.1038/tp.2015.144

234. Fallah H, Sayad A, Ranjbaran F, Talebian F, Ghafouri-Fard S, Taheri M. IFNG/IFNG-AS1 expression level balance: implications for autism spectrum disorder. *Metab Brain Dis.* (2020) 35:327–33. doi: 10.1007/s11011-019-00510-4

235. DeWitt JJ, Grepo N, Wilkinson B, Evgrafov OV, Knowles JA, Campbell DB. Impact of the autism-associated long noncoding RNA MSNP1AS on neuronal architecture and gene expression in human neural progenitor cells. *Genes.* (2016) 7:76. doi: 10.3390/genes7100076

236. Kerin T, Ramanathan A, Rivas K, Grepo N, Coetzee GA, Campbell DB. A noncoding RNA antisense to moesin at 5p14.1 in autism. *Sci Transl Med.* (2012) 4:128ra40. doi: 10.1126/scitranslmed.3003479

237. Bilinovich SM, Lewis K, Grepo N, Campbell DB. The long noncoding RNA RPS10P2-AS1 is implicated in autism spectrum disorder risk and modulates gene expression in human neuronal progenitor cells. *Front Genet.* (2019) 10:970. doi: 10.3389/fgene.2019.00970

238. Parikshak NN, Swarup V, Belgard TG, Irimia M, Ramaswami G, Gandal MJ, et al. Genome-wide changes in lncRNA, splicing, and regional gene expression patterns in autism. *Nature.* (2016) 540:423–7. doi: 10.1038/nature20612

239. Taheri M, Honarmand Tamizkar K, Omrani S, Arsang-Jang S, Ghafouri-Fard S, Omrani MD. MEG3 lncRNA is over-expressed in autism spectrum disorder. *Metab Brain Dis.* (2021) 36:2235–42. doi: 10.1007/s11011-021-00764-x

240. Sayad A, Omrani MD, Fallah H, Taheri M, Ghafouri-Fard S. Aberrant expression of long non-coding RNAs in peripheral blood of autistic patients. *J Mol Neurosci.* (2019) 67:276–81. doi: 10.1007/s12031-018-1240-x

241. Luo T, Liu P, Wang XY, Li LZ, Zhao LP, Huang J, et al. Effect of the autism-associated lncRNA Shank2-AS on architecture and growth of neurons. *J Cell Biochem.* (2018) 120:1754–62. doi: 10.1002/jcb.27471

242. Frye RE, Rose S, McCullough S, Bennuri SC, Porter-Gill PA, Dweep H, et al. MicroRNA expression profiles in autism spectrum disorder: role for miR-181 in immunomodulation. *J Pers Med.* (2021) 11:922. doi: 10.3390/jpm11090922

243. Sun W, Poschmann J, Cruz-Herrera Del Rosario R, Parikshak NN, Hajan HS, Kumar V, et al. Histone acetylome-wide association study of autism spectrum disorder. *Cell.* (2016) 167:1385–97.e11. doi: 10.1016/j.cell.2016.10.031

244. Tordjman S, Somogyi E, Coulon N, Kermarrec S, Cohen D, Bronsard G, et al. Gene × Environment interactions in autism spectrum disorders: role of epigenetic mechanisms. *Front Psychiatry.* (2014) 5:53. doi: 10.3389/fpsyt.2014.00053

245. Ahmad SF, Nadeem A, Ansari MA, Bakheet SA, Attia SM, Zoheir KM, et al. Imbalance between the anti- and pro-inflammatory milieu in blood leukocytes of autistic children. *Mol Immunol.* (2017) 82:57–65. doi: 10.1016/j.molimm.2016.12.019

246. Ebrahimi Meimand S, Rostam-Abadi Y, Rezaei N. Autism spectrum disorders and natural killer cells: a review on pathogenesis and treatment. *Expert Rev Clin Immunol.* (2021) 17:27–35. doi: 10.1080/1744666X.2020.1850273

247. Yang S, Liu ZW, Wen L, Qiao HF, Zhou WX, Zhang YX. Interleukin-1beta enhances NMDA receptor-mediated current but inhibits excitatory synaptic transmission. *Brain Res.* (2005) 1034:172–9. doi: 10.1016/j.brainres.2004.11.018

248. Careaga M, Rogers S, Hansen RL, Amaral DG, Van de Water J, Ashwood P. Immune endophenotypes in children with autism spectrum disorder. *Biol Psychiatry.* (2017) 81:434–41. doi: 10.1016/j.biopsych.2015.08.036

249. Bilbo SD, Block CL, Bolton JL, Hanamsagar R, Tran PK. Beyond infection - maternal immune activation by environmental factors, microglial development, and relevance for autism spectrum disorders. *Exp Neurol.* (2018) 299 (Pt. A):241–51. doi: 10.1016/j.expneurol.2017.07.002

Glossary

ADA, adenosine deaminase; AKT2, AKT serine/threonine kinase 2; AKT3, AKT serine/threonine kinase 3; ApaI, abbreviation of a *VDR* gene polymorphism; ASD, autism spectrum disorder; BAFF, B-cell activating factor; BsmI, abbreviation of a *VDR* gene polymorphism; CAMK2A, calcium/calmodulin dependent protein kinase II alpha; CAMK2B, calcium/calmodulin dependent protein kinase II beta; CCR3, C-C motif chemokine receptor 3; CD, cluster of differentiation; Cj1137c, glycosyltransferase; COX, cyclooxygenases; CSF, cerebrospinal fluid; CSTB, Cystatin B; CXCR, C-X-C motif chemokine receptor; CYB, cytochrome B; DETs, differentially expressed transcripts; DNA, deoxyribonucleic acid; DNAm, DNA methylation; DRP1, dynamin 1 like; EGFR, epidermal growth factor receptor; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; FIS1, fission, mitochondrial 1; FokI, abbreviation of a *VDR* gene polymorphism; FRα, folate receptor alpha; GBS, group B streptococcus; GI, gastrointestinal system; GM-CSF, granulocyte-macrophage colony-stimulating factor; GSTA1, glutathione S-transferase alpha 1; GSTM1, glutathione S-transferase mu 1; GSTP1, glutathione S-transferase pi 1; GSTs, glutathione transferases; GSTT1, glutathione S-transferase theta 1; H2O2, hydrogen peroxide; HIGD2A, HIG1 hypoxia inducible domain family member 2A; HLA, human leukocyte antigen; HLA-A, major histocompatibility complex, class I, A; HLA-DRB1, HLA class II histocompatibility antigen, DRB1 beta chain; HOCl, hypochlorous acid; HSP70i, inducible heat shock protein 70; IFN, interferon; Ig, immunoglobulins; IL, interleukin; iNOS, inducible nitric oxide synthase; JAK1, Janus kinase 1; kfiC, glycosyltransferase; LCL, lymphoblastoid cell line; lncRNA, long non-coding ribonucleic acid; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MCP, macrophage chemoattractant protein; MFN1, mitofusin 1; MFN2, mitofusin 2; MHC, major histocompatibility complex; miRNA, micro ribonucleic acid; MPO, myeloperoxidase; MRC1, mannose receptor C-type 1; mRNA, messenger ribonucleic acid; mTOR, mammalian target of rapamycin kinase; NADH, the reduced form of nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; ND4L, NADH:ubiquinone oxidoreductase core subunit 4L; NDDs, neurodevelopmental disorders; NF1, neurofibromin 1; NK, natural killer; NO, nitric oxide; NOSs, NO synthases; NOX, nicotinamide adenine dinucleotide phosphate oxidase; NR4A1, nuclear receptor subfamily 4 group A member 1; OPA1, OPA1 mitochondrial dynamin like GTPase; PBMCs, peripheral blood mononuclear cells; PRKAA2, protein kinase AMP-activated catalytic subunit alpha 2; PTEN, phosphatase and tensin homolog; RNA, ribonucleic acid; RNAseq, RNA sequencing; RNS, reactive nitrogen species; ROS, reactive oxygen species; S6K1, ribosomal protein S6 kinase beta-1; SCID, severe combined human immune deficiency; SNVs, single nucleotide variants; SOD, superoxide dismutase; STAT, signal transducer and activator of transcription; TaqI, abbreviation of an *VDR* gene polymorphism; TCR, T cell receptor; TGF, transforming growth factor; Th, helper cells; THRIL, TNF and HNRNPL related immunoregulatory long non-coding RNA; TLR4, toll like receptor 4; TNF, tumor necrosis factor; Tnfsf13b, TNF superfamily member 13b gene; mTORC1, mechanistic target of rapamycin complex 1; TSC1, TSC complex subunit 1; TSC2, TSC complex subunit 2; UCP2, uncoupling protein 2; UQCRC1, ubiquinol-cytochrome c reductase core protein 1; VDR, vitamin D receptor; VFGM, virulence factor-related gut microbiota; wlaN, beta-1,3 galactosyltransferase; ZNF322, zinc finger protein 322.

frontiers | Frontiers in Psychiatry

TYPE Original Research
PUBLISHED 02 November 2022
DOI 10.3389/fpsyt.2022.1037503

OPEN ACCESS

EDITED BY
Martina Micai,
National Institute of Health (ISS), Italy

REVIEWED BY
Zhaoxia Zhang,
Chongqing Medical University, China
Liu Zhen Min,
Chongqing Medical University, China
Mang Sun,
Second Affiliated Hospital of Chongqing Medical University, China
Xudong Huang,
Massachusetts General Hospital and Harvard Medical School, United States

*CORRESPONDENCE
Yu Zhou
zhouy178@stu.cqmu.edu.cn
Jing Gao
jinggao@yzu.edu.cn

SPECIALTY SECTION
This article was submitted to Autism, a section of the journal Frontiers in Psychiatry

RECEIVED 06 September 2022
ACCEPTED 17 October 2022
PUBLISHED 02 November 2022

CITATION
Zhou Y and Gao J (2022) Why not try to predict autism spectrum disorder with crucial biomarkers in cuproptosis signaling pathway?
Front. Psychiatry 13:1037503.
doi: 10.3389/fpsyt.2022.1037503

Why not try to predict autism spectrum disorder with crucial biomarkers in cuproptosis signaling pathway?

Yu Zhou[1,2]* and Jing Gao[1,2]*

[1]Department of Child Rehabilitation Division, Huai'an Maternal and Child Health Care Center, Huai'an, China, [2]Affiliated Hospital of Yang Zhou University Medical College, Huai'an Maternal and Child Health Care Center, Huai'an, China

The exact pathogenesis of autism spectrum disorder (ASD) is still unclear, yet some potential mechanisms may not have been evaluated before. Cuproptosis is a novel form of regulated cell death reported this year, and no study has reported the relationship between ASD and cuproptosis. This study aimed to identify ASD in suspected patients early using machine learning models based on biomarkers of the cuproptosis pathway. We collected gene expression profiles from brain samples from ASD model mice and blood samples from humans with ASD, selected crucial genes in the cuproptosis signaling pathway, and then analysed these genes with different machine learning models. The accuracy, sensitivity, specificity, and areas under the receiver operating characteristic curves of the machine learning models were estimated in the training, internal validation, and external validation cohorts. Differences between models were determined with Bonferroni's test. The results of screening with the Boruta algorithm showed that FDX1, DLAT, LIAS, and ATP7B were crucial genes in the cuproptosis signaling pathway for ASD. All selected genes and corresponding proteins were also expressed in the human brain. The k-nearest neighbor, support vector machine and random forest models could identify approximately 72% of patients with ASD. The artificial neural network (ANN) model was the most suitable for the present data because the accuracy, sensitivity, and specificity were 0.90, 1.00, and 0.80, respectively, in the external validation cohort. Thus, we first report the prediction of ASD in suspected patients with machine learning methods based on crucial biomarkers in the cuproptosis signaling pathway, and these findings may contribute to investigations of the potential pathogenesis and early identification of ASD.

KEYWORDS

cuproptosis, autism spectrum disorder, biomarkers, machine learning, artificial neural network

Introduction

Autism spectrum disorder (ASD) is defined as a group of neurodevelopmental psychiatric disorders characterized by deficits in social interactions, interpersonal communications, and repetitive and stereotyped behaviors and can accompany other disorders, such as intellectual and language disorders (1). Although ASD can be diagnosed as early as 18–24 months of age, a significant proportion of children are not identified until the school years (2, 3). Early identification of ASD in children could improve developmental outcomes and quality of life through early intervention.

The genetic influence of autism is complex and possibly related to environmental factors (4). ASD has been found to be associated with many physiological abnormalities, including reactive oxygen species (ROS), mitochondrial dysfunction, intracellular calcium ion level regulation and even the gut microbiota (5–7). However, there is no established biomarker for ASD diagnosis. Thus, in the past, some physiological processes and biomarkers for ASD and diagnosis may have been ignored.

A recent study published in *Science* by Tsvetkov et al. showed that intracellular copper (Cu) induced a novel form of cell death (8), named cuproptosis. Cuproptosis is mainly regulated by ferredoxin 1 (FDX1)-mediated mitochondrial proteotoxic stress. The authors indicated that FDX1 could reduce Cu^{2+} to Cu^{+} and promote the lipoylation and aberrant oligomerization of DLAT, which is involved in the regulation of the mitochondrial tricarboxylic acid cycle. Glutathione (GSH) blocks cuproptosis by chelating intracellular Cu. In addition, lipoic acid synthetase (LIAS) decreases cell sensitivity to cuproptosis by blocking the lipoylation of proteins. Solute carrier family 31 member 1 (SLC31A1) and ATPase copper transporting beta (ATP7B) affect cuproptosis sensitivity by regulating the level of intracellular Cu^{+}. However, no study has revealed the relationship between ASD and crucial genes for cuproptosis thus far.

Predicting the incidence of disease has been a challenging task in the past. In recent years, the development of machine learning methods has allowed us to envision a future of improved health care through the investigation of biomedical profiles and patient datasets (9). A recent study showed that the use of machine learning methods in Alzheimer's disease shows promise for the identification of novel molecular characterizations (10), while those methods are not still being investigated in ASD.

Hence, we aimed to investigate some novel biomarkers in the cuproptosis signaling pathway for ASD through the use of machine learning algorithms. To support our goals, we collected gene expression profiles from brain tissue samples from ASD model mice and peripheral blood samples from humans with ASD. Then, we selected crucial genes in the cuproptosis signaling pathway for ASD and verified these features with different machine learning algorithms.

Materials and methods

Data collection

The gene expression data of ASD mouse brain samples were obtained from the Gene Expression Omnibus (GEO) database (GSE72149 and GSE81501). The gene expression data of peripheral blood samples from 20 children with ASD and 20 healthy control children were also obtained (GSE26415). All genes in the expression profiles were annotated as unique gene symbols, and expression values were transformed by log2. Then, expression values were normalized with the "limma" package in R software to achieve consistency and comparability between arrays. The differentially expressed genes (DEGs) were screened by the "limma" package according to a previous study (11). If the p-value was < 0.01 between arrays, the corresponding gene was considered a DEG.

Visualization of crucial genes in the cuproptosis signaling pathway

We selected six crucial genes of cuproptosis regulation reported as candidate biomarkers in a previous study, including FDX1, DLAT, LIAS, GSH, ATP7B, and SLC31A1 (8). Selected genes were visualized in a heatmap created with the "pheatmap" package in R software.

Screened risk factor genes in the cuproptosis signaling pathway

The FDX1, DLAT, LIAS, GSH, ATP7B, and SLC31A1 expression data were evaluated by the Boruta algorithm. The Boruta method, which has shown reasonable reliability for feature selection in many fields, and is considered one of the most powerful algorithms for analyzing large data sets (12–14). This method was built around the random forest classifier to determine the relevance and importance of in relation to the target variables (15). Thus, we used the Boruta algorithm to select risk features in the present study.

We next divided the gene expression data of ASD mice into a training cohort (70%) and an internal validation cohort (30%), and the peripheral blood gene expression profiles of humans with ASD were used as an external validation cohort.

Expression of selected genes and subcellular localization in the human brain

All selected risk genes in the cuproptosis signaling pathway were detected in the Human Protein Atlas database

(Version: 21.1).[1] This database maps all human proteins in cells, tissues, and organs using an integration of various omics technologies, including antibody-based imaging, mass spectrometry-based proteomics, transcriptomics, and systems biology (16). This database has been used in many studies (17–19). The expression levels of four selected genes were measured in different parts of the human brain, and protein expression analysis was used to determine the locations of protein expression in cells.

Verification with different machine learning methods

The risk factor genes in the cuproptosis signaling pathway screened with the Boruta algorithm were verified by five frequently used machine learning methods, including k-nearest neighbor (KNN), naive Bayesian (NB), support vector machine (SVM) with polynomial kernel, random forest (RF), and artificial neural network (ANN). All five machine learning models were trained in the training cohort and verified in the internal validation cohort and external validation cohort.

k-nearest neighbor performs classification by assigning a point to the class that is most prevalent out of the k points closest to it (20). The k parameter was set between 2 and 20 in the present study, and the optimized k value was chosen (usually an odd number). KNN was performed with the "kknn" package in R.

Naive Bayesian is conducted based on Bayes' theorem and finds the probability that an input with some features belongs to a certain class (21). NB was conducted by the "e1071" package in R software.

Support vector machine performs input data as feature vectors and calculates them in a space with the same dimensionality, divides the data points into two categories, and finally selects the optimal hyperplane (22). SVM was performed by the "e1071" package in R software.

Random forest is made up of decision trees with slight differences. RF can classify input data into the most common classifications based on constituent decision trees (23). The optimized number of trees was selected for the next validation, and RF was pruned to combat their tendency to overfit. RF was conducted by the "randomForest" package.

The ANN was made up of several layers of neurons and could loosely mimic the learning method in human brains (24). The number of hidden layers was set to five to six in the present study, and the sigmoid function was used as the standard activation method. ANN was performed with the "neuralnet" package in R software.

1 https://www.proteinatlas.org

Statistical analysis

The true condition was set to ASD or control in different cohorts. The prediction accuracy and its 95% confidence interval (CI) and kappa statistic values were calculated in the training, internal validation, and external validation cohorts for all models. For repeatability, a fixed seed number was set before cross validation. Receiver operating characteristic (ROC) curves were plotted for the internal validation cohort and external validation cohort, and the area under the curve (AUC) was calculated to examine the performance of different machine learning models.

The "resamples" package in R was used to analyse and visualize the performance of each model after cross validation. Differences between paired machine learning methods were determined with Bonferroni's test (25).

Results

Data normalization and visualization

Twenty brain expression datasets of ASD and control mice (10 each) were collected from GSE72149 and GSE81501. Twenty peripheral blood gene expression profiles of children with ASD and 20 age- and sex-matched peripheral blood gene expression profiles of healthy controls were collected from GSE26415. The flowchart of the data analysis is shown in **Figure 1**. As shown in **Figure 2**, the expression data were normalized between the arrays in each dataset.

Visualization of crucial genes in the cuproptosis signaling pathway and the selection of risk features for autism spectrum disorder

The selected expression arrays in each cohort and crucial genes in the cuproptosis signaling pathway were visualized with a heatmap (**Figure 2C**). The results of Boruta analysis showed that FDX1, DLAT, LIAS, and ATP7B were identified as feature genes, and other genes were classified as unimportant feature genes in the present data.

Expression of selected genes and the location of proteins in the human brain

The expression profiles of humans with ASD were obtained from blood; however, whether these risk genes are expressed in the human brain is still unclear. Based on Human Protein Atlas immunofluorescence analysis, FDX1, and DLAT were located in

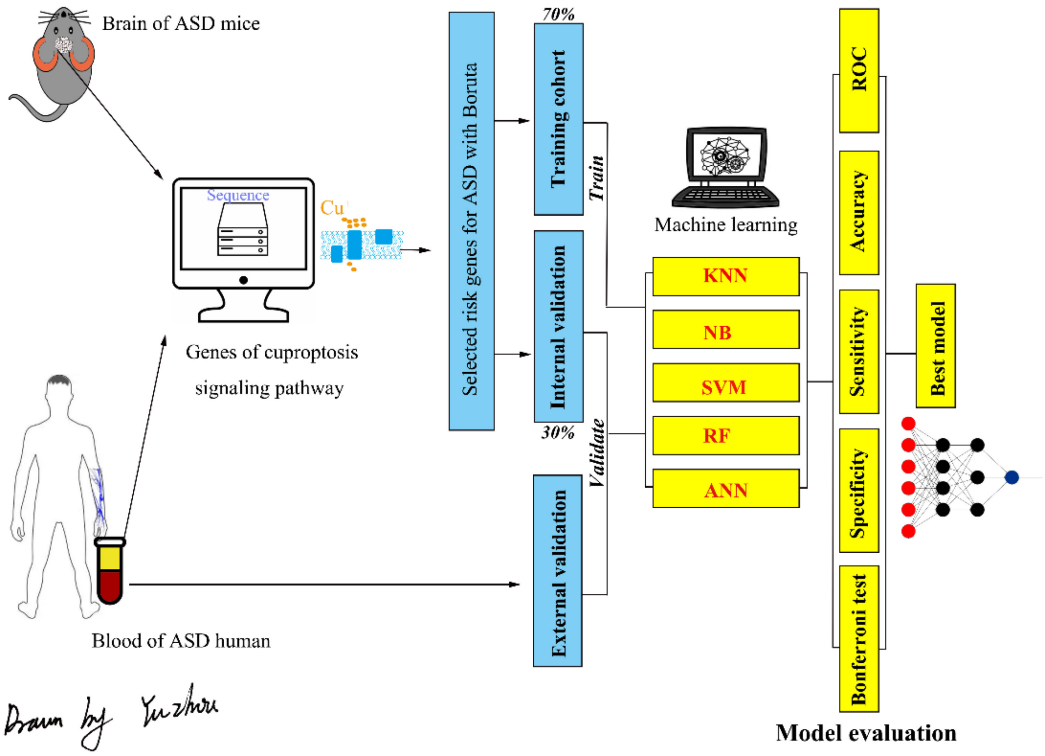


FIGURE 1
The flowchart of the present study. ASD, autism spectrum disorder; KNN, k-nearest neighbor; NB, naive Bayesian; SVM, support vector machine; RF, random forest; ANN, artificial neural network.

mitochondria, ATP7B was expressed in the Golgi apparatus, and LIAS could be detected in mitochondria and the nucleoplasm (Figure 3). In addition, the four selected genes were all expressed in the main parts of the brain. Thus, these four genes could be detected in the brains of mice and both the blood and the brains of humans.

Modeling by k-nearest neighbor

The optimized k value was set as 11 (Figure 4A). In the training cohort, the accuracy was 0.76 (95% CI, 0.60–0.88), and the sensitivity and specificity were 0.80 and 0.72, respectively. In the internal validation cohort, the accuracy was 0.67 (95% CI, 0.51–0.87); the sensitivity and specificity were 0.80 and 0.50, respectively; and the AUC was 0.650 (Table 1 and Figure 5A). The accuracy, sensitivity, and specificity were 0.73 (95% CI, 0.56–0.86), 0.75 and 0.70, respectively (Table 1), and the AUC was 0.725 in the external validation cohort (Figure 5B).

Modeling by naive Bayesian

The results showed that the accuracy of NB was 0.64 (95% CI, 0.50–0.78), the sensitivity was 0.95, and the specificity was 0.36 in the training dataset. The accuracy was 0.56 (95% CI, 0.41–0.78) and 0.55 (95% CI, 0.40–0.70) in the internal validation cohort and external validation cohort, respectively. The sensitivity of the internal validation cohort and external validation cohort was 1.00, but the specificity was zero in the internal validation cohort and only 0.1 in the external validation cohort (Table 1). The AUC values were 0.500 and 0.550 in the internal validation cohort and external validation cohort, respectively (Figures 4D, 5C).

Modeling by support vector machine

The number of support vectors was 25 with the best SVM model in the present study, and the performance of the SVM is shown in Figure 4B. With the best SVM model, accuracy, sensitivity, and specificity were 0.89 (95% CI, 0.74–0.96), 0.85 and 0.91, respectively, in the training cohort (Table 1). In the internal validation cohort, the accuracy, sensitivity, specificity and AUC were 0.68 (95% CI, 0.42–0.87), 0.80, 0.51, and 0.660, respectively (Table 1 and Figure 5E). The accuracy, sensitivity, specificity, and AUC were 0.75 (95% CI, 0.59–0.87), 0.75, 0.75, and 0.750, respectively, in the external validation cohort (Table 1 and Figure 5F).

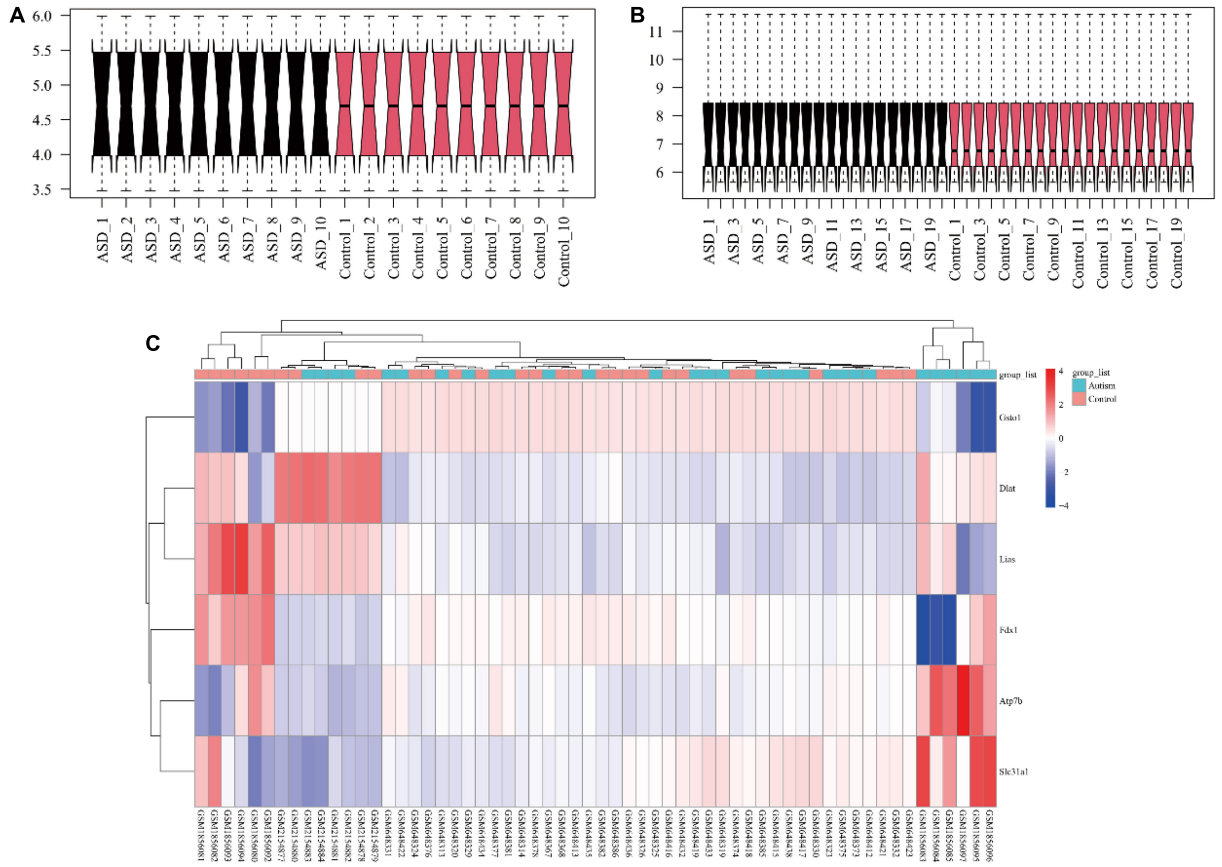


FIGURE 2
The visualization of gene expression profiles. The gene expression data were normalized between arrays of mouse brain **(A)** and human blood **(B)**. The crucial genes in the cuproptosis signaling pathway and the selected arrays of each dataset were visualized in a heatmap **(C)**. ASD, autism spectrum disorder.

Modeling by random forest

The RF was performed with an optimized tree number (Figure 4C). The accuracy, sensitivity and specificity in the training dataset with RF were 0.83 (95% CI, 0.69–0.93), 0.95, and 0.73, respectively. The accuracy, sensitivity, specificity and AUC were 0.72 (95% CI, 0.52–0.90), 0.70, 0.75, and 0.725 in the internal validation cohort, respectively (Table 1 and Figure 5G). In the external validation cohort, the accuracy, sensitivity, specificity and AUC were 0.75 (95% CI, 0.59–0.87), 0.85, 0.65, and 0.750, respectively (Table 1 and Figure 5H).

Modeling by artificial neural network

We first trained the ANN model in the training cohort (Figure 4D). After 43,703 steps, the accuracy was 1.00 (95% CI, 0.92–1.00), and the sensitivity and specificity were 1.00 and 1.00, respectively (Table 1). Then, the parameters of the ANN model that passed in the training cohort were applied in the internal validation cohort and external validation cohort. The results showed that the accuracy, sensitivity, specificity and AUC of the model were 0.78 (95% CI, 0.62–0.94), 1.00, 0.61, and 0.800, respectively, in the internal validation cohort (Table 1 and Figure 5I). In the external validation cohort, the accuracy, sensitivity, specificity, and AUC were 0.90 (95% CI, 0.76–0.97), 1.00, 0.80, and 0.900, respectively (Table 1 and Figure 5J).

Evaluation of different machine learning models and the selection of the most suitable model

We evaluated the different machine learning models with the "resamples" function in R software after cross validation. The 95% CIs of the accuracy and kappa values after cross validation in each model are visualized in Figure 6A. Paired comparisons of the different models showed that the accuracy was significantly different between ANN and NB (Bonferroni's test, $p < 0.05$) (Figure 6B). Although there was no significant

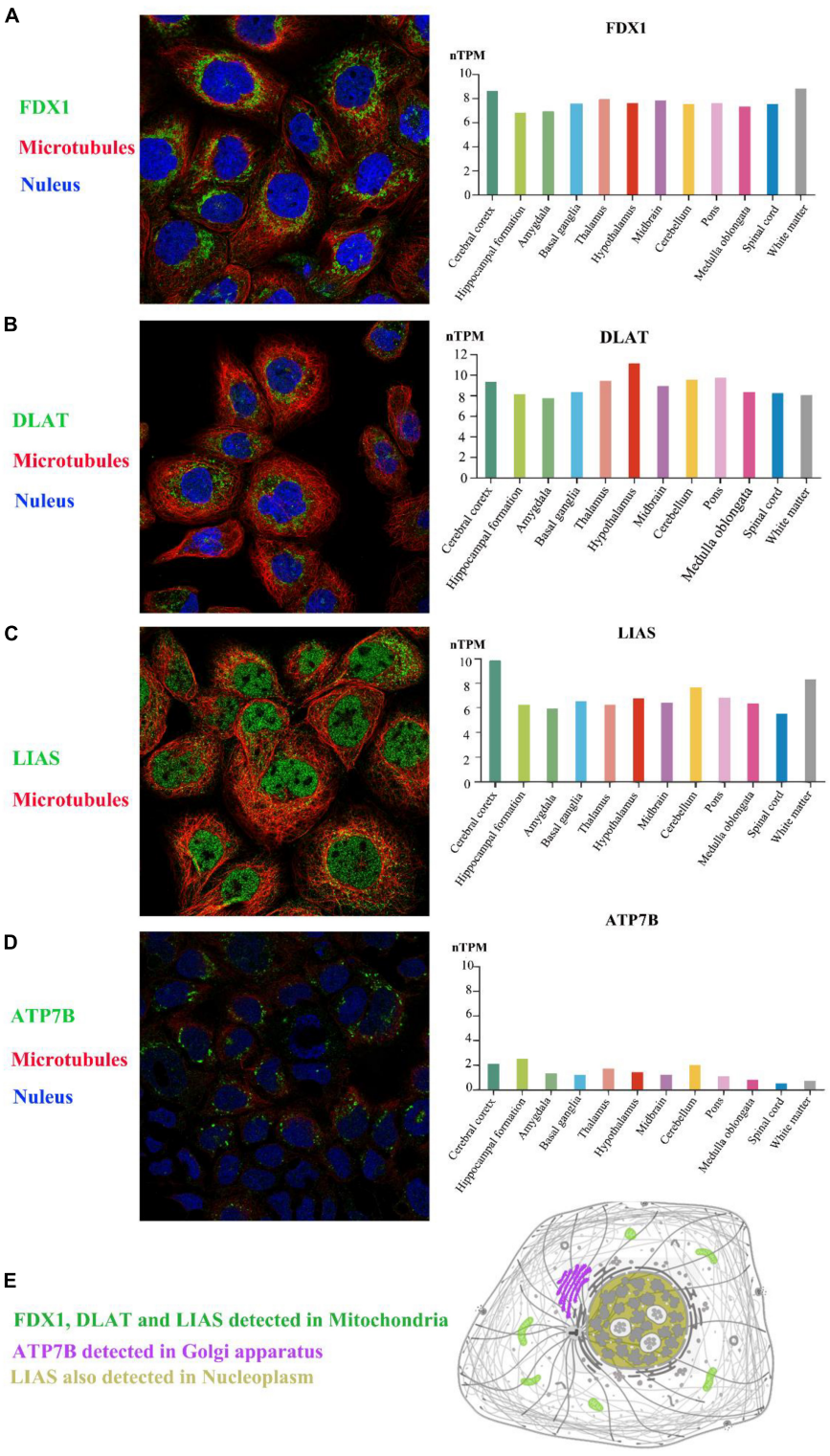


FIGURE 3
The expression of selected genes in the brain. Based on the Human Protein Atlas, FDX1, DLAT, LIAS, and ATP7B could all be detected in 12 brain regions. In the A-431 cell line, FDX1 protein and DLAT protein were located in mitochondria **(A,B)**, and LIAS protein was located in mitochondria and the nucleoplasm **(C)**. ATP7B protein was also expressed in the Golgi apparatus in the CACO-2 cell line **(D)**. The schematic graph shows the main location of each protein in cells **(E)**. The target proteins, nuclei and microtubules were stained green, blue, and red, respectively. nTPM, normalized transcript expression values.

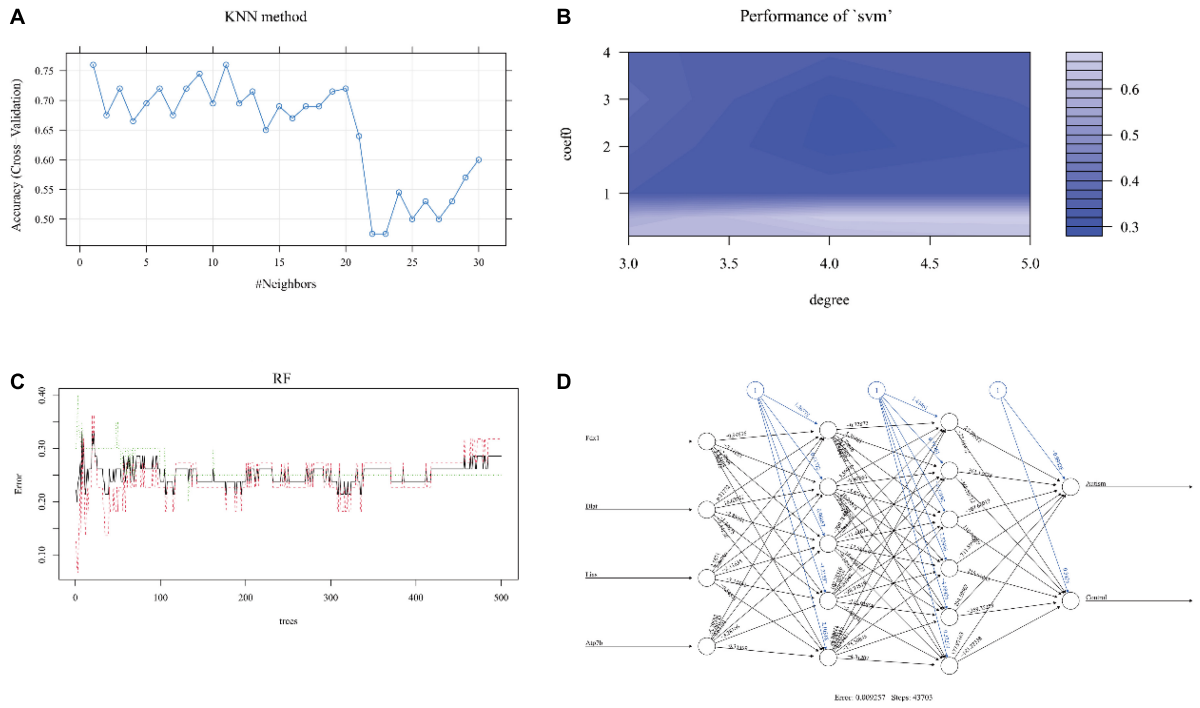


FIGURE 4
The performance of each machine learning model. The relation between the number of neighbors (k value) and accuracy in KNN **(A)**. The degree and coefficient of SVM are shown in panel **(B)**. In RF, the interrelation between the number of trees and model error is shown in panel **(C)**. After 43,703 steps, the error was 0.009257 in the ANN model **(D)**. KNN, k-nearest neighbor; SVM, support vector machine; RF, random forest; ANN, artificial neural network.

difference between any of the other machine learning models ($p > 0.05$), we considered ANN to be the most suitable model for ASD prediction because of the high accuracy, sensitivity, specificity, and AUC, especially in the external validation cohort.

Discussion

The prevalence of ASD has risen from 2 to 4 in 1,000 population to around 1% in large-scale population surveys (26). In clinical practice, we find most children are diagnosed between 2 and 3 years old. Briefly, ASD is much more common than previously believed, yet clinicians are often still confused regarding the early identification of ASD and its pathological mechanisms (27). The selection of novel potential biomarkers is crucial for the early identification and early treatment of children with ASD.

Cuproptosis is a new form of programmed cell death that is unlike apoptosis, pyroptosis, necroptosis, and ferroptosis (28). We selected the expression profiles of crucial genes in the cuproptosis signaling pathway from the brains of ASD mice and the peripheral blood of humans with ASD. The results of screening with the Boruta algorithm indicated that FDX1, DLAT, LIAS, and ATP7B were crucial genes in the cuproptosis signaling pathway for ASD in the present data. The results showed that ANN was the most suitable machine learning model for ASD prediction based on cuproptosis-related genes for the present cohort. This is the first study investigating biomarkers of the cuproptosis signaling pathway for ASD through the use of a machine learning algorithm.

Over the last 3 years, the Boruta algorithm has been used in many fields for feature selection, and it has shown reliability and stability with different evaluation methods (29–31). We also used the Boruta algorithm for the screening of risk genes for ASD in the cuproptosis signaling pathway, and we found that FDX1, DLAT, LIAS, and ATP7B were risk genes. Next, we found that these four selected genes were also expressed in the human brain, mainly in the mitochondria and Golgi apparatus, based on Human Protein Atlas immunofluorescence analysis. Thus, these four risk genes were closely related to brain function and cellular metabolism.

A previous study found that zinc-copper rhythmicity was disrupted in children with ASD (32). However, the previous mechanism could not exactly explain ASD, which may be because the cuproptosis signaling pathway in the cell cycle was reported just this year. FDX1 was found to be involved in copper-dependent cell death and could rescue cells from death by regulating mitochondrial metabolism (33). In this study, the expression of FDX1 was decreased in mice and humans with ASD (**Figure 2**). In addition, we found that FDX1 was expressed

in mitochondria. The abnormal expression of FDX1 in ASD could cause a decrease in the expression of Fe–S cluster proteins and inhibit steroidogenesis (34). Abnormal steroid hormone levels have been found to contribute to the likelihood of autism (35). FDX1 deletion could inhibit DLAT lipoylation (28).

DLAT was another crucial risk gene in the cuproptosis signaling pathway for ASD identified by Boruta analysis in the present study. DLAT was specifically related to depression and anxiety in a chronic mild stress rat model (36). 6-Phosphogluconate dehydrogenase mutation led to reduced RNA and increased ROS by DLAT regulation (37). In addition, copper could induce the accumulation of DLA and activate the mitochondrial tricarboxylic acid cycle (8), which is consistent with our finding in the present study that DLAT is located in mitochondria. Thus, further studies should closely focus on the regulation of FDX1 and DLAT for mitochondrial function in ASD.

Lipoic acid synthetase is a protein target of lipoylation, and LIAS mutation has been described as being related to a defect in mitochondrial energy metabolism (38). In the present study, we found that LIAS expression was increased and was located in both the nucleoplasm and mitochondria. Previous studies found that mutations in LIAS were associated with non-ketotic hyperglycinaemia-like early-onset convulsions and encephalopathy combined with a defect in mitochondrial energy metabolism, and LIAS overexpression inhibited oxidative stress and inflammation (38–40). Therefore, we deduce that the accumulation of LIAS is not only related to Fe-S cluster synthesis and copper circulation but also indicates that oxidative stress levels may be increased in ASD patients.

TABLE 1 Accuracy, sensitivity, and specificity of each machine learning model.

Model types	Cohorts	Accuracy (95% CI)	Sensitivity	Specificity
KNN	Training cohort	0.76 (0.60–0.88)	0.80	0.72
	Internal validation	0.67 (0.51–0.87)	0.80	0.50
	External validation	0.73 (0.56–0.86)	0.75	0.70
NB	Training cohort	0.64 (0.50–0.78)	0.95	0.36
	Internal validation	0.56 (0.41–0.78)	1.00	0.00
	External validation	0.55 (0.40–0.70)	1.00	0.10
SVM	Training cohort	0.89 (0.74–0.96)	0.85	0.91
	Internal validation	0.68 (0.42–0.87)	0.80	0.51
	External validation	0.75 (0.59–0.87)	0.75	0.75
RF	Training cohort	0.83 (0.69–0.93)	0.95	0.73
	Internal validation	0.72 (0.52–0.90)	0.70	0.75
	External validation	0.75 (0.59–0.87)	0.85	0.65
ANN	Training cohort	1.00 (0.92–1.00)	1.00	1.00
	Internal validation	0.78 (0.62–0.94)	1.00	0.61
	External validation	0.90 (0.76–0.97)	1.00	0.80

The brain expression level of ATP7B was lower than that of other crucial genes based on the Human Protein Atlas; ATP7B plays an essential role in human physiology in the brain and liver. The deletion of ATP7B in cells and animals could decrease copper toxicity in Wilson's disease (41). Copper homeostasis has been found to be associated with Alzheimer's disease and Parkinson's disease (42, 43). However, no study has revealed the role of this crucial regulatory gene in the copper concentration in ASD patients, and we hypothesize that ATP7B is another promising target for ASD research.

Although four crucial genes in the cuproptosis signaling pathway were screened, their power to predict ASD in suspected patients still needs to be investigated.

We next employed five machine learning methods for testing. The results showed that the accuracy of KNN, SVM, and RF was approximately 70% and up to 90% with the ANN model in the external validation cohort. Previous studies also show SVM, KNN, and RF have a decent prediction value for ASD (44, 45). While those studies are not verified in external validation cohorts, it is crucial to test the performance of prediction models in external validation cohorts. In the present study, each model was trained in the training cohort and validated in the internal cohort and external cohort.

However, the NB model showed poor overall performance and significantly poorer performance than the ANN model ($p < 0.05$). Some other studies have also found that the performance of NB was poor in comparison to other methods (24, 46). Additionally, NB's poor performance might have been caused by the limited number of samples in the present study. Therefore, the NB method was not suitable for the present study.

The accuracies of KNN, SVM, and RF for ASD prediction did not differ much in the present study. In addition, sensitivity and specificity were also similar in the KNN, SVM, and RF models in the external validation cohort. Thus, KNN, SVM, and RF with selected genes in the cuproptosis signaling pathway have a similar ability to predict ASD in suspected patients.

Artificial neural network (ANN) was identified as the most suitable method for ASD prediction in the present study. For developing the DrugMiner web tool, Jamali et al. found that ANN outperformed NB, KNN, RF, and SVM (47). In addition,

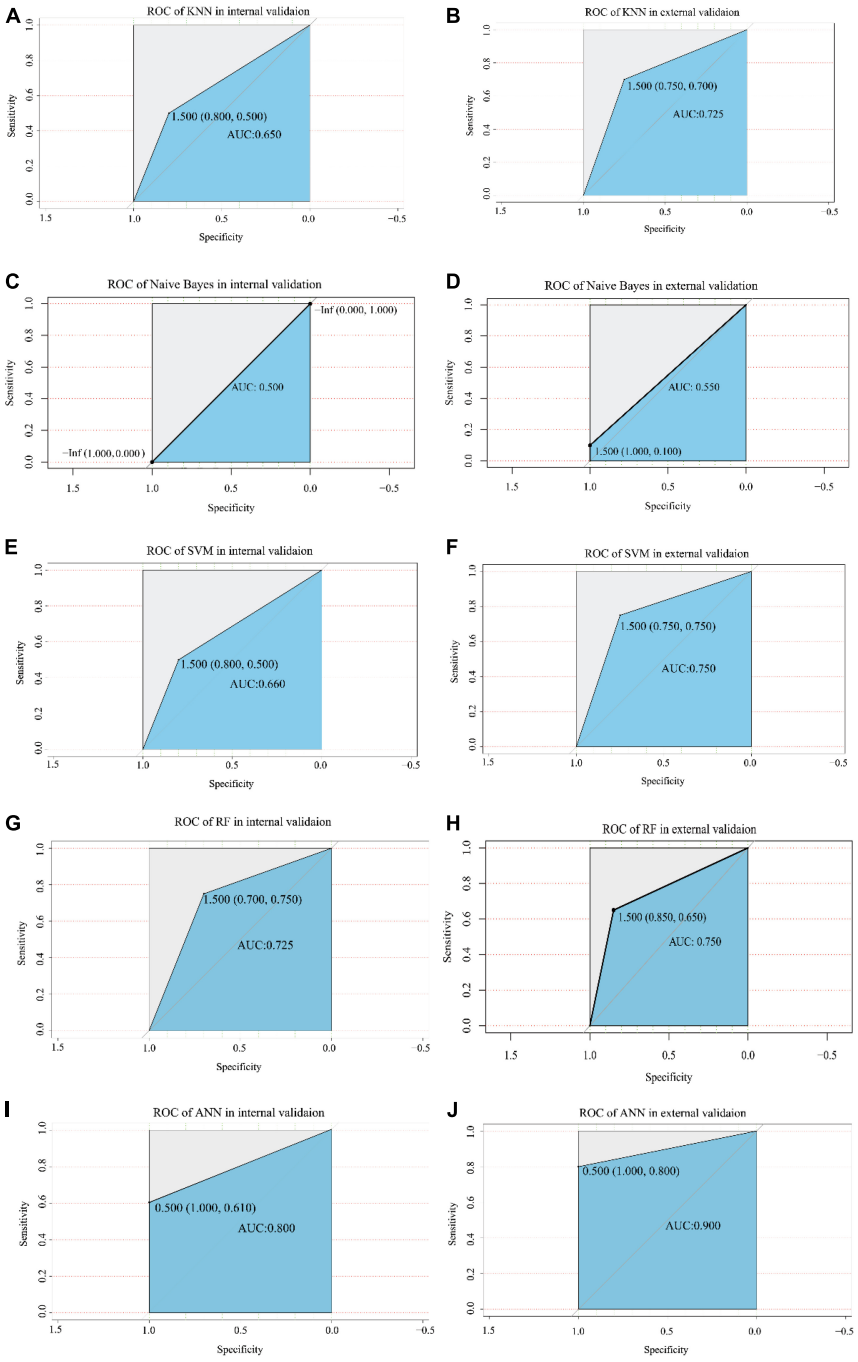


FIGURE 5
The ROC analysis of each model in the internal validation and external validation cohorts. The X-axis and Y-axis represent specificity and sensitivity, respectively. The AUC values are indicated in the blue area, including KNN model **(A,B)**, Naive Bayes model **(C,D)**, SVM model **(E,F)**, RF model **(G,H)**, and ANN model **(I,J)**. The value of the cut-off point is shown at the inflection point. ROC, receiver operating characteristic; AUC, area under the curve; KNN, k-nearest neighbor; SVM, support vector machine; RF, random forest; ANN, artificial neural network.

in reviews of machine learning methods, the authors also indicated that ANNs will be the dominant method in the field of biomedical science (24, 48).

Thus, detecting the expression levels of FDX1, DLAT, LIAS, and ATP7B in blood could predict the risk of ASD with ANN. These four risk factor genes could also be developed as microarrays for clinical examination. Future basic and experimental studies could also investigate the underlying pathophysiological mechanisms of the risk genes for ASD screened in the present study.

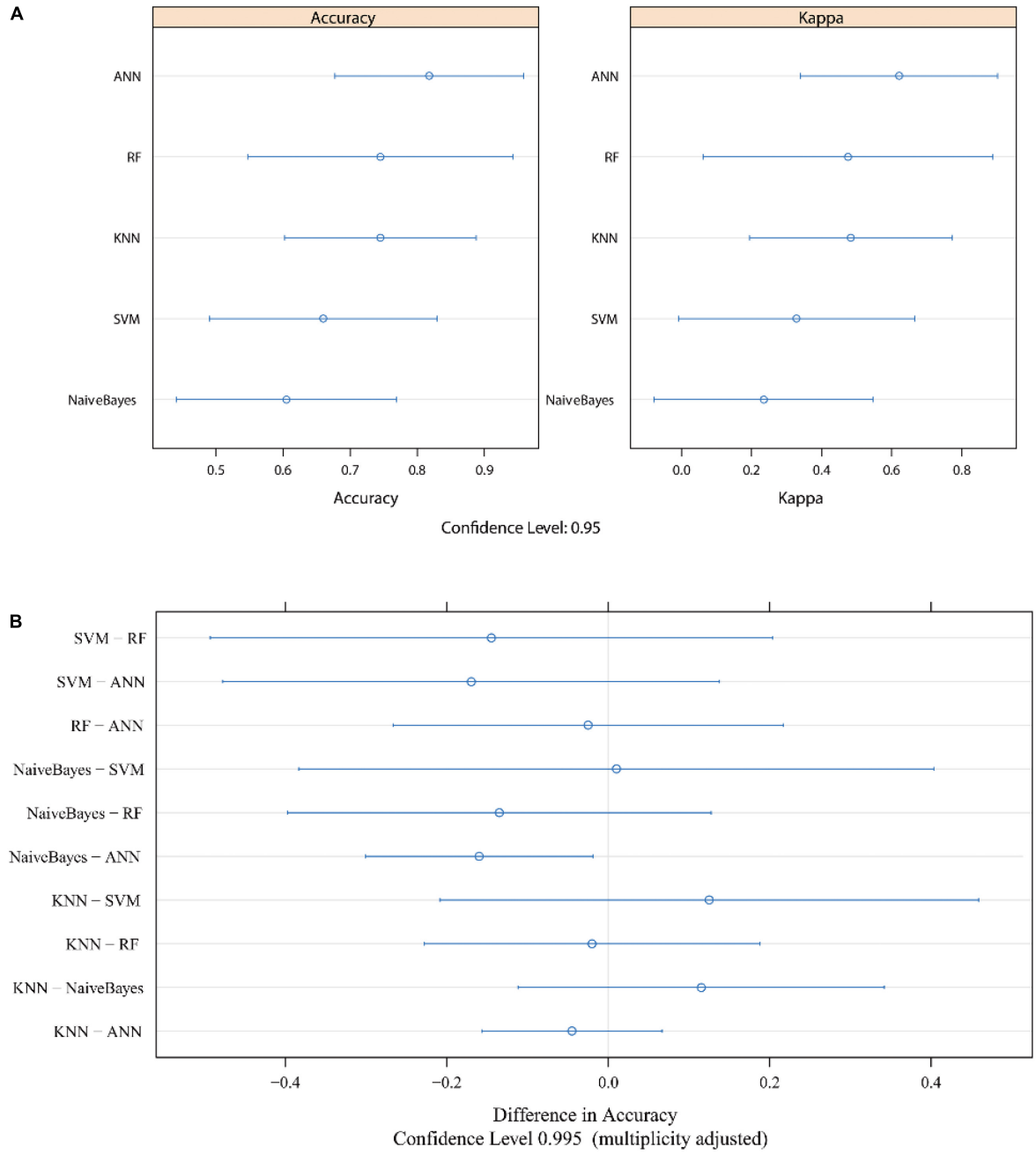


FIGURE 6
The evaluation of each model after cross validation. The accuracy and Kappa value are shown in panel **(A)**. Bonferroni's test results are shown in panel **(B)**. The median numbers are represented by dots, and lines indicate the confidence level. ROC, receiver operating characteristic; AUC, area under the curve; KNN, k-nearest neighbor; SVM, support vector machine; RF, random forest; ANN, artificial neural network.

Furthermore, there are some limitations to the present study. The current study has a limited number of samples. The results need to be validated in a large sample size. Additionally, a prospective cohort study would be needed to detect the conclusions. However, we provided reliable machine learning methods, and four genes in the cuproptosis pathway that may be crucial for identifying mechanisms in autistic children.

Conclusion

In the present study, on the basis of the results of screening with the Boruta algorithm, we selected FDX1, DLAT, LIAS, and ATP7B as crucial genes in the cuproptosis signaling pathway for ASD. The crucial risk genes were expressed in the brains of not only mice but also humans. ANN was the

most suitable model for ASD prediction in the present study. We first reported that biomarkers in the cuproptosis-related signaling pathway had good power to predict ASD in suspected patients through different machine learning methods, which indicated that the cuproptosis signaling pathway may play a crucial role in ASD. The findings of the present study could contribute to the early identification of ASD in children and provide novel inspirations for investigations of the causes and treatments of ASD.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: https://www.ncbi.nlm.nih.gov/gds with the accession numbers GSE72149, GSE81501, and GSE26415.

Author contributions

YZ: conceptualization, methodology, software, data curation, and writing—original draft preparation. JG: visualization, investigation, supervision, software, validation, writing—reviewing and editing, and required funding. Both authors read and approved the manuscript.

Funding

This study was financially supported by The Plan of Innovation Capacity Building and Key Laboratory Construction of the Science and Technology Bureau (Grant No. HAP202104) and the Maternal and Child Health Research Project of the Jiangsu Provincial Health Commission (Grant No. F202062).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Daghsni M, Rima M, Fajloun Z, Ronjat M, Brusés JL, M'rad R, et al. Autism throughout genetics: perusal of the implication of ion channels. *Brain Behav.* (2018) 8:e00978. doi: 10.1002/brb3.978

2. Daniels AM, Halladay AK, Shih A, Elder LM, Dawson G. Approaches to enhancing the early detection of autism spectrum disorders: a systematic review of the literature. *J Am Acad Child Adolesc Psychiatry.* (2014) 53:141–52. doi: 10.1016/j.jaac.2013.11.002

3. Berg KL, Acharya K, Shiu CS, Msall ME. Delayed diagnosis and treatment among children with autism who experience adversity. *J Autism Dev Disord.* (2018) 48:45–54. doi: 10.1007/s10803-017-3294-y

4. Wang YM, Qiu MY, Liu Q, Tang H, Gu HF. Critical role of dysfunctional mitochondria and defective mitophagy in autism spectrum disorders. *Brain Res Bull.* (2021) 168:138–45. doi: 10.1016/j.brainresbull.2020.12.022

5. Castora FJ. Mitochondrial function and abnormalities implicated in the pathogenesis of ASD. *Prog Neuropsychopharmacol Biol Psychiatry.* (2019) 92:83–108. doi: 10.1016/j.pnpbp.2018.12.015

6. Rossignol DA, Frye RE. Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis. *Mol Psychiatry.* (2012) 17:290–314. doi: 10.1038/mp.2010.136

7. Ye D, Tester DJ, Zhou W, Papagiannis J, Ackerman MJ. A pore-localizing CACNA1C-E1115K missense mutation, identified in a patient with idiopathic QT prolongation, bradycardia, and autism spectrum disorder, converts the L-type calcium channel into a hybrid nonselective monovalent cation channel. *Heart Rhythm.* (2019) 16:270–8. doi: 10.1016/j.hrthm.2018.08.030

8. Tsvetkov P, Coy S, Petrova B, Dreishpoon M, Verma A, Abdusamad M, et al. Copper induces cell death by targeting lipoylated TCA cycle proteins. *Science.* (2022) 375:1254–61. doi: 10.1126/science.abf0529

9. Goecks J, Jalili V, Heiser LM, Gray JW. How machine learning will transform biomedicine. *Cell.* (2020) 181:92–101. doi: 10.1016/j.cell.2020.03.022

10. Muraoka S, DeLeo AM, Sethi MK, Yukawa-Takamatsu K, Yang Z, Ko J, et al. Proteomic and biological profiling of extracellular vesicles from Alzheimer's disease human brain tissues. *Alzheimers Dement.* (2020) 16:896–907. doi: 10.1002/alz.12089

11. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res.* (2015) 43:e47. doi: 10.1093/nar/gkv007

12. Guindo ML, Kabir MH, Chen R, Liu F. Potential of Vis-NIR to measure heavy metals in different varieties of organic-fertilizers using Boruta and deep belief network. *Ecotoxicol Environ Saf.* (2021) 228:112996. doi: 10.1016/j.ecoenv.2021.112996

13. Gomes Marques de Freitas A, Almir Cavalcante Minho L, Elizabeth Alves de Magalhães B, Nei Lopes Dos Santos W, Soares Santos L, Augusto de Albuquerque Fernandes S. Infrared spectroscopy combined with random forest to determine tylosin residues in powdered milk. *Food Chem.* (2021) 365:130477. doi: 10.1016/j.foodchem.2021.130477

14. Sun Y, Zhang Q, Yang Q, Yao M, Xu F, Chen W. Screening of gene expression markers for Corona virus disease 2019 through Boruta MCFS feature selection. *Front Public Health.* (2022) 10:901602. doi: 10.3389/fpubh.2022.901602

15. Degenhardt F, Seifert S, Szymczak S. Evaluation of variable selection methods for random forests and omics data sets. Brief. *Bioinformatics.* (2019) 20:492–503. doi: 10.1093/bib/bbx124

16. Colwill K, Gräslund S. A roadmap to generate renewable protein binders to the human proteome. *Nat Methods.* (2011) 8:551–8. doi: 10.1038/nmeth.1607

17. Karlsson M, Zhang C, Méar L, Zhong W, Digre A, Katona B, et al. A single-cell type transcriptomics map of human tissues. *Sci Adv.* (2021) 7:eabh2169. doi: 10.1126/sciadv.abh2169

18. Bergman-Larsson J, Gustafsson S, Méar L, Huvila J, Tolf A, Olovsson M, et al. Combined expression of HOXA11 and CD10 identifies endometriosis versus normal tissue and tumors. *Ann Diagn Pathol.* (2022) 56:151870. doi: 10.1016/j.anndiagpath.2021.151870

19. Pattanaik B, Hammarlund M, Mjörnstedt F, Ulleryd MA, Zhong W, Uhlén M, et al. Polymorphisms in alpha 7 nicotinic acetylcholine receptor gene, CHRNA7, and its partially duplicated gene, CHRFAM7A, associate with increased inflammatory response in human peripheral mononuclear cells. *FASEB J.* (2022) 36:e22271. doi: 10.1096/fj.202101898R

20. Shen M, Xiao Y, Golbraikh A, Gombar VK, Tropsha A. Development and validation of k-nearest-neighbor QSPR models of metabolic stability of drug candidates. *J Med Chem.* (2003) 46:3013–20. doi: 10.1021/jm020491t

21. Jiang M, Ma Y, Guo S, Jin L, Lv L, Han L, et al. Using machine learning technologies in pressure injury management: systematic review. *JMIR Med Inform.* (2021) 9:e25704. doi: 10.2196/25704

22. Winters-Hilt S, Merat S. SVM clustering. *BMC Bioinformatics.* (2007) 8(Suppl. 7):S18. doi: 10.1186/1471-2105-8-S7-S18

23. Wong SL, Zhang LV, Tong AH, Li Z, Goldberg DS, King OD, et al. Combining biological networks to predict genetic interactions. *Proc Natl Acad Sci USA.* (2004) 101:15682–7. doi: 10.1073/pnas.0406614101

24. Carpenter KA, Huang X. Machine learning-based virtual screening and its applications to Alzheimer's drug discovery: a review. *Curr Pharm Des.* (2018) 24:3347–58. doi: 10.2174/1381612824666180607124038

25. Yu H, Wu H, Wang W, Jolly S, Jin JY, Hu C, et al. Machine learning to build and validate a model for radiation pneumonitis prediction in patients with non-small cell lung cancer. *Clin Cancer Res.* (2019) 25:4343–50. doi: 10.1158/1078-0432.CCR-18-1084

26. Zhou H, Xu X, Yan W, Zou X, Wu L, Luo X, 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) 36:961–71. doi: 10.1007/s12264-020-00530-6

27. Mottron L, Bzdok D. Autism spectrum heterogeneity: fact or artifact. *Mol Psychiatry.* (2020) 25:3178–85. doi: 10.1038/s41380-020-0748-y

28. Wang Y, Zhang L, Zhou F. Cuproptosis: a new form of programmed cell death. *Cell Mol Immunol.* (2022) 19:867–8. doi: 10.1038/s41423-022-00866-1

29. Pezzuto F, Lunardi F, Vedovelli L, Fortarezza F, Urso L, Grosso F, et al. P14/ARF-positive malignant pleural mesothelioma: a phenotype with distinct immune microenvironment. *Front Oncol.* (2021) 11:653497. doi: 10.3389/fonc.2021.653497

30. Maeda-Gutiérrez V, Galván-Tejada CE, Cruz M, Galván-Tejada JI, Gamboa-Rosales H, García-Hernández A, et al. Risk-profile and feature selection comparison in diabetic retinopathy. *J Pers Med.* (2021) 11:1327. doi: 10.3390/jpm11121327

31. Lei J, Sun T, Jiang Y, Wu P, Fu J, Zhang T, et al. Risk identification of bronchopulmonary dysplasia in premature infants based on machine learning. *Front Pediatr.* (2021) 9:719352. doi: 10.3389/fped.2021.719352

32. Curtin P, Austin C, Curtin A, Gennings C, Arora M, Tammimies K, et al. Dynamical features in fetal and postnatal zinc-copper metabolic cycles predict the emergence of autism spectrum disorder. *Sci Adv.* (2018) 4:eaat1293. doi: 10.1126/sciadv.aat1293

33. Tsvetkov P, Detappe A, Cai K, Keys HR, Brune Z, Ying W, et al. Mitochondrial metabolism promotes adaptation to proteotoxic stress. *Nat Chem Biol.* (2019) 15:681–9. doi: 10.1038/s41589-019-0291-9

34. Palandri A, L'hôte D, Cohen-Tannoudji J, Tricoire H, Monnier V. Frataxin inactivation leads to steroid deficiency in flies and human ovarian cells. *Hum Mol Genet.* (2015) 24:2615–26. doi: 10.1093/hmg/ddv024

35. Baron-Cohen S, Tsompanidis A, Auyeung B, Nørgaard-Pedersen B, Hougaard DM, Abdallah M, et al. Foetal oestrogens and autism. *Mol Psychiatry.* (2020) 25:2970–8. doi: 10.1038/s41380-019-0454-9

36. Tang M, Huang H, Li S, Zhou M, Liu Z, Huang R, et al. Hippocampal proteomic changes of susceptibility and resilience to depression or anxiety in a rat model of chronic mild stress. *Transl Psychiatry.* (2019) 9:260.

37. Shan C, Elf S, Ji Q, Kang HB, Zhou L, Hitosugi T, et al. Lysine acetylation activates 6-phosphogluconate dehydrogenase to promote tumor growth. *Mol Cell.* (2014) 55:552–65. doi: 10.1016/j.molcel.2014.06.020

38. Habarou F, Hamel Y, Haack TB, Feichtinger RG, Lebigot E, Marquardt I, et al. Biallelic mutations in LIPT2 cause a mitochondrial lipoylation defect associated with severe neonatal encephalopathy. *Am J Hum Genet.* (2017) 101:283–90. doi: 10.1016/j.ajhg.2017.07.001

39. Baker PRII, Friederich MW, Swanson MA, Shaikh T, Bhattacharya K, Scharer GH, et al. Variant non ketotic hyperglycinemia is caused by mutations in LIAS, BOLA3 and the novel gene GLRX5. *Brain.* (2014) 137(Pt. 2):366–79. doi: 10.1093/brain/awt328

40. Zhao Y, Xu G, Li H, Chang M, Guan Y, Li Y, et al. Overexpression of endogenous lipoic acid synthase attenuates pulmonary fibrosis induced by crystalline silica in mice. *Toxicol Lett.* (2020) 323:57–66. doi: 10.1016/j.toxlet.2020.01.023

41. Polishchuk EV, Merolla A, Lichtmannegger J, Romano A, Indrieri A, Ilyechova EY, et al. Activation of autophagy, observed in liver tissues from patients with Wilson disease and from ATP7B-deficient animals, protects hepatocytes from copper-induced apoptosis. *Gastroenterology.* (2019) 156:1173.e–89.e. doi: 10.1053/j.gastro.2018.11.032

42. Squitti R, Ventriglia M, Simonelli I, Bonvicini C, Costa A, Perini G, et al. Copper imbalance in Alzheimer's disease: meta-analysis of serum, plasma, and brain specimens, and replication study evaluating ATP7B gene variants. *Biomolecules.* (2021) 11:790. doi: 10.3390/biom11070960

43. Montes S, Rivera-Mancia S, Diaz-Ruiz A, Tristan-Lopez L, Rios C. Copper and copper proteins in Parkinson's disease. *Oxid Med Cell Longev.* (2014) 2014:147251. doi: 10.1155/2014/147251

44. Oh DH, Kim IB, Kim SH, Ahn DH. Predicting autism spectrum disorder using blood-based gene expression signatures and machine learning. *Clin Psychopharmacol Neurosci.* (2017) 15:47–52. doi: 10.9758/cpn.2017.15.1.47

45. Lin PI, Moni MA, Gau SS, Eapen V. Identifying subgroups of patients with autism by gene expression profiles using machine learning algorithms. *Front Psychiatry.* (2021) 12:637022. doi: 10.3389/fpsyt.2021.637022

46. Lenselink EB, Ten Dijke N, Bongers B, Papadatos G, van Vlijmen HWT, Kowalczyk W, et al. Beyond the hype: deep neural networks outperform established methods using a ChEMBL bioactivity benchmark set. *J Cheminform.* (2017) 9:45. doi: 10.1186/s13321-017-0232-0

47. Jamali AA, Ferdousi R, Razzaghi S, Li J, Safdari R, Ebrahimie E. DrugMiner: comparative analysis of machine learning algorithms for prediction of potential druggable proteins. *Drug Discov Today.* (2016) 21:718–24. doi: 10.1016/j.drudis.2016.01.007

48. Albaradei S, Thafar M, Alsaedi A, Van Neste C, Gojobori T, Essack M, et al. Machine learning and deep learning methods that use omics data for metastasis prediction. *Comput Struct Biotechnol J.* (2021) 19:5008–18. doi: 10.1016/j.csbj.2021.09.001

frontiers | Frontiers in Psychiatry

TYPE Original Research
PUBLISHED 08 March 2023
DOI 10.3389/fpsyt.2023.1088727

OPEN ACCESS

EDITED BY
Valentina Riva,
Eugenio Medea (IRCCS), Italy

REVIEWED BY
Aldina Venerosi,
National Institute of Health (ISS), Italy
Martina Micai,
National Institute of Health (ISS), Italy

*CORRESPONDENCE
Guillermo Benito-Ruiz
✉ guillermo.benito@autismo.org.es

SPECIALTY SECTION
This article was submitted to Autism, a section of the journal Frontiers in Psychiatry

RECEIVED 03 November 2022
ACCEPTED 14 February 2023
PUBLISHED 08 March 2023

CITATION
Vidriales-Fernández R, Plaza-Sanz M, Hernández-Layna C, Verde-Cagiao M, Benito-Ruiz G and Carvajal-Molina F (2023) Characterizing the physical and mental health profile of children, adolescents and adults with autism spectrum disorder in Spain. *Front. Psychiatry* 14:1088727. doi: 10.3389/fpsyt.2023.1088727

Characterizing the physical and mental health profile of children, adolescents and adults with autism spectrum disorder in Spain

Ruth Vidriales-Fernández[1], Marta Plaza-Sanz[1], Cristina Hernández-Layna[1], María Verde-Cagiao[1], Guillermo Benito-Ruiz[1,2]* and Fernando Carvajal-Molina[3]

[1]Confederación Autismo España, Madrid, Spain, [2]Facultad de Ciencias de la Salud, Universidad Isabel I, Burgos, Spain, [3]Facultad de Psicología, Departamento de Psicología Biológica y de la Salud, Universidad Autónoma de Madrid, Madrid, Spain

Introduction: Autistic men and women are more likely to experience health issues than the general population, although the available epidemiological studies addressing co-occurrence conditions are limited. This is the first Spanish epidemiologic study addressing the health profile and poor-health exacerbating factors in individuals of all ages with autism spectrum disorder (ASD).

Methods: We analyzed 2,629 registries extracted from Autism Spain's sociodemographic registry (November 2017–May 2020). A descriptive health data analysis was conducted to assess the prevalence of other conditions associated to ASD in the Spanish population. Nervous system disorders (12.9%), mental health diagnoses (17.8%), and other comorbidities (25.4%) were reported. Men-to-women ratio was 4:1.

Results: Women, elder individuals and those with intellectual disability (ID) were at an increased risk of health comorbidities and psychopharmacological exposure. Women were also more prone to severe intellectual and functional impairment. Nearly all individuals had difficulties in their adaptative functioning, especially those with ID (50% of the population). Almost half of the sample received psychopharmacological treatments starting from infancy and early childhood, mostly antipsychotics and anticonvulsants.

Discussion: This study represents an important first approach to the health status of autistic people in Spain and can contribute to the development of public policies and innovative health strategies.

KEYWORDS

autism spectrum disorder, health conditions, epidemiological factors, mental health, psychopharmacological treatment, Spain

1. Introduction

Autism spectrum disorders (ASD) encompass several neurodevelopmental chronic conditions with early childhood onset that may be, or not, accompanied with an intellectual disability (ID) or language impairment (1, 2). The prevalence of ASD is currently estimated at 1%, although that estimation is variable, reflecting complex and dynamic

interactions between patterns of community awareness, service capacity, help seeking, and sociodemographic factors (3–7). In absolute terms, ASD affects 28.3 million people worldwide, and it is three to four times more prevalent in men than women (8). In Spain, rough estimates point to the existence of approximately half a million people of all ages with ASD (9, 10), but there is no official statistical data available.

Currently, available and official population databases in Spain consider only broad health categories such as developmental (11, 12) or mental disorders (13, 14), thereby providing highly inaccurate or outdated information related to autism. Other sources, like the last national health survey, reported an estimated prevalence of autism or ASD (0.6%) for the first time, though considering only children aged 3–14 and no further health data (15).

Cognitive and behavioral symptoms of ASD have a severe and life-long impact on the quality of life (QoL) and personal outcomes of people living with this condition (16); World Health Organization (2). Moreover, compared to the general population, premature mortality is at increased risk among autistic people due to their health comorbidities and other accidental factors (17–20). In this regard, more than 70% of people on the autism spectrum have some kind of neurological, gastrointestinal or immune co-occurring disorder, among others (21–23), and they are at significant risk of stroke, seizure and chronic diseases like obesity, diabetes, dyslipidemia, hypertension, coronary heart disease, and cancer (24, 25).

People with ASD are also more prone to mental health issues than neurotypicals (24, 26). A cohort-based study found that 70% of children and adolescents on the autism spectrum had one or more co-occurring mental health conditions, and 41% of them presented two or more (27). According to a recent meta-analysis, the most frequent ASD-associated mental health disorders in all ages are: Attention-deficit hyperactivity disorder (ADHD), anxiety disorder, sleep-wake disturbances, disruptive behaviors, impulse-control, and conduct disorder, depressive disorder, obsessive-compulsive disorder (OCD), bipolar disorder and those within the schizophrenia spectrum (26).

Several barriers and disadvantaging factors may compound the aforementioned ASD-related health disparities and limit people's access to healthcare services (28, 29). Specifically, some studies suggest that the existence of concomitant ID and being a women predispose to a worse health profile (24, 26, 30–34). In addition, people on the autism spectrum may experience accelerated aging and age-related diseases at younger ages compared to the general population (32, 35).

Despite this evidence, most health problems beyond ASD-related symptoms have been overlooked for decades. Only a few studies have systematically addressed them in the last years (22, 26, 36), and fewer still have included adult individuals (24, 30, 33, 37). Thus, the lack of research on health outcomes is a significant barrier to promoting the QoL of people on the autism spectrum. It is also an obstacle to improving health care systems and developing evidence-based policies.

Here, we present the first Spanish epidemiologic study describing the health status of a large cohort of children, adolescents, and adults with ASD, including physical and mental health co-occurring conditions. We aim to analyse how their health is influenced by sex, age, and concurrent ID.

2. Materials and methods

2.1. Design and participants

We conducted an observational retrospective analytic study with a cohort of autistic individuals in Spain based on demographic and health data from a national registry collected by Autism Spain (38). Autism Spain is the leading charity related to ASD in Spain. It brings together 151 non-profitable organizations from all over the country that support people on the autism spectrum and their families to achieve equal opportunities and guarantee their QoL.

Autism Spain's ASD registry contains information about people with a confirmed clinical diagnosis of ASD of any age. Most of them are users or members of autism supportive associations linked to Autism Spain and receive support from them (psychosocial, educational, occupational, juridical, and administrative, among others). The data collection period fell between the date of the first and last entries into the registry (November 2017 and May 2020).

Forty-one ASD associations participated in this study and provided data related to 2,623 autistic people. Six participants contributed independently to the registry. Inclusion criteria were: (a) having a confirmed clinical diagnosis of any pervasive developmental disorder (PDD) (according to the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), the International Classification of Diseases 10th Revision (ICD-10 or earlier diagnostic criteria) or ASD (according to the recent DSM fifth edition) (2, 16, 39), ICD-10 codes: F84.0, F84.2, F84.3, F84.5, F84.9 (**Figure 1**) and (b) providing informed consent to participate. Diagnoses from health care centers or authorized non-medical centers with ASD diagnostic services were included in the analysis. All registered individuals fulfilled these criteria and therefore provided data that were subsequently analysed. No drop-out events or experimental deaths were reported.

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the Autonomous University of Madrid (CEI-105-2039). All individuals provided written informed consent to participate and allowed the publication of the results. Personal data were handled according to the current General Data Protection Regulation 2016/679 of the European Parliament (EU-GPDR) and the Council of 27 April 2016 and with the Spanish Organic Law 3/2018 of 5 December 2018 on Data Protection and Guarantee of Digital Rights. Due to the retrospective nature of this study, there was no influence on any medical decision, including treatment prescription.

2.2. Description of the variables and outcome measures

Data from the registries was analysed according to the following sociodemographic and health variables:

Sociodemographics

- Sex and age. Boys and girls and adolescents (age 18 or younger) and adults (older than 18).

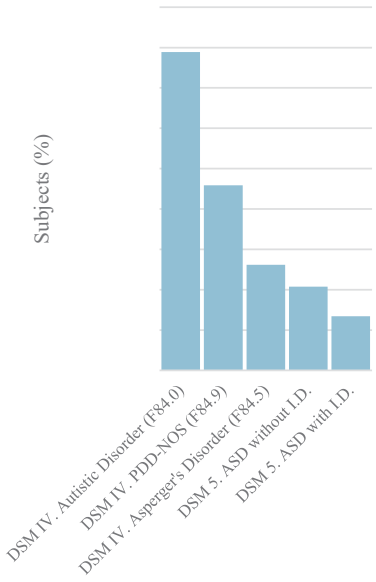


FIGURE 1
Distribution of study participants (N = 2,563) diagnosed according to DSM-IV or ICD-10 criteria for pervasive developmental disorder (PDD) and according to DSM 5 criteria for ASD. ASD, autism spectrum disorder; DSM-IV, American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; DSM-5, American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition; ICD-10, International Classification of Diseases 10th Revision.

- Health data. Health information is based on the clinical data included in health records and reports provided by the participants. Only those health conditions formally diagnosed by a specialized health practitioner (both in the Spanish public Health Care System or in private authorized health care services) according to international classification systems [DSM-IV (16; or ICD-10 (40)] were included in the registry.
- Diagnosis of any disorder within the autism spectrum (hereinafter "ASD"): according to DSM-IV (39), DSM-5 (16), or ICD-10 (2).
- Co-occurring conditions: divided into mental health disorders and other general medical conditions with a clinical confirmed diagnosis.
  Those conditions with a stronger pathophysiological link to ASD, such as genetic (21) and nervous system disorders, (ICD-10 codes: G40.90, Q05, G80, P04.3), especially epilepsy (41–43), were grouped apart.
- Intellectual Disability: corresponding to an intelligence quotient (IQ) score <76 estimated with any standardized intelligence test (Weschler Adult Intelligence Scale IV, Wechsler Intelligence Scale for Children Revised and IV, Wechsler Preschool and Primary Scale of Intelligence III and IV, Wechsler Non-verbal Scale of Ability, Kaufman Brief Intelligence Test, Kaufman Assessment Battery for Children). ID level was then classified as profound (IQ < 20), severe (IQ = 20–40), moderate (IQ = 41–55), mild (IQ = 56–75), border (IQ = 76–85), or absent (IQ > 85), according to ICD-10 subcategories (2).
- Psychopharmacological treatment: only currently prescribed psychiatric drugs were considered.
- Functional disability: defined by a degree of general disability of ≥33% according to the official certificate issued by the Institute of Social Services and the Elderly (IMSERSO) of the Spanish Ministry of Social Rights and 2030 Agenda. This is a summary score of the individual's functional limitations due to physical, mental, intellectual, or sensorial impairments. When reaching a percentage of ≥25%, social factors that limit equal, effective, and full participation in society, are added to the calculations. Those provided with a certificate equal or upper to a 33% percentage are eligible to apply for a government subsidy (44). This study rated functional disability in daily living skills according to the following percentage ranges: ≥75, 65–74, 33–64, and <33%.

Participants' information was collected through a data entry questionnaire linked to the *Autism Spain's* registry (available as **Supplementary material**). Affiliated members of *Autism Spain* had access only to their own registers. in each autism-support association the questionnaire was filled in by a designated professional that the Confederation had previously trained to contrast the information in the medical and social reports. The Confederation did not fix or save any copies of these documents.

Outcome measures in this study were the frequency of sociodemographic variables, clinical diagnosis, mental and physical health comorbidities, and psychiatric psychopharmacological prescription in the study population. Also, the distribution of health-related variables was analysed: neurological, chronic health diseases, and mental health disorders, as well as psychopharmacological treatment according to sex, age, and intellectual disability; diagnosis of ASD according to sex; ID level according to sex and age; and degree of functional disability according to sex and ID.

2.3. Data analysis

Participant data were analysed by descriptive statistics. Absolute and relative frequencies were used to describe categorical variables. The continuous ones were expressed by the mean and standard deviation (SD). Inferential tests were conducted to compare variables according to sex, age, and ID level: Student's t-test (continuous variables) and Chi-square test (categorical variables). Statistical significance was set at $P < 0.05$. When possible, point-estimates of effect-size (odds ratio for variables with two categories and Cramér's V for variables with more than two categories) with 95% confidence intervals (CI) for each inferential test were also conducted. Data analysis was performed by IBM's Statistical Package for the Social Sciences (SPSS®) for Windows version 24 (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.).

3. Results

3.1. Sociodemographics of the study sample

Autism Spain's registry contained information about 2,629 individuals with a confirmed diagnosis of ASD. Mean age was

TABLE 1 Sociodemographic characteristics of autism spectrum disorder (ASD).

	Total subjects
Sex (male), *n* (%)	2,158 (82.1)
Age, years[1]	
Mean (SD)	16.2 (10.7)
Age range, *n* (%)[1]	
≤18 years (non-adults)	1,825 (69.6)
>18 years (adults)	798 (30.4)
0–5 years	312 (11.9)
6–11 years	779 (29.7)
12–17 years	734 (28.0)
18–25 years	374 (14.3)
>25 years	424 (16.2)

Unless otherwise indicated, percentages refer to the total study population (N = 2,629). [1]N = 2,623; n missing = 6.

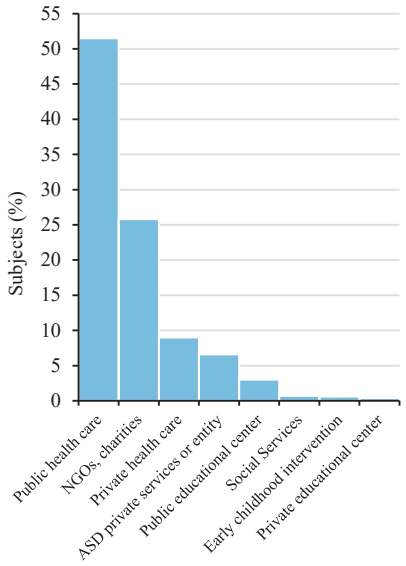


FIGURE 2
Location of diagnosis of the study participants (N = 2,563). NGO, non-governmental organization.

significantly higher for women than for men (18.4; SD = 12.1 vs. 15.8; SD = 10.3, respectively, $P < 0.0001$). Almost one third of the study population were adults (N = 798; 30.4%), and approximately half of them (N = 424; 16.2%) were older than 25 and up to 60 years old (see Table 1).

3.2. Autism spectrum disorder diagnosis

We found that mean age at diagnosis time was 7.5 years (SD = 6.5), and almost all participants (N = 2,344; 95.8%) had received it before age 21. Public health care (N = 1,320; 51.5%) and diagnostic services at non-governmental organizations related to ASD (N = 662; 25.8%) were the most common diagnostic sites

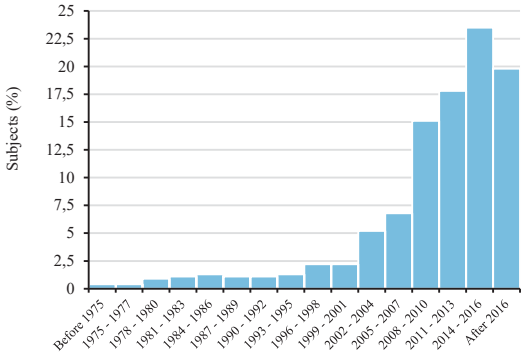


FIGURE 3
Percentages of autism spectrum disorder (ASD) diagnoses between before 1975 and after 2016.

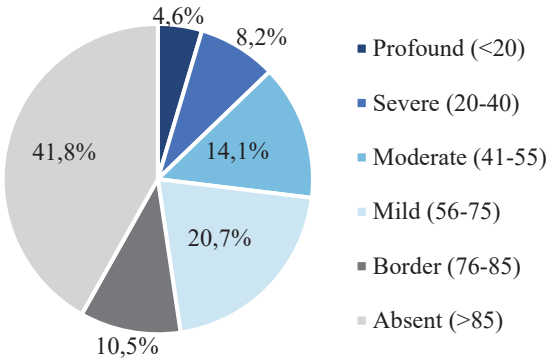


FIGURE 4
Distribution of intellectual disability severity degree of the study participants. The level of intellectual disability (ID) according to the intelligence quotient (IQ) test score. ID corresponds to an IQ test score <76.

(Figure 2). The number of diagnoses showed an increasing trend during the last two decades and especially between the 2005–2007 (N = 167; 6.8%) and the 2008–2010 (N = 370; 15.1%) periods (see Figure 3).

3.3. Occurrence of intellectual disability and recognition of supporting needs

Around a third of the study participants (N = 885; 33.7%) had been tested with an IQ formal test. Mean IQ score was 76.1 (SD = 30.9) for the whole study population, and it was higher in men than in woman (78.2; SD = 30.3 vs. 66.8; SD = 32.0, respectively, $P < 0.0001$). Approximately half of the individuals had no ID accompanying the ASD diagnosis (ICD-10 Codes: F70, F71. F72, F73, F78, and F79) (N = 463; 52.3%), while the other half (N = 422; 47.7%) presented this concurrent condition in different severity levels (Figure 4).

Adaptative skills had been officially assessed for most of the study population (N = 2,219; 84.4%). In most cases some degree of functional impairment that provided access to public support was identified (N = 2,181; 98.3%).

3.4. Genetic and nervous system disorders, other co-occurring physical and mental conditions, and psychopharmacological treatment

Among those with any confirmed genetic disorders (N = 134; 5.1%), Fragile X Syndrome (ICD-10 code: Q99.2) (N = 9; 0.3%) and Down Syndrome (ICD-10 codes: Q90.X) (N = 7; 0.3%) were the most frequent ones (Table 2). Other identified genetic disorders (N = 118; 4.5%) corresponded to a wide range of distinct chromosomic anomalies. A total of 338 (12.9%) individuals had a nervous system disorder. Epilepsy and recurrent epileptic seizures (ICD-10 codes G40.X) (N = 288; 11.0%) were the most frequently reported in this category (see Table 2).

Around one fifth (N = 467; 17.8%) of the studied sample had one or more mental health diagnoses apart from ASD with or without ID, namely: impulse-control and disruptive behavior disorder (ICD-10 code: F63) (N = 304; 11.6%), anxiety disorder (ICD-10 code: F41) (N = 179; 6.8%), attention deficit and hyperactivity disorder (ICD-10 code: F90.X) (N = 151; 5.8%), obsessive compulsive disorder (ICD-10 codes: F42.X) (N = 117; 4.5%), and eating disorders (ICD-10 codes: F50.X) (N = 109; 4.1%), among others (see Table 2). Almost half (N = 1,187; 45.2%) of the participants were currently taking psychopharmacological drugs, mainly antipsychotics (N = 763; 29.0%) and anticonvulsants (N = 355; 13.5%) (Figure 5).

Other general medical conditions were found in 668 (25.4%) people. In some cases, two (N = 138; 5.2%), three (N = 53; 2.0%), or more than three (N = 33; 1.3%) comorbidities were reported for the same individual. The main ones were sleep-wake disturbances (ICD-10 code: F51.3) (N = 175; 6.7%), dermatitis and eczema (ICD-10 code: L30.9) (N = 123; 4.7%), overweight and obesity (ICD-10 code: E66.X) (N = 111; 4.2%), and intestinal disorders (ICD-10 codes: K55-K63) (N = 107; 4.1%) (see Table 2).

TABLE 2 Genetic, nervous system, and other comorbid disorders.

	Total subjects (N = 2,629)
Genetic disorders, *n* (%)	
Fragile X syndrome (Q99.2)	9 (0.3)
Don syndrome (Q90.X)	7 (0.3)
Nervous system disorders, *n* (%)	
Epilepsy and recurrent epileptic seizures (G40.X)	288 (11.0)
Spina bifida (Q05.X) and other congenital anomalies of the nervous system (Q00-07)	25 (1.0)
Cerebral palsy (G 80.X)	11 (0.4)
Fetal alcohol syndrome (Q86.0)	5 (0.2)
Mental health disorders, *n* (%)	
Impulse-control and disruptive behavior disorder (F63)	304 (11.6)
Anxiety disorder (F41)	179 (6.8)
Attention deficit and hyperactivity disorder (F90.X)	151 (5.8)
Obsessive compulsive disorder (F42.X)	117 (4.5)
Eating disorders (F50.X)	109 (4.1)
Depressive disorders (F32.X)	75 (2.9)
Personality disorders (F60.X)	22 (0.8)
Phonological disorder (R47.X)	12 (0.5)
General medical conditions, *n* (%)	
Sleep-wake disturbances (F51.3)	175 (6.7)
Dermatitis and eczema (L30.9)	123 (4.7)
Overweight and obesity (E66.X)	111 (4.2)
Intestinal disorders (K55-K63)	107 (4.1)
Dentofacial anomalies and other mandibular disorders (M26-M27)	43 (1.6)
Thyroid gland disorders (E03.X, E05.X)	36 (1.4)
Gastric ulcer (K25.X), gastritis (K29.0-7) or esophagitis (K20.X)	20 (0.8)
Visual problems (H53-H54)	21 (0.8)
Other metabolic disorders (E70-E90)	19 (0.7)
Vasomotor (J30.0) and allergic rhinitis (J30.9)	17 (0.6)
Hearing loss (H90-H91)	14 (0.5)
Kidney disease (N18.X) or other affected urinary tract system organs (N30-N39)	14 (0.5)
Diabetes (E08-E013)	12 (0.5)
Urticaria (L50.X)	8 (0.3)

International Classification of Diseases 10th Revision (ICD-10) codes between brackets.

3.5. Health disparities according to sex, age, and cognitive functioning

Women presented more severe forms of ID (moderate-to-severe levels) than the male subgroup (P = 0.0002; Table 3). The degree of functional impairment in daily living skills was also differently distributed according to sex (P < 0.0001). The percentage of women with this type of difficulties almost doubled that of men (see Table 3).

Proportionally, more women than men endured one or more nervous system disorders (OR = 0.49; 95% CI = 0.38–0.64; P < 0.0001) and other medical conditions (OR = 0.56; 95% CI = 0.45-0.70; P < 0.0001). Mental health co-occurring disorders showed the same trend, although not reaching statistical significance (OR = 0.81; 95% CI = 0.65–1.01; P = 0.0565), and they also received more psychopharmacological treatments than men (OR = 0.78; 95% CI = 0.64-0.95; P = 0.0129) (see Table 3).

The occurrence of multiple nervous system (P < 0.0001), mental health (P < 0.0001), and other health comorbid disorders (P < 0.0001) as well as the number of individuals with psychopharmacological prescriptions (P < 0.0001) increased with age. However, the prescription of melatonin (P < 0.0001) and methylphenidate (P < 0.0001) showed the reverse tendency, reaching its highest in infancy and childhood, and childhood and adolescence, respectively, (see Table 4).

Intellectual disability was associated with a greater risk of neurological disorders (OR = 0.16; 95% CI = 0.10–0.26; P < 0.0001) and other medical co-occurring conditions (OR = 0.35; 95% CI = 0.26–0.48; P < 0.0001) (see Table 5). Mental health

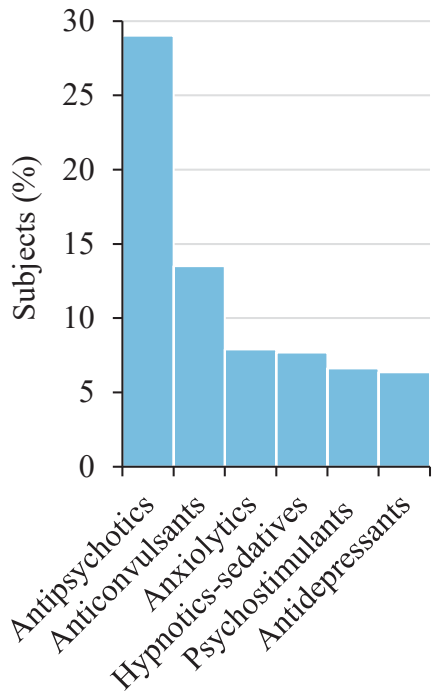


FIGURE 5
Current psychopharmacological treatment: therapeutic groups. Only those with ≥ 5% of users are shown (*N* = 2,629). Antipsychotics (risperidone, aripiprazole), anticonvulsants (valproates, sulfamates, carboxamides), anxiolytics (benzodiazepines), hypnotic-sedatives (melatonin), psychostimulants (methylphenidate), and antidepressants (SSRIs). Only those with ≥ 1% of users are shown (*N* = 2,629). SSRIs, selective serotonin reuptake inhibitors.

comorbidities impacted differently depending on the ID level: impulse-control and disruptive behavior (OR = 0.30; 95% CI = 0.19–0.47; $P < 0.0001$) and OCD (OR = 0.39; 95% CI = 0.19–0.80; $P = 0.0076$) were more common in the group with ID, whereas ADHD and personality disorders had been diagnosed to a greater extent among those without ID (OR = 2.14; 95% CI = 1.46–3.13; $P < 0.0001$) (Table 5). Psychopharmacological drug prescription was generally higher in the ID group (OR = 0.34; 95% CI = 0.26–0.45; $P < 0.0001$) except for methylphenidate, which showed the reverse tendency (OR = 3.51; 95% CI = 1.95–6.32) (Table 5).

Finally, we found that the levels of intellectual and functional disabilities were highly correlated ($P < 0.0001$). More individuals with ID had been certified with the highest percentages of disability in proportion to those without ID (Table 5).

4. Discussion

For the first time in our country, we have characterized the health status of the largest autism dataset in Spain. We found that the overall health status of people with ASD in Spain is compromised by different factors. The mean age at ASD diagnosis was 7.5 years old and it was generally obtained through the public health system or non-governmental organizations specialized in autism care. Up to 25% of the sample presented nervous system disorders, mental health associated conditions and other comorbidities apart from ASD. Around 50% of them received psychopharmacological treatment. Being a woman, advanced age and the presence of ID were identified as potential exacerbating factors of health problems. Also, those with ID had poorer adaptive functioning, and women were more prone to severe intellectual and functional impairment.

4.1. Autism diagnosis

The average age at ASD diagnosis of the individuals in our sample corresponds to the estimated ranges in Europe between 1990 and 2012, which varied from 3 to 10 years of age. However, most up-to-date studies show that in recent years (2012–2019) it has decreased and oscillates rather between 3.5 and 5 years (45). Therefore, according to our data, it seems that in Spain age at diagnosis is still relatively late compared to the European standard.

A men-to-women ratio of 4:1 was observed in our sample, in line with traditional epidemiologic reports (46, 47). However, in the last decades, estimates have pointed to a reduced 3:1 ratio (8, 48, 49). Several authors have suggested the presence of a sex bias in ASD diagnosis, including more wrong, delayed or missed diagnoses in autistic women (50–52). This could be partially explained by a male-biased understanding of the condition, the existence of sex differences in clinical presentations and the lack of instruments and procedures to effectively recognize them (53, 54). Consequently, the prevalence of ASD in women may still be underestimated, also in the Spanish context.

4.2. Health comorbidities

Only a couple of genetic syndromes (Down and Fragile X) were identified, which explained less than 1% of the cases in our study. Recent evidence suggests that complex polygenic mechanisms and environmental factors contribute approximately the same to the etiology of autism (55, 56).

Regarding other pathologies accompanying ASD, we suspect that the prevalence of nervous system disorders, mental health diagnoses and other comorbidities in our sample may be underestimated since it falls far below that of other countries (around 70%) (21–23, 57). This may be due to considerable differences in health care systems or to the study design criteria (we only considered comorbid clinical diagnoses confirmed by a health care professional according to DSM or ICD international classification systems).

However, the categories found in greater representation in this study do correspond to those usually reported by other European studies on the health of people with autism (3, 58).

4.3. Pharmacological prescriptions

Regarding pharmacological treatments, we found that psychiatric polypharmacy was significantly higher in participants with ASD and ID, as well as in the women and elderly subgroups. In addition, the number of individuals with psychopharmacological prescriptions was higher than the total number of neurological and

TABLE 3 Intellectual and functional disability, nervous system, and mental health disorders, general medical conditions and psychopharmacological treatment according to sex.

	Men	Women	Size effects: Cramer's V or OR (IC 95%)	*P*-value
Level of ID according to the IQ test score, *n* (%)	724 (100.0)	161 (100.0)	0.158	0.0002
Profound (<20)	28 (3.9)	13 (8.1)	–	–
Severe (20–40)	56 (7.7)	17 (10.6)	–	–
Moderate (41–55)	89 (12.3)	36 (22.4)	–	–
Mild (56–75)	152 (21.0)	31 (19.3)	–	–
Border (76–85)	79 (10.9)	14 (8.7)	–	–
Absent (>85)	320 (44.2)	50 (31.1)	–	–
Degree of functional disability, *n* (%)	1,820 (100.0)	399 (100.0)	0.147	<0.0001
<33%	32 (1.8)	6 (1.5)	–	–
33–64%	1,059 (58.2)	174 (43.6)	–	–
65–74%	297 (16.3)	57 (14.3)	–	–
≥75%	432 (23.7)	162 (40.6)	–	–
Nervous system disorders, *n* (%)	2,158 (100.0)	471 (100.0)	–	–
Individuals with ≥1 nervous system disorder	242 (11.2)	96 (20.4)	0.49 (0.38–0.64)	<0.0001
Epilepsy and recurrent epileptic seizures (G40.X)	205 (9.5)	83 (17.6)	0.49 (0.37–0.65)	<0.0001
Spina bifida (Q05.X) and other congenital anomalies of the nervous system (Q00-07)	16 (0.7)	9 (1.9)	0.38 (0.17–0.87)	0.0178
Individuals who do not declare nervous system disorders	1,916 (88.8)	375 (79.6)	–	<0.0001
Mental health disorders, *n* (%)	2,158 (100.0)	471 (100.0)	–	–
Individuals with ≥1 mental health disorder	532 (24.7)	136 (28.9)	0.81 (0.65–1.01)	0.0565
Number	2,158 (100.0)	471 (100.0)	0.06	0.048
0	1,626 (75.3)	335 (71.1)	–	–
1	362 (16.8)	85 (18.0)	–	–
2	106 (4.9)	27 (5.7)	–	–
3	35 (1.6)	17 (3.6)	–	–
>3	29 (1.3)	7 (1.5)	–	–
Type	–	–	–	–
Impulse-control and disruptive behaviour disorders (F63)	232 (10.8)	72 (15.3)	0.67 (0.50–0.89)	0.0053
Anxiety disorders (F41)	131 (6.1)	48 (10.2)	0.57 (0.40–0.81)	0.0013
Obsessive compulsive disorder (F42.X)	93 (4.3)	24 (5.1)	0.84 (0.53–1.33)	0.4536
Eating disorders (F50.X)	87 (4.0)	22 (4.7)	0.86 (0.53–1.38)	0.5283
Depressive disorders (F32.X)	52 (2.4)	23 (4.9)	0.48 (0.29–0.79)	0.0035
Others	210 (9.7)	32 (6.8)	1.48 (1.01–2.18)	0.0458
Attention deficit and hyperactivity disorder (F90.X)	136 (64.8)	15 (46.9)	2.04 (1.19–3.52)	–
Personality disorders (F60.X)	19 (9.0)	3 (9.4)	1.39 (0.41–4.70)	–
Phonological disorder (R47.X)	11 (5.2)	1 (3.1)	2.41 (0.31–18.7)	–
General medical conditions, *n* (%)	2,158 (100.0)	471 (100.0)	–	–
Individuals with ≥1 comorbid condition	465 (21.5)	155 (32.9)	0.56 (0.45–0.70)	<0.0001
Sleep-wake disturbances (F51.3)	126 (5.8)	49 (10.4)	0.53 (0.38–0.75)	0.0003
Overweight and obesity (E66.X)	79 (3.7)	32 (6.8)	0.52 (0.34–0.80)	0.0022
Intestinal disorders (K55-K63)	78 (3.6)	29 (6.2)	0.57 (0.37–0.89)	0.0114
Dentofacial anomalies and other mandibular disorders (M26-M27)	28 (1.3)	15 (3.2)	0.40 (0.21–0.75)	0.0034

(Continued)

TABLE 3 (Continued)

	Men	Women	Size effects: Cramer's V or OR (IC 95%)	*P*-value
Individuals who do not declare general medical conditions	1693 (78.4)	316 (67.1)	–	0.0001
Psychopharmacological treatment, *n* (%)	2,158 (100.0)	471 (100.0)	–	–
Individuals currently prescribed with ≥1 psychiatric drug	950 (44.0)	237 (50.3)	0.78 (0.64–0.95)	0.0129
Antipsychotics	627 (29.1)	136 (28.9)	1.01 (0.81–1.26)	0.9379
Anticonvulsants	262 (12.1)	93 (19.7)	0.56 (0.43–0.73)	<0.0001
Anxiolytics	161 (7.5)	47 (10.0)	0.73 (0.52–1.02)	0.0666
Melatonin	149 (6.9)	42 (8.9)	0.76 (0.53–1.08)	0.1273
Antidepressants	133 (6.2)	34 (7.2)	0.84 (0.57–1.25)	0.3948
Methylphenidate	121 (5.6)	16 (3.4)	1.69 (0.99–2.87)	0.0506

Condition of disability is defined by a degree of functional disability of ≥33%. Condition of ID corresponds to an IQ test score <76. OR only shown for dichotomous variables. International Classification of Diseases 10th Revision (ICD-10) codes between brackets.

psychiatric confirmed diagnoses that require drug administration. Also, we noticed a sudden rise in the number of children (≥6 years old) under psychotropic treatment that was not mirrored by in the number of mental health co-occurring confirmed diagnoses. Likewise, the higher proportion of youngsters treated with methylphenidate than the actual number of ADHD cases in the sample is as well intriguing.

According to international guidelines and recommendations, antipsychotics, antidepressants, and anticonvulsants should not be prescribed to manage any core autism symptomatology (59). A case-by-case analysis should be performed to exclude or confirm any unnecessary or inappropriate interventions with those psychiatric medications. Instead, their use should be restricted to cases of concerning behaviors, i.e., when it jeopardizes QoL and/or safety (self or others') (60, 61). Despite these recommendations, some authors claim that people with autism, ID or both, are currently overprescribed, especially with antipsychotics and antidepressants (62, 63), which may have deleterious effects on their physical and mental health throughout their lifespan (64). Our study results also point to this presumed overprescription of psychopharmacological treatments that increase with age and, possibly, in the absence of the indicated clinical conditions too. In this regard, rethinking psychiatric prescription protocols is imperative, perhaps by encouraging evidence-based psycho-educative practices, complementary or alternative to psychopharmacological prescriptions, and promoting good practices in their follow-up.

4.4. Poor health risk factors related to autism: Sex, age, intellectual disability

As in the present study, the ASD-associated medical conditions identified so far belong to a wide range of medical areas and are subject to both sex- and age-related disparities (24, 30, 33). Compared to the men ASD subpopulation, we observed that women were significantly more prone to neurological or other health disorders (epilepsy, spina bifida, sleep-wake disturbances, intestinal disorders, overweight, obesity, and dental abnormalities) and most likely to one or more mental health co-occurring conditions too (particularly, impulse control and conduct disorders, anxiety, and depression). Several reasons may explain these sex-based differences, such as the delays or errors in ASD diagnosis mentioned above and thus a lack of understanding and support for their needs (50–52). Other physiological factors such as hormonal imbalances, which may imply severe physical and mental complications for women, could also intervene (31, 34), but more research is needed to achieve solid conclusions. Finally, being a woman was also associated with higher ID levels and more complex support needs. Those women with milder supporting needs are less represented in our sample, as they are in similar studies (3, 65). Research on their reality and priorities should increase, in order to improve their QoL and the community response to their needs.

On the other hand, most of the analysed cooccurring conditions showed, as expected, an increasing trend with age. The percentage of individuals with more than one neurological disorder, mental health disorder or general condition already doubled or tripled in the >25 years-old group compared to younger ages. Considering that no adults in our study population were older than 60, our findings led us to suspect premature comorbidity onset in ASD compared to the general population. In connection to this result, emerging studies have suggested an association between ASD and accelerated aging after reporting the early onset of age-related disorders such as seizure, hypertension, hyperlipidemia, and chronic kidney disease, especially in the presence of ID. This health status decline is accompanied by progressive less autonomy, poorer adaptive skills and the use of polypharmacy (32, 35).

Mental health issues can, as well, profoundly affect QoL at younger ages. According to a recent European longitudinal study, depression and anxiety symptoms in children and adolescents with ASD significantly reduced their perception of wellbeing (66). The same age group is at an increased risk of an anxiety disorder (67, 68), which is even higher in the presence of ADHD (67). Reciprocally, the coexistence of ADHD and anxiety has been related to poorer adaptive skills in autistic children (67). In our results, we observed that school- and high school-stage ASD participants had already received a diagnosis of anxiety, although its prevalence increased at older ages together with the prescription of antidepressants and anxiolytics. On the contrary, ADHD was

TABLE 4 Intellectual disability, nervous system, and mental health disorders, general medical conditions and psychopharmacological treatment according to age groups.

	0–5 years	6–11 years	12–17 years	18–25 years	>25 years	*P*-value
Level of ID according to the IQ test score, *n* (%)	54 (100.0)	249 (100.0)	290 (100.0)	137 (100.0)	154 (100.0)	<0.0001
Profound (<20)	2 (3.7)	4 (1.6)	5 (1.7)	7 (5.1)	23 (14.9)	–
Severe (20–40)	–	7 (2.8)	11 (3.8)	11 (8.0)	44 (28.6)	–
Moderate (41–55)	13 (24.1)	26 (10.4)	36 (12.4)	24 (17.5)	26 (16.9)	–
Mild (56–75)	17 (31.5)	49 (19.7)	73 (25.2)	29 (21.2)	15 (9.7)	–
Border (76–85)	7 (13.0)	32 (12.9)	35 (12.1)	10 (7.3)	9 (5.8)	–
Absent (>85)	15 (27.8)	131 (52.6)	130 (44.8)	56 (40.9)	37 (24.0)	–
Nervous system disorders, *n* (%)	312 (100.0)	779 (100.0)	734 (100.0)	374 (100.0)	424 (100.0)	–
Individuals with ≥1 nervous system disorder	9 (2.9)	54 (6.9)	71 (9.7)	48 (12.8)	156 (36.8)	<0.0001
Epilepsy and recurrent epileptic seizures (G40.X)	6 (1.9)	41 (5.3)	56 (7.6)	43 (11.5)	142 (33.5)	<0.0001
Individuals who do not declare nervous system disorders	303 (97.1)	725 (93.06)	663 (90.3)	326 (87.2)	268 (63.2)	<0.0001
Mental health disorders, *n* (%)	312 (100.0)	779 (100.0)	734 (100.0)	374 (100.0)	424 (100.0)	–
Individuals with ≥1 mental health disorder	27 (8.7)	147 (18.9)	186 (25.3)	106 (28.3)	200 (47.2)	<0.0001
Number	312 (100.0)	779 (100.0)	734 (100.0)	374 (100.0)	424 (100.0)	<0.0001
0	285 (91.3)	632 (81.1)	548 (74.7)	268 (71.7)	224 (52.8)	
1	19 (6.1)	120 (15.4)	135 (18.4)	69 (18.4)	103 (24.3)	
2	5 (1.6)	17 (2.2)	34 (4.6)	20 (5.3)	56 (13.2)	
3	3 (1.0)	7 (0.9)	9 (1.2)	8 (2.1)	25 (5.9)	
>3	–	3 (0.4)	8 (1.1)	9 (2.4)	16 (3.8)	
Type	–	–	–	–	–	–
Impulse-control and disruptive behaviour disorders (F63)	10 (3.2)	56 (7.2)	65 (8.9)	45 (12.0)	127 (30.0)	<0.0001
Anxiety disorders (F41)	3 (1.0)	25 (3.2)	39 (5.3)	38 (10.2)	73 (17.2)	<0.0001
Obsessive compulsive disorder (F42.X)	6 (1.9)	13 (1.7)	24 (3.3)	22 (5.9)	52 (12.3)	<0.0001
Depressive disorders (F32.X)	–	3 (0.4)	13 (1.8)	15 (4.0)	43 (10.1)	<0.0001
Others	6 (100.0)	62 (100.0)	99 (100.0)	34 (100.0)	41 (100.0)	<0.0001
Phonological disorder (R47.X)	2 (33.3)	5 (8.1)	4 (4.0)	1 (2.9)	–	
Attention deficit and hyperactivity disorder (F90.X)	1 (16.7)	43 (69.4)	73 (73.7)	23 (67.6)	11 (26.8)	
Personality disorders (F60.X)	–	1 (1.6)	4 (4.0)	4 (11.8)	13 (31.7)	
General medical conditions, *n* (%)	312 (100.0)	779 (100.0)	734 (100.0)	374 (100.0)	424 (100.0)	–
Individuals with ≥ 1 comorbid condition	44 (14.1)	161 (20.7)	130 (17.7)	74 (19.8)	211 (49.8)	<0.0001
Sleep-wake disturbances (F51.3)	14 (4.5)	38 (4.9)	31 (4.2)	18 (4.8)	74 (17.5)	<0.0001
Overweight and obesity (E66.X)	–	12 (1.5)	18 (2.5)	21 (5.6)	60 (14.2)	<0.0001
Intestinal disorders (K55-K63)	12 (3.8)	28 (3.6)	29 (4.0)	9 (2.4)	29 (6.8)	0.0217
Dentofacial anomalies and other mandibular disorders (M26-M27)	3 (1.0)	13 (1.7)	8 (1.1)	2 (0.5)	17 (4.0)	0.0005
Thyroid gland disorders (E03.X, E05.X)	2 (0.6)	10 (1.3)	6 (0.8)	4 (1.1)	14 (3.3)	0.005
Gastric ulcer (K25.X), gastritis (K29.0-7) or esophagitis (K20.X)	1 (0.3)	4 (0.5)	3 (0.4)	2 (0.5)	10 (2.4)	0.0018
Metabolic disorders (E70-E90)	1 (0.3)	7 (0.9)	2 (0.3)	1 (0.3)	8 (1.9)	0.0161
Individuals who do not declare general medical conditions	–	–	–	–	–	–
Psychopharmacological treatment, *n* (%)	312 (100.0)	779 (100.0)	734 (100.0)	374 (100.0)	424 (100.0)	–

(Continued)

TABLE 4 (Continued)

	0–5 years	6–11 years	12–17 years	18–25 years	>25 years	*P*-value
Individuals currently prescribed with ≥1 psychiatric drug	64 (20.5)	300 (38.5)	333 (45.4)	180 (48.1)	306 (72.2)	<0.0001
Antipsychotics	20 (6.4)	174 (22.3)	207 (28.2)	127 (34.0)	233 (55.0)	<0.0001
Anticonvulsants	7 (2.2)	34 (4.4)	74 (10.1)	76 (20.3)	163 (38.4)	<0.0001
Anxiolytics	1 (0.3)	6 (0.8)	22 (3.0)	36 (9.6)	142 (33.5)	<0.0001
Melatonin	37 (11.9)	79 (10.1)	44 (6.0)	18 (4.8)	13 (3.1)	<0.0001
Antidepressants	–	12 (1.5)	41 (5.6)	43 (11.5)	70 (16.5)	<0.0001
Methylphenidate	3 (1.0)	51 (6.5)	69 (9.4)	8 (2.1)	6 (1.4)	<0.0001
Individuals who do not declare psychopharmacological treatment	289 (92.6)	554 (71.1)	451 (1.4)	225 (60.1)	146 (34.4)	<0.0001

Condition of ID corresponds to an IQ test score <76. ID, intellectual disability; IQ, intelligence quotient; OCD, obsessive-compulsive disorder. International Classification of Diseases 10th Revision (ICD-10) codes between brackets.

more frequently reported in children, which may be due to elder individuals' under- or delayed diagnosis (69).

The risk for health-related issues also depends on the presence of ID (70, 71). Global estimates on the prevalence of cognitive impairment in ASD greatly vary across publications. The percentage found in our study (47.7% of tested individuals) falls between the estimated range of 40–61% (72), although newer evidence suggests that it can go down to 30% (31). Few studies have addressed the influence of ID on the health status of ASD individuals. In the meta-analysis performed by Lai and colleagues, heterogeneity in prevalence estimates of mental comorbidities in ASD was associated with intellectual functioning, besides other variables (26). Another publication of the same year found a high physical and mental comorbidity burden in adults with ASD and ID (mean age = 42.9) that was comparable to that of the general and older geriatric population (mean age = 79) from the same hospital (32). In our comparative analysis, an extensive range of physical and mental comorbidities were associated with the presence of ID, except for personality disorders and ADHD, possibly because they are challenging to diagnose in this context.

4.4.1. Study limitations

Due to the retrospective nature of our research, there are some related limitations that must be taken into account when interpreting the results.

Although only clinically confirmed health issues according to international classifications had been included, changes in diagnosis criteria of those coexistent conditions over time and missing data could have biased our results.

Also, the sample representativeness is limited because most of the participants where related to autism organizations or specialized supporting services. We were not able to obtain enough information from autistic people who were not related to that network, so it is not clear whether our results can be generalized to the whole ASD population in Spain, even being coincident, in some extent, with those obtained in similar studies in Europe (3, 58).

This sampling bias may be especially relevant for women and older participants. As already mentioned, we have observed from our registry that the socio-economic, educational and health context of women applicants is usually more disadvantageous compared with male counterparts and globally, adults (especially elderly persons) with ASD are underrepresented in research, and so they are in the present study.

Finally, comparisons with the non-ASD population could not be made, and for some analyses, such as the distribution of genetic disorders, our sample size was too small to allow making any inferences.

4.4.2. Future research

According to our findings and the related scientific literature, it is urgent to enhance the autistic community access to health, improving prevention, identification and management of the conditions that affect their physical and emotional wellbeing. This should include up-to-date training for health professionals regarding ASD, as well as providing resources and guidance to prevent, diagnose and treat comorbidities in a timely and effective manner, including more routine health check-ups and promoting healthy lifestyle changes.

In the future, it will be as well necessary to explore how the aforementioned and other co-occurring conditions impact autistic people's QoL, their emotional well-being and adaptive functioning, and are subject to sex-based differences.

To increase sample representativeness, further epidemiologic studies with large cohorts are also needed, especially with elder adults and those who are not related to the ASD specialized organizations or do not usually receive any support services. There is a lack of scientific information on the health profile of people with ASD who do not have access to those networks, probably because they have less information and opportunities to participate in health research too. The specific ASD conditions that favor membership in associations could also be linked to health variables, or maybe these organizations support their members in a way that influences their health needs (73). Data from typical controls such as health records, registries and surveys will also be necessary to compare the prevalence of comorbidities in ASD to the general population and pinpoint any associated risk factors.

From a public health perspective, other risk factors such as poor nutritional habits, little physical activity, long-term psychopharmacological treatments and institutionalization increase health problems morbidity among autistic people (28). It has also been suggested that children, adolescents and young

TABLE 5 Functional disability, nervous system, and mental health disorders, general medical conditions and psychopharmacological treatment according to intellectual quotient groups.

	IQ ≥ 76	IQ < 76	Size effects: Cramer's V or OR (IC 95%)	*P*-value
Degree of functional disability, *n* (%)	352 (100.0)	399 (100.0)	0.117	<0.0001
<33%	22 (6.3)	1 (0.3)	–	–
33–64%	280 (79.5)	162 (40.6)	–	–
65–74%	43 (12.2)	67 (16.8)	–	–
≥75%	7 (2.0)	169 (42.4)	–	–
Nervous system disorders, *n* (%)	463 (100.0)	422 (100.0)	–	–
Individuals with ≥1 nervous system disorder	20 (4.3)	94 (22.3)	0.16 (0.10–0.26)	<0.0001
Epilepsy and recurrent epileptic seizures G40.X)	11 (2.4)	85 (20.1)	0.10 (0.05–0.18)	<0.0001
Mental health disorders, *n* (%)	463 (100.0)	422 (100.0)	–	–
Individuals with ≥1 mental health disorder	141 (30.5)	134 (31.8)	0.94 (0.71–1.25)	0.6764
Type	–	–	–	–
Impulse-control and disruptive behavior disorders (F63)	27 (5.8)	73 (17.3)	0.30 (0.19–0.47)	<0.0001
Obsessive compulsive disorder (F42.X)	11 (2.4)	25 (5.9)	0.39 (0.19–0.80)	0.0076
Others	96 (100.0)	46 (100.0)	2.14 (1.46–3.13)	<0.0001
Attention deficit and hyperactivity disorder (F90.X)	73 (76.0)	25 (54.3)	2.97 (1.85–4.78)	–
Personality disorders (F60.X)	10 (10.4)	4 (8.7)	2.31 (0.72–7.41)	–
General medical conditions, *n* (%)	463 (100.0)	422 (100.0)	–	–
Individuals with ≥1 comorbid condition	85 (18.4)	165 (39.1)	0.35 (0.26–0.48)	<0.0001
Overweight and obesity (E66.X)	17 (3.7)	31 (7.3)	0.48 (0.26–0.88)	0.0159
Sleep-wake disturbances (F51.3)	12 (2.6)	60 (14.2)	0.16 (0.09–0.30)	<0.0001
Intestinal disorders (K55-K63)	9 (1.9)	31 (7.3)	0.25 (0.12–0.53)	0.0001
Dentofacial anomalies and other mandibular disorders (M26-M27)	4 (0.9)	19 (4.5)	0.18 (0.06-0.55)	0.0007
Gastric ulcer (K25.X), gastritis (K29.0-7) or esophagitis (K20.X)	0 (0.0)	8 (1.9)	–	0.0029
Psychopharmacological treatment, *n* (%)	–	–	–	–
Individuals currently prescribed with ≥ 1 psychiatric drug	149 (32.2)	246 (58.3)	0.34 (0.26–0.45)	<0.0001
Antipsychotics	68 (14.7)	172 (40.8)	0.25 (0.18–0.35)	<0.0001
Methylphenidate	53 (11.4)	15 (3.6)	3.51 (1.95–6.32)	<0.0001
Anticonvulsants	16 (3.5)	96 (22.7)	0.12 (0.07–0.21)	<0.0001
Melatonin	13 (2.8)	33 (7.8)	0.34 (0.18–0.66)	0.0008
Anxiolytics	12 (2.6)	50 (11.8)	0.20 (0.10-0.38)	<0.0001

Condition of disability is defined by a degree of functional disability of ≥33%. Condition of intellectual disability (ID) corresponds to an IQ test score <76. OR only shown for dichotomous variables. CI, confidence interval; IQ, intelligence quotient; OCD, obsessive-compulsive disorder; OR, odds ratio. International Classification of Diseases 10th Revision (ICD-10) codes between brackets.

adults on the spectrum are more vulnerable to health comorbidities than non-autistic population (22, 24, 25, 74). Finally, difficulties in accessing health care services have also been described for people with ASD, translating into diagnostic and treatment delays (26, 28, 29). All of these areas are amenable to further investigation.

Although the conclusions are still speculative, there seems to exist an association with maternal lifestyle and subsequent diseases (obesity, diabetes, epilepsy and antiepileptic drugs, among others), exposure to specific nutrients and pollutants during pregnancy, advanced parental age and birth complications associated with neonatal hypoxia/ischemia (56, 75–77). Unfortunately, those risk conditions were not included in the registry, and more research is needed to explore their contribution to ASD etiology.

Even though autistic people utilize more health care resources (outpatient visits, emergency room services and hospitalization), they are also more likely to report unmet medical needs, low satisfaction regarding the medical attention received and poor inter-personal communication with health care providers (29). There is an urgent need to increase research that captures the autistic community perspective about those challenges and barriers.

Qualitative and quantitative mixed methods should be applied to identify the key factors that affect the health status and wellbeing

of autistic people, taking advantage of the strengths of both approaches and enriching the research results (78, 79).

Also, improving participatory research is necessary, where people on the autism spectrum have an active role in prioritizing the research objectives and how to reach them, and provide feedback to be subsequently analysed and interpreted (80, 81).

To contribute to these efforts, our study has described the health challenges that require urgent awareness and at least some of the main factors (age, sex, and ID) that increase the vulnerability of people on the autism spectrum and deteriorate their wellbeing and QoL. There is a need to determine what factors besides ID contribute to shaping adaptive functioning in autism and QoL, for instance, mental and physical health status, social and workplace inclusion, access to educational, social participation, specialized support resources, and equality of opportunities. The extent to which global health status influences all these outcomes, especially in adulthood and middle age and beyond, remains to be clarified, and it is one of the main priorities considered in the public policies related to ASD around the world (3, 62).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of the Universidad Autónoma de Madrid (CEI-105-2039). Written informed consent to participate in this study was provided by the participants or their legal guardian/next of kin.

Author contributions

RV-F, MP-S, and CH-L contributed to the study conception and design, and were involved in the material preparation, acquisition, analysis, and interpretation of the data. RV-F and MP-S involved in the drafting of the manuscript. GB-R involved in the analysis and interpretation of the data. CH-L, MV-C, GB-R, and FC-M made substantial contributions to the final draft of the manuscript. All authors read and approved the final manuscript for its publication.

Funding

This study was funded by the Ministry of Social Rights and 2030 Agenda (grant number 198/2020). The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article, or the decision to submit it for publication.

Acknowledgments

We acknowledge the contributions of specific colleagues, institutions, or agencies that aided the efforts of the authors.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2023.1088727/full#supplementary-material

References

1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, Text Revision (DSM-5-TR)*. Washington, DC: American Psychiatric Association (2022).

2. World Health Organization [WHO]. *International Classification of Diseases 10th Revision - ICD-10*. Geneva: World Health Organization (1992).

3. Zeidan J, Fombonne E, Scorah J, Ibrahim A, Durkin MS, Saxena S, et al. Global prevalence of autism: a systematic review update. *Autism Res.* (2022) 15:778–90. doi: 10.1002/aur.2696

4. Chiarotti F, Venerosi A. Epidemiology of autism spectrum disorders: a review of worldwide prevalence estimates since 2014. *Brain Sci.* (2020) 10:274. doi: 10.3390/brainsci10050274

5. Lord C, Brugha TS, Charman T, Cusack J, Dumas G, Frazier T, et al. Autism spectrum disorder. *Nat Rev Dis Primers.* (2020) 6:5. doi: 10.1038/s41572-019-0138-4

6. Autism Europe. *Prevalence rate of autism – Autism Europe. Recuperado 20 de diciembre de*. Brussels: Autism Europe (2021).

7. Autism Spectrum Disorders in the European Union [ASDEU]. *ASDEU - Findings*. Dwarka: Autism Spectrum Disorders in the European Union (2021).

8. Institute for Health Metrics and Evaluation [IHME], The Global Burden of Disease Study [GBD]. *Autism spectrum disorders — Level 3 cause*. Seattle: Institute for Health Metrics and Evaluation (2019).

9. Instituto Nacional de Estadística [INE]. *Population in Spain*. Madrid: Instituto Nacional de Estadística (2021).

10. Morales-Hidalgo P, Roigé-Castellví J, Hernández-Martínez C, Voltas N, Canals J. Prevalence and characteristics of autism spectrum disorder among spanish school-age children. *J Autism Dev Disord.* (2018) 48:3176–90. doi: 10.1007/s10803-018-3581-2

11. Institute of Social Services and the Elderly [IMSERSO]. *Persons with Disabilities National Database*. Madrid: Institute of Social Services and the Elderly (2019).

12. Ministry of Education and Vocational Training. *EDUCAbase. Non-university Education. Educational support needs. 2019/2020*. Dar es Salaam: Ministry of Education and Vocational Training (2020).

13. Spanish National Institute of Statistics [INE]. *Disability, Independence and Dependency Situations Survey (DIDSS)*. Paseo de la Castellana: Spanish National Institute of Statistics (2008).

14. Spanish National Institute of Statistics [INE]. *Disabilities, Independence and Dependency Situations Survey (DIDSS). Methodology*. Paseo de la Castellana: Spanish National Institute of Statistics (2010).

15. Ministry of Health. *2017 Spanish Health Survey. Main results*. New Delhi: Ministry of Health (2018).

16. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5)*. Washington, DC: American Psychiatric Association (2013).

17. Bilder D, Botts EL, Smith KR, Pimentel R, Farley M, Viskochil J, et al. Excess mortality and causes of death in autism spectrum disorders: a follow up of the 1980s Utah/UCLA autism epidemiologic study. *J Autism Dev Disord.* (2013) 43:1196–204. doi: 10.1007/s10803-012-1664-z

18. Gillberg C, Billstedt E, Sundh V, Gillberg IC. Mortality in autism: a prospective longitudinal community-based study. *J Autism Dev Disord.* (2010) 40:352–7. doi: 10.1007/s10803-009-0883-4

19. Pickett JA, Paculdo DR, Shavelle RM, Strauss DJ. 1998-2002 Update on «Causes of death in autism». *J Autism Dev Disord.* (2006) 36:287–8. doi: 10.1007/s10803-005-0066-x

20. Woolfenden S, Sarkozy V, Ridley G, Coory M, Williams K. A systematic review of two outcomes in autism spectrum disorder – epilepsy and mortality. *Dev Med Child Neurol.* (2012) 54:306–12. doi: 10.1111/j.1469-8749.2012.04223.x

21. Lai M-C, Lombardo MV, Baron-Cohen S. Autism. *Lancet.* (2014) 383:896–910. doi: 10.1016/S0140-6736(13)61539-1

22. Muskens JB, Velders FP, Staal WG. Medical comorbidities in children and adolescents with autism spectrum disorders and attention deficit hyperactivity disorders: a systematic review. *Eur Child Adolesc Psychiatry.* (2017) 26:1093–103. doi: 10.1007/s00787-017-1020-0

23. Theoharides TC, Tsilioni I, Patel AB, Doyle R. Atopic diseases and inflammation of the brain in the pathogenesis of autism spectrum disorders. *Transl Psychiatry.* (2016) 6:e844–844. doi: 10.1038/tp.2016.77

24. Croen LA, Zerbo O, Qian Y, Massolo ML, Rich S, Sidney S, et al. The health status of adults on the autism spectrum. *Autism.* (2015) 19:814–23. doi: 10.1177/1362361315577517

25. Tyler CV, Schramm SC, Karafa M, Tang AS, Jain AK. Chronic disease risks in young adults with autism spectrum disorder: forewarned is forearmed. *Am J Intellect Dev Disabil.* (2011) 116:371–80. doi: 10.1352/1944-7558-116.5.371

26. Lai M-C, Kassee C, Besney R, Bonato S, Hull L, Mandy W, et al. Prevalence of co-occurring mental health diagnoses in the autism population: a systematic review and meta-analysis. *Lancet Psychiatry.* (2019) 6:819–29. doi: 10.1016/S2215-0366(19)30289-5

27. Simonoff E, Pickles A, Charman T, Chandler S, Loucas T, Baird G. Psychiatric Disorders in Children With Autism Spectrum Disorders: Prevalence, Comorbidity, and Associated Factors in a Population-Derived Sample. *J Am Acad Child Adolesc Psychiatry.* (2008) 47:921–9. doi: 10.1097/CHI.0b013e318179964f

28. Bishop-Fitzpatrick L, Kind AJH. A scoping review of health disparities in autism spectrum disorder. *J Autism Dev Disord.* (2017) 47:3380–91. doi: 10.1007/S10803-017-3251-9

29. Mason D, Ingham B, Urbanowicz A, Michael C, Birtles H, Woodbury-Smith M, et al. a systematic review of what barriers and facilitators prevent and enable physical healthcare services access for autistic adults. *J Autism Dev Disord.* (2019) 49:3387–400. doi: 10.1007/S10803-019-04049-2

30. DaWalt LS, Taylor JL, Movaghar A, Hong J, Kim B, Brilliant M, et al. Health profiles of adults with autism spectrum disorder: Differences between women and men. *Autism Res.* (2021) 14:1896–904. doi: 10.1002/AUR.2563

31. Howlin P. Adults with autism: changes in understanding since DSM-111. *J Autism Dev Disord.* (2021) 51:4291–308. doi: 10.1007/s10803-020-04847-z

32. Miot S, Akbaraly T, Michelon C, Couderc S, Crepiat S, Loubersac J, et al. Comorbidity burden in adults with autism spectrum disorders and intellectual disabilities—a report from the EFAAR (Frailty Assessment in Ageing Adults With Autism Spectrum and Intellectual Disabilities) Study. *Front Psychiatry.* (2019) 10:617. doi: 10.3389/FPSYT.2019.00617/BIBTEX

33. Rydzewska E, Hughes-McCormack LA, Gillberg C, Henderson A, MacIntyre C, Rintoul J, et al. General health of adults with autism spectrum disorders – A whole country population cross-sectional study. *Res Autism Spect Disord.* (2019) 60:59–66. doi: 10.1016/j.rasd.2019.01.004

34. Simantov T, Pohl A, Tsompanidis A, Weir E, Lombardo MV, Ruigrok A, et al. Medical symptoms and conditions in autistic women. *Autism.* (2021) 26:373–88. doi: 10.1177/13623613211022091

35. Fortuna RJ, Robinson L, Smith TH, Meccarello J, Bullen B, Nobis K, et al. Health conditions and functional status in adults with autism: a cross-sectional evaluation. *J Gen Intern Med.* (2015) 31:77–84. doi: 10.1007/s11606-015-3509-x

36. Lindly OJ, Chan J, Levy SE, Parker RA, Kuhlthau KA. Service use classes among school-aged children from the autism treatment network registry. *Pediatrics.* (2020) 145(Suppl. 1):S140–50. doi: 10.1542/peds.2019-1895Q

37. Micai M, Ciaramella A, Salvitti T, Fulceri F, Fatta LM, Poustka L, et al. Autistic Adult Health and Professional Perceptions of It: Evidence From the ASDEU Project. *Front Psychiatry.* (2021) 12:689. doi: 10.3389/FPSYT.2021.614102

38. Confederación Autismo España. *Participa en nuestros estudios de investigación*. Madrid: Confederación Autismo España (2014).

39. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR)*. Washington, DC: American Psychiatric Association (2000).

40. World Health Organization [WHO]. *The ICD-10 Classification of Mental and Behavioural Disorders*. Geneva: World Health Organization (1993).

41. Bozzi Y, Provenzano G, Casarosa S. Neurobiological bases of autism-epilepsy comorbidity: a focus on excitation/inhibition imbalance. *Eur J Neurosci.* (2018) 47:534–48. doi: 10.1111/EJN.13595

42. Jacob J. Cortical interneuron dysfunction in epilepsy associated with autism spectrum disorders. *Epilepsia.* (2016) 57:182–93. doi: 10.1111/epi.13272

43. Pan PY, Bölte S, Kaur P, Jamil S, Jonsson U. Neurological disorders in autism: a systematic review and meta-analysis. *Autism.* (2021) 25:812–30. doi: 10.1177/1362361320951370

44. Institute of Social Services and the Elderly [IMSERSO]. *Degree of disability. Personal Autonomy and Dependence*. Madrid: Institute of Social Services and the Elderly (2020).

45. Van 't Hof M, Tisseur C, van Berckelear-Onnes I, van Nieuwenhuyzen A, Daniels AM, Deen M, et al. Age at autism spectrum disorder diagnosis: a systematic review and meta-analysis from 2012 to 2019. *Autism*. (2021) 25:862–73. doi: 10.1177/1362361320971107

46. Dworzynski K, Ronald A, Bolton P, Happé F. How different are girls and boys above and below the diagnostic threshold for autism spectrum disorders? *J Am Acad Child Adolesc Psychiatry.* (2012) 51:788–97. doi: 10.1016/J.JAAC.2012.05.018

47. Rutter M. Autism research: lessons from the past and prospects for the future. *J Autism Dev Disord.* (2005) 35:241–57. doi: 10.1007/s10803-004-2003-9

48. Egerton J, Carpenter B. *Girls and Autism: Flying Under the Radar. A quick guide to supporting girls with autism spectrum conditions*. London: Nasen (2016).

49. Loomes R, Hull L, Mandy WPL. What is the male-to-female ratio in autism spectrum disorder? a systematic review and meta-analysis. *J Am Acad Child Adolesc Psychiatry.* (2017) 56:466–74. doi: 10.1016/j.jaac.2017.03.013

50. Begeer S, Mandell D, Wijnker-Holmes B, Venderbosch S, Rem D, Stekelenburg F, et al. Sex differences in the timing of identification among children and adults with autism spectrum disorders. *J Autism Dev Disord.* (2013) 43:1151–6. doi: 10.1007/s10803-012-1656-z

51. Gould J, Ashton-Smith J. Missed diagnosis or misdiagnosis? Girls and women on the autism spectrum. *Good Autism Pract.* (2011) 12:34–41.

52. Hiller RM, Young RL, Weber N. Sex differences in pre-diagnosis concerns for children later diagnosed with autism spectrum disorder. *Autism.* (2016) 20:75–84. doi: 10.1177/1362361314568899

53. Beggiato A, Peyre H, Maruani A, Scheid I, Rastam M, Amsellem F, et al. Gender differences in autism spectrum disorders: Divergence among specific core symptoms. *Autism Res.* (2017) 10:680–9. doi: 10.1002/AUR.1715

54. Rynkiewicz A, Schuller B, Marchi E, Piana S, Camurri A, Lassalle A, et al. An investigation of the 'female camouflage effect' in autism using a computerized ADOS-2 and a test of sex/gender differences. *Mol Autism.* (2016) 7:10. doi: 10.1186/s13229-016-0073-0

55. Lyall K, Croen L, Daniels J, Fallin MD, Ladd-Acosta C, Lee BK, et al. The changing epidemiology of autism spectrum disorders. *Annu Rev Public Health.* (2017) 38:81–102. doi: 10.1146/annurev-publhealth-031816-044318

56. Modabbernia A, Velthorst E, Reichenberg A. Environmental risk factors for autism: an evidence-based review of systematic reviews and meta-analyses. *Mol Autism.* (2017) 8:13. doi: 10.1186/s13229-017-0121-4

57. Pehlivanidis A, Papanikolaou K, Mantas V, Kalantzi E, Korobili K, Xenaki LA, et al. Lifetime co-occurring psychiatric disorders in newly diagnosed adults with attention deficit hyperactivity disorder (ADHD) or/and autism spectrum disorder (ASD). *BMC Psychiatry.* (2020) 20:423. doi: 10.1186/S12888-020-02828-1

58. Li YA, Chen ZJ, Li XD, Gu MH, Xia N, Gong C, et al. Epidemiology of autism spectrum disorders: Global burden of disease 2019 and bibliometric analysis of risk factors. *Front Pediatr.* (2022) 10:972809. doi: 10.3389/fped.2022.972809

59. 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.* (2021) 19:33. doi: 10.1186/S12955-021-01669-0

60. National Institute for Health and Care Excellence [NICE]. *Autism spectrum disorder in adults: diagnosis and management*. Ra'anana: NICE (2012).

61. National Institute for Health and Care Excellence [NICE]. *Autism spectrum disorder in under 19s: support and management. Clinical guideline [CG170]*. Ra'anana: NICE (2013).

62. Micai M, Ciaramella A, Salvitti T, Fulceri F, Fatta LM, Poustka L, et al. Intervention services for autistic adults: an asdeu study of autistic adults, carers, and professionals' experiences. *J Autism Dev Disord.* (2021) 2021:1–17. doi: 10.1007/S10803-021-05038-0

63. Royal College of Psychiatrists. *Position Statement PS05/21: Stopping the overprescribing of people with intellectual disability, autism or both (STOMP) and supporting treatment and appropriate medication in paediatrics (STAMP).* London: Royal College of Psychiatrists (2021).

64. Ritter C, Hewitt K, McMorris CA. Psychotropic polypharmacy among children and youth with autism: a systematic review. *J Child Adolesc Psychopharmacol.* (2021) 31:244–58. doi: 10.1089/cap.2020.0110

65. Lai MC, Szatmari P. Sex and gender impacts on the behavioural presentation and recognition of autism. *Curr Opin Psychiatry.* (2020) 33:117–23. doi: 10.1097/YCO.0000000000000575

66. Oakley BFM, Tillmann J, Ahmad J, Crawley D, San José Cáceres A, Holt R, et al. How do core autism traits and associated symptoms relate to quality of life? Findings from the Longitudinal European Autism Project. *Autism.* (2021) 25:389–404. doi: 10.1177/1362361320959959

67. Avni E, Ben-Itzchak E, Zachor DA. The presence of comorbid ADHD and anxiety symptoms in autism spectrum disorder: clinical presentation and predictors. *Front Psychiatry.* (2018) 9:717. doi: 10.3389/fpsyt.2018.00717

68. van Steensel FJA, Bögels SM, Perrin S. Anxiety disorders in children and adolescents with autistic spectrum disorders: a meta-analysis. *Clin Child Fam Psychol Rev.* (2011) 14:302–17. doi: 10.1007/s10567-011-0097-0

69. Lau-Zhu A, Fritz A, McLoughlin G. Overlaps and distinctions between attention deficit/hyperactivity disorder and autism spectrum disorder in young adulthood: Systematic review and guiding framework for EEG-imaging research. *Neurosci Biobehav Rev.* (2019) 96:93–115. doi: 10.1016/j.neubiorev.2018.10.009

70. May ME, Kennedy CH. Health and problem behavior among people with intellectual disabilities. *Behav Anal Pract.* (2010) 3:4–12. doi: 10.1007/BF03391759

71. van Schrojenstein Lantman-De Valk HM. Health problems in people with intellectual disability in general practice: a comparative study. *Family Pract.* (2000) 17:405–7. doi: 10.1093/fampra/17.5.405

72. 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) 3:104–15. doi: 10.1007/S40474-016-0085-7

73. Micai M, Fulceri F, Salvitti T, Romano G, Poustka L, Diehm R, et al. Autistic adult services availability, preferences, and user experiences: results from the autism spectrum disorder in the european union survey. *Front psychiatry.* (2022) 13:919234. doi: 10.3389/fpsyt.2022.919234

74. Sala R, Amet L, Blagojevic-Stokic N, Shattock P, Whiteley P. Bridging the gap between physical health and autism spectrum disorder. *Neuropsychiatr Dis Treat.* (2020) 16:1605–18. doi: 10.2147/NDT.S251394

75. Cortese M, Moster D, Wilcox AJ. Term birth weight and neurodevelopmental outcomes. *Epidemiology.* (2021) 32:583–90. doi: 10.1097/EDE.0000000000001350

76. Hisle-Gorman E, Susi A, Stokes T, Gorman G, Erdie-Lalena C, Nylund CM. Prenatal, perinatal, and neonatal risk factors of autism spectrum disorder. *Pediatr Res.* (2018) 84:190–8. doi: 10.1038/PR.2018.23

77. Lyall K, Schmidt RJ, Hertz-Picciotto I. Maternal lifestyle and environmental risk factors for autism spectrum disorders. *Int J Epidemiol.* (2014) 43:443–64.

78. Lockwood Estrin G, Milner V, Spain D, Happé F, Colvert E. Barriers to autism spectrum disorder diagnosis for young women and girls: a systematic review. *Rev J Autism Dev Disord.* (2021) 8:454–70. doi: 10.1007/s40489-020-00225-8

79. David N, Dückert S, Gewohn P, König H, Rahlff P, Erik F, et al. Mixed-methods investigation of barriers and needs in mental healthcare of adults with autism and recommendations for future care (BarrierfreeASD): study protocol. *BMJ open.* (2022) 12:e061773. doi: 10.1136/bmjopen-2022-061773

80. den Houting J. *Participatory and inclusive autism Research Practice Guides*. Brisbane: Autism CRC (2021).

81. Keating CT. Participatory autism research: how consultation benefits everyone. *Front Psychol.* (2021) 12:713982. doi: 10.3389/fpsyg.2021.713982

frontiers | Frontiers in Psychiatry

TYPE Original Research
PUBLISHED 30 March 2023
DOI 10.3389/fpsyt.2023.1116239

OPEN ACCESS

EDITED BY
David Saldaña,
Sevilla University,
Spain

REVIEWED BY
George Golovko,
University of Texas Medical Branch at Galveston,
United States
Guo Min,
Shanghai Institute for Biomedical and Pharmaceutical Technologies,
China

*CORRESPONDENCE
James Cheng-Chung Wei
jccwei@gmail.com
Jing-Yang Hung
wchinyang@gmail.com

†These authors share co-first authorship

SPECIALTY SECTION
This article was submitted to Autism, a section of the journal Frontiers in Psychiatry

RECEIVED 05 December 2022
ACCEPTED 28 February 2023
PUBLISHED 30 March 2023

CITATION
Lee Y-F, Wu M-C, Ma KS-K, Huang J-Y and Wei JC-C (2023) Association of early childhood constipation with the risk of autism spectrum disorder in Taiwan: Real-world evidence from a nationwide population-based cohort study. *Front. Psychiatry* 14:1116239. doi: 10.3389/fpsyt.2023.1116239

Association of early childhood constipation with the risk of autism spectrum disorder in Taiwan: Real-world evidence from a nationwide population-based cohort study

Yi-Feng Lee[1,2†], Meng-Che Wu[2,3,4†], Kevin Sheng-Kai Ma[5,6,7], Jing-Yang Huang[8,9*] and James Cheng-Chung Wei[9,10,11*]

[1]Division of Neonatology, Children's Medical Center, Taichung Veterans General Hospital, Taichung, Taiwan, [2]School of Medicine, Chung Shan Medical University, Taichung, Taiwan, [3]Division of Gastroenterology, Children's Medical Center, Taichung Veterans General Hospital, Taichung, Taiwan, [4]Department of Post-Baccalaureate Medicine, College of Medicine, National Chung Hsing University, Taichung, Taiwan, [5]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, United States, [6]Center for Global Health, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, United States, [7]Department of Orthodontics and Dentofacial Orthopedics, Henry M. Goldman School of Dental Medicine, Boston University, Boston, MA, United States, [8]Center for Health Data Science, Chung Shan Medical University Hospital, Taichung, Taiwan, [9]Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan, [10]Division of Allergy, Immunology and Rheumatology, Chung Shan Medical University Hospital, Taichung, Taiwan, [11]Graduate Institute of Integrated Medicine, China Medical University, Taichung, Taiwan

Background: Autism spectrum disorder (ASD) is a neurodevelopmental problem that presents with limited interests, repetitive behaviors, and deficits in reciprocal communication and social interactions. Mounting evidence indicates that an imbalanced gut microbiota contributes to autism *via* the gut-brain axis. Constipation may result in alteration of the gut microbiota. The clinical influence of constipation on ASD has not been fully researched. Thus, in this study we aimed to evaluate whether early childhood constipation influenced the risk of developing ASD using a nationwide population-based cohort study.

Methods: We identified 12,935 constipated children aged 3years or younger from the National Health Insurance Research Database (NHIRD) in Taiwan from 1997 to 2013. Non-constipated children were also selected from the database and propensity score matching of age, gender, and underlying comorbidities was conducted with a ratio of 1:1. Kaplan–Meier analysis was applied to determine different levels of constipation severity and cumulative incidence of autism. Subgroup analysis was also applied in this study.

Results: The incidence rate of ASD was 12.36 per 100,000 person-months in the constipation group, which was higher than the rate of 7.84 per 100,000 person-months noted in the non-constipation controls. Constipated children had a significantly higher risk of autism when compared to the non-constipation group (crude relative risk=1.458, 95% CI=1.116–1.904; adjusted hazard ratio=1.445, 95% CI=1.095–1.907).

Moreover, among constipated children, a higher number of laxative prescriptions, male gender, constipation during infancy, and atopic dermatitis were significantly associated with higher risks of ASD when compared to the non-constipation group.

Conclusion: Constipation in early childhood was correlated with a significantly increased risk of ASD. Clinicians should pay attention to the possibility of ASD in constipated children. Further research is necessary to study the possible pathophysiological mechanisms of this association.

KEYWORDS

constipation, autism, microbiota, national cohort study, infant constipation, autistic spectrum disorder, National Health Insurance Research Database, gut-brain axis

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental problem characterized by persistent deficits in reciprocal communication and social interaction. Children with ASD often present with limited interests, repetitive behavior, and varying levels of intellectual disability (1). In a recent meta-analysis, a higher prevalence was noted in boys compared with girls, with a male-to-female ratio of about 3 to 1 (2). A male-to-female ratio of 7 to 1 was found among 13,000 autistic patients in Taiwan, according to data from the Ministry of Health and Welfare in 2018 (3). Despite the increased prevalence of ASD in the past few decades, the underlying etiological factors remain unclear and there seems to be complicated interactions between genetic and environmental factors. These factors and the resultant variety of symptoms mean that the therapeutic targets are complex. Efforts are underway to find genetic or pathophysiological pathways shared by animal models and humans in order to identify a common method for better treatment targeting (4). The roles of prenatal exposure to risk factors, such as maternal-specific drug use, prenatal steroid exposure, old parental age, and use of antibiotics in the prevention of autism have been investigated in recent years (5–7). The latest studies have focused on biological molecular mechanisms, such as short chain fatty acid, which is considered to be a key connection between gut microbiota and mental diseases, brain-derived neurotrophic factor (BDNF), lipopolysaccharide (LPS), indole, as well as other immunological biomarkers appear to be involved in the biochemical mechanism of autism (8–12). However, exposure to risk factors and the mechanisms of autism in young children, especially in toddlers and infants have not been well studied yet.

Constipation is a common problem in the pediatric population. Even if constipation does not pose a serious threat to health, the related symptoms often have a highly detrimental effect on quality of life (QoL) in children, such as physical pain, emotional distress, social interaction, school life, and vitality (13). If left untreated, there may be persistent follow-up visits to the outpatient department (OPD), emergent hospital visits, and increased cost of health burden (14). In recent studies, constipation was shown to increase the risk of worsening renal function, childhood nocturnal enuresis, Parkinson's disease, and allergic rhinitis, among other conditions (15–18). As for the treatment of constipation, prokinetic agents, osmo-or non-osmotic laxative agents, and probiotics are now used to relieve constipation and to regulate the bacterial flora in the intestinal tract (19, 20). Researchers have investigated the impact of constipation on multiple systems in our body (21). Dysbiosis may be the shared pathway between those disorders. Furthermore, the phenomenon of altered gut microbiome, if discovered to be a marked risk factor of autism, is potentially more treatable, analyzable, and preventable, compared with other disease risk factors and exposures, such as antenatal exposure, maternal metabolic condition, metabolic disorders in newborn, and genetic expression.

Dysbiosis, known as an imbalance in the composition or function of the microbial species in the intestinal tract, is believed to be one of the results of or an aggravating factor in constipation (19). It has been shown to induce alterations in the body, and to affect systemic pathways, which in turn have an impact on our central nervous system (CNS) (22, 23). The "microbiome-gut-brain axis" is known to play an important role in the pathogenesis of neurodevelopmental diseases, such as ASD (24).

Interestingly, in children with ASD, constipation is known to be a clinically significant gastrointestinal symptom, and severity of constipation has been shown to be correlated with autistic symptoms. (25–27). This correlation serves as a reminder that constipation may alter the gut microenvironment by significantly elevating the amount of specific kinds of bacteria, lowering bacterial diversity, and inducing an abnormal level of short chain fatty acids, phenomena which have been proven to be associated with ASD (28–32). Although the microbiome-gut-brain axis and its mechanisms, as well as the relationships between risk factors and diseases, including related treatments, have been extensively studied, there are no data focusing on the association between early-aged pediatric constipation and subsequent risk of an ASD diagnosis. We thus hypothesized that early childhood constipation might lead to a higher ASD risk *via* the microbiome-gut-brain axis and investigated the association between early childhood constipation and ASD by assessing a population-based retrospective, nationwide cohort from Taiwan's National Health Institute Research Database (NHIRD).

Materials and methods

Data sources and study design

This was a retrospective cohort study using data collected from the National Health Insurance Research Database (NHIRD). The National Health Insurance (NHI) program provides healthcare for more than 99% of Taiwan's population of around 23 million people. The NHIRD comprises all NHI claims data, including not only medical records of treatments and diagnoses from the outpatient department, but also documents recorded during hospitalization and in the emergency room (33). Eligible pediatric patients diagnosed

with constipation during 1997–2013 were identified from the Longitudinal Health Insurance Database (LHID), which is a subset of one million subjects randomly sampled from the 23 million NHI beneficiaries in the NHIRD. Patients in the LHID are not statistically different from those in the NHIRD in terms of demographic distribution.

To guarantee the validity of these data, both autism and constipation required confirmation by not less than two OPD records or at least one inpatient discharge diagnosis, and all diagnoses were peer-reviewed. The sampled data were de-identified. The study was approved by the Institutional Review Board of Chung Shan Medical University and Hospital in Taichung, Taiwan (Approval number CS15134).

Case definition for the constipation cohort and selection of comparisons

We retrieved insurance claim records from the LHID for the period 1997 to 2013. A diagnosis of constipation in pediatric patients was defined as an ICD-9-CM code 564.0 in two or more OPD visits or in at least one inpatient discharge note for children younger than 3 years old during the period 2001 to 2011, so as to validate the diagnosis of autism and constipation. The number of autism cases identified after early-aged constipation was compared with that in non-constipated children. We defined the first date of constipation diagnosis +1 year as the index date. For early-aged children with constipation, their history of exposure to laxatives (ATC code: A06A, A02AA02) was identified between constipation diagnosis and the index date, that was used to estimate the severity levels of constipation and the effect on the development of autism. The enrolled children were followed up until the diagnosis of autism, 31 December 2013, or withdrawal from the national health insurance system, whichever happened prior to constipation. In order to ensure that every autistic condition developed after constipation, we excluded the following children: (1) pediatric patients with missing demographics, (2) born before 2001, (3) children diagnosed with autism before the index date, (4) those whose diagnoses were made after 2011.

In order to reduce potential selection bias to a minimum, propensity score matching was utilized in this study to select non-constipation children, matching for age, gender, underlying comorbidities, and index year, in order to minimize the influences of potential confounders. The propensity score was estimated by logistic regression, and was used to match the constipation and non-constipation individuals at a ratio of 1:1. Absolute standardized difference (AbSD) was calculated to ensure that the selection of the constipation numbers and the matched non-constipation controls was not biased. When AbSD was 0.1 or less, the features of both groups could be regarded as similar. Therefore, propensity score matching was used to balance the differences of baseline features and underlying comorbidities between these two groups. Using propensity score matching, participants without current or a history of constipation were selected as controls accordingly. As antibiotics are known to have serious effects on microbiome composition, antibiotics exposure (ATC code: J01, A07A) of more than 28 days within 1 year before the index date was propensity controlled and sensitivity analyzed.

Outcome measurements in the constipation cohort

As the primary outcome of the constipation cohort, the diagnosis of autism was retrieved using the ICD-9-CM code 299.x for ASD at two outpatient visits or in at least one inpatient discharge note. To identify the children who had higher risk of autism following constipation, subgroup analyses and multiple Cox regression of age, gender, socioeconomic status, urbanization, hospitalized stays, underlying comorbidities, and co-medication were conducted to determine any associations. Ages of constipation onset were stratified to 0–1, 1–2, and 2–3 (<3) years.

Covariates and comorbidities adjusted by propensity score matching

The baseline features included age, sex, and underlying comorbidities, such as asthma (ICD-9-CM = 493), atopic dermatitis (ICD-9-CM = 691), preterm or low birth weight (ICD-9-CM = 765), neonatal infections (ICD-9-CM = 771), congenital malformations (ICD-9-CM = 740–759), allergic rhinitis (ICD-9-CM = 477), urticaria (ICD-9-CM = 708), intestinal infectious diseases (ICD-9-CM = 001–009), noninfective enteritis and colitis (ICD-9-CM = 555–558), and metabolic conditions [ICD-9-CM = 250, 260–269, 270–279, 774, 775 (including diabetes mellitus, nutritional deficiency, and inborn error disorders)], as well as use of co-medications that include the use of antihistamines (ATC code = R06, D04AA) and antibiotics (ATC code = J01, A07A) for ≥28 days within 1 year before the index date. The comorbidities which were noted between birth and the index date for at least one hospitalization or two outpatient visits were analyzed. After propensity score matching, the effects of the comorbidities and co-medications on the outcome were better controlled and the differences were minimized.

Statistical analysis

Overall, using a large, national health insurance database, we retrieved data from early-aged (up to 3 years old) constipated children longitudinally, set a one-year washout period to minimize possible causes other than constipation, and compared the study group to the age-and sex-matched non-constipated children in order to determine whether early constipation increases the risk of a subsequent diagnosis of autism. The balance of baseline demographics and underlying comorbidities between the constipation group and the non-constipation group were evaluated by and presented with AbSD. In this step of analysis, the possible confounding factors were all controlled and matched. Other than basic personal and geographic data, comorbidities such as allergic diseases, infectious problems, inflammatory conditions, metabolic disorders, and certain drug exposures that were believed to have a potential impact on the mechanism of either constipation or autism were all included in the analysis. Next, the severity of constipation was stratified into three categories based on the numbers of prescriptions for laxative agents. Kaplan–Meier analysis was applied either before and after PSM to assess the cumulative incidence of autism, including the dose-defined

severity analysis, and determine the causal relationship between constipation and autism. Log-rank test was utilized to calculate the significance of differences between the groups. A Cox proportional hazard model was applied to evaluate the hazard ratio of autism between the study groups. In the next step, multiple Cox regression and subgroup analysis were applied to identify the interaction within groups or between other factors and the study group. Finally, due to the marked effects that exposure to antibiotics may have on microbiome composition, which could strongly confound our primary outcome, sensitivity analysis was performed to exclude antibiotics exposure prior to the index date. The statistical software used was SAS version 9.4 (Statistical Analysis Software 9.4, SAS Institute Inc., Cary, North Carolina, United States).

Results

Basic demographics of the study subjects

A total of 12,935 children who were newly diagnosed with constipation were identified from the LHID. Patients who had been diagnosed with ASD before the index date ($n = 13$) were excluded (Figure 1). There were 12,922 constipation patients and 25,844 non-constipated patients who were matched individually by sex and birth year and selected for further analysis (Figure 1). After propensity score matching of these potential confounding factors, 12,469 subjects with constipation and an equal number of subjects without constipation were selected for inclusion in the final cohort (Table 1). Patients with constipation had a higher prevalence of asthma (14.46 vs. 10.50%, with AbSD = 0.120), atopic dermatitis (48.68 vs. 36.71%, with AbSD = 0.244), allergic rhinitis (27.60 vs. 17.88%, with AbSD = 0.131), urticaria (21.40 vs. 16.30%, with AbSD = 0.131), intestinal infectious diseases (47.11% vs. 37.63%, with AbSD = 0.193), noninfective enteritis and colitis (74.25% vs. 63.25%, with AbSD = 0.239), and metabolic conditions (28.39% vs. 19.49%, with AbSD = 0.210), as well as greater prevalence rates of antihistamines use (74.95 vs. 56.48%, with AbSD = 0.397), and antibiotics use (17.46 vs. 11.12%, with AbSD = 0.182) before propensity score matching; whereas the other comorbidities and risk factors for ASD, including preterm or low birth weight, neonatal infections, and congenital malformations of brain, were not different between the constipation and non-constipation groups (AbSD < 0.1). With respect to the personal data between the age-and sex-matched non-constipation and constipation group, geographic area distribution (AbSD = 0.130) and hospitalized stays (AbSD = 0.230) seemed to be comparable between the constipation and non-constipation group; whereas the socioeconomic status (represented by insured unit type), and urbanization did not show any differences (AbSD < 0.1). After propensity score matching, there were no statistically significant differences in any of the aforementioned possible confounding risk factors, nor were there any differences in underlying comorbidities between the constipation group and the non-constipation group (all AbSD < 0.1; Table 1).

Risk of autism in patients who had constipation

Among the 12,922 patients with constipation, 125 cases developed autism after constipation onset, which was identified in over 1,011,294 observed person-months. The incidence rate (IR) of autism was significantly higher in constipated children than in the non-constipation controls (12.36 vs. 7.84 per 100,000 person-months). Children who had constipation showed a significantly

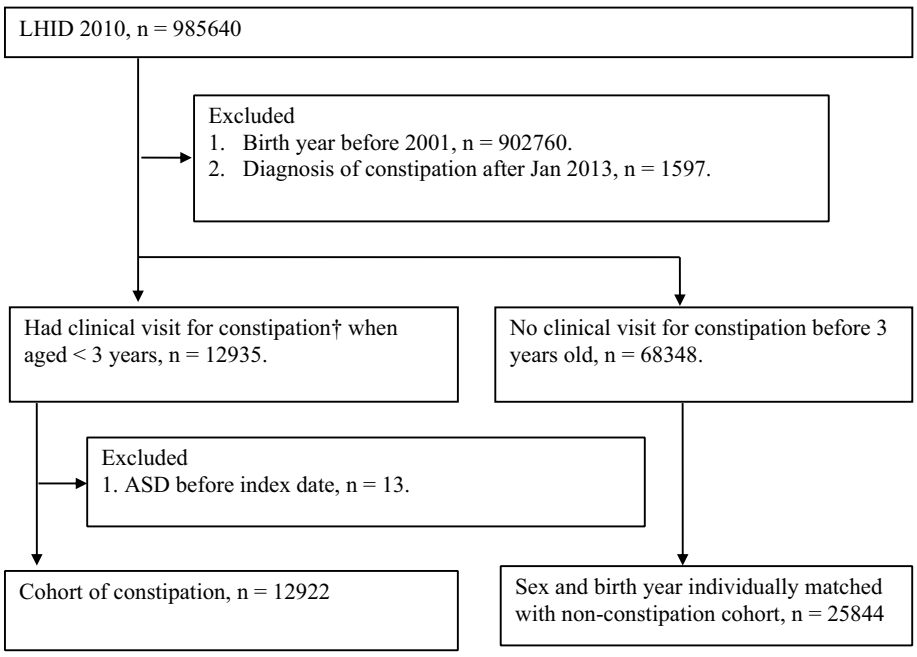


FIGURE 1
study flow chart. † Patients had constipation (ICD-9564.0, two or more OPD visits or at least one inpatient discharge note) when aged <3years from 2001 to 2011.

TABLE 1 Baseline characteristics among study groups.

	Age- and sex-matched			PSM		
	Non-constipation	Constipation	AbSD	Non-constipation	Constipation	AbSD
N	25,844	12,922		12,469	12,469	
Sex			0.000			0.003
Female	13,738 (53.16%)	6,869 (53.16%)		6,623 (53.12%)	6,643 (53.28%)	
Male	12,106 (46.84%)	6,053 (46.84%)		5,846 (46.88%)	5,826 (46.72%)	
Age at diagnosis	0.974 ± 0.743	0.974 ± 0.743	0.000	0.983 ± 0.740	0.976 ± 0.743	0.009
0–1	7,484 (28.96%)	3,742 (28.96%)		3,524 (28.26%)	3,590 (28.79%)	
1–2	11,558 (44.72%)	5,779 (44.72%)		5,636 (45.20%)	5,587 (44.81%)	
2–3	6,802 (26.32%)	3,401 (26.32%)		3,309 (26.54%)	3,292 (26.40%)	
Insured unit type			0.054			0.058
Government	1,348 (5.22%)	614 (4.75%)		552 (4.43%)	597 (4.79%)	
Privately held company	11,872 (45.94%)	6,064 (46.93%)		5,878 (47.14%)	5,848 (46.90%)	
Agricultural organizations	1873 (7.25%)	1,093 (8.46%)		1,063 (8.53%)	1,049 (8.41%)	
Low-income	81 (0.31%)	35 (0.27%)		24 (0.19%)	35 (0.28%)	
Non-labor force	2,522 (9.76%)	1,168 (9.04%)		1,076 (8.63%)	1,128 (9.05%)	
Others	8,148 (31.53%)	3,948 (30.55%)		3,876 (31.09%)	3,812 (30.57%)	
Urbanization			0.090			0.000
Urban	15,515 (60.03%)	7,249 (56.10%)		7,085 (56.82%)	7,090 (56.86%)	
Suburban	7,999 (30.95%)	4,284 (33.15%)		4,166 (33.41%)	4,145 (33.24%)	
Rural	2,330 (9.02%)	1,389 (10.75%)		1,218 (9.77%)	1,234 (9.90%)	
Geographic area			0.130			0.069
Taipei	9,356 (36.36%)	4,080 (31.79%)		3,973 (31.86%)	4,011 (32.17%)	
North	4,282 (16.64%)	1952 (15.21%)		1917 (15.37%)	1909 (15.31%)	
Central	4,833 (18.78%)	2,745 (21.39%)		2,636 (21.14%)	2,654 (21.28%)	
South	3,313 (12.87%)	1857 (14.47%)		1810 (14.52%)	1785 (14.32%)	
Kaohsiung/Pingtung	3,423 (13.30%)	1923 (14.98%)		1898 (15.22%)	1845 (14.80%)	
East	528 (2.05%)	279 (2.17%)		235 (1.88%)	265 (2.13%)	
Baseline hospitalized stays			0.230			0.041
0 days	22,050 (85.32%)	9,772 (75.62%)		9,728 (78.02%)	9,636 (77.28%)	
1–6 days	2,751 (10.64%)	2,169 (16.79%)		1953 (15.66%)	1993 (15.98%)	
≥7 days	1,043 (4.04%)	981 (7.59%)		788 (6.32%)	840 (6.74%)	
Baseline comorbidities						
Asthma	2,713 (10.50%)	1869 (14.46%)	0.120	1,657 (13.29%)	1732 (13.89%)	0.018
Atopic dermatitis	9,488 (36.71%)	6,291 (48.68%)	0.244	6,032 (48.38%)	5,919 (47.47%)	0.018
Preterm or low birth weight	614 (2.38%)	395 (3.06%)	0.042	343 (2.75%)	372 (2.98%)	0.014
Neonatal infections	1,283 (4.96%)	924 (7.15%)	0.092	804 (6.45%)	837 (6.71%)	0.011
Congenital malformations	2,880 (11.14%)	1858 (14.38%)	0.097	1,683 (13.50%)	1722 (13.81%)	0.009
Allergic rhinitis	4,622 (17.88%)	3,566 (27.60%)	0.233	3,158 (25.33%)	3,248 (26.05%)	0.017
Urticaria	4,212 (16.30%)	2,765 (21.40%)	0.131	2,550 (20.45%)	2,580 (20.69%)	0.006
Intestinal infectious diseases	9,725 (37.63%)	6,087 (47.11%)	0.193	5,738 (46.02%)	5,737 (46.01%)	0.000
Noninfective enteritis and colitis	16,346 (63.25%)	9,594 (74.25%)	0.239	9,297 (74.56%)	9,173 (73.57%)	0.023
Metabolic conditions	5,038 (19.49%)	3,668 (28.39%)	0.210	3,222 (25.84%)	3,362 (26.96%)	0.025
Baseline co-medication						
Antihistamines	14,597 (56.48%)	9,685 (74.95%)	0.397	9,306 (74.63%)	9,252 (74.20%)	0.010
Antibiotics	2,875 (11.12%)	2,256 (17.46%)	0.182	1967 (15.78%)	2042 (16.38%)	0.016

AbSD, absolute standardized difference.

higher risk of autism compared with the non-constipation group (crude relative risk = 1.458, 95% CI = 1.116–1.904; adjusted HR = 1.431, 95% CI = 1.083–1.891, after adjusting for the confounders; Table 2). Likewise, when stratifying the severity of constipation into constipation without the need of laxatives (n = 4,813), with one or two laxative prescriptions (n = 6,553), and with over three laxative prescriptions (n = 1,556), it was noted that patients with more severe constipation, i.e., those receiving one or two laxative prescriptions (IR = 12.85; crude relative risk = 1.528, 95% CI = 1.099–2.123; adjusted HR = 1.517, 95% CI = 1.082–2.128) or over three laxative prescriptions (IR = 19.89; crude relative risk = 2.300, 95% CI = 1.387–3.815; adjusted HR = 2.379, 95% CI = 1.409–3.990) had a significantly higher severity-related risk of developing autism compared with the non-constipation group. The findings were similar in the PSM cohort (Table 2).

The log-rank test in the Kaplan–Meier curve analysis revealed that the cumulative risk of autism in the constipation group was significantly higher than in the non-constipation group (Figure 2); moreover, the findings were supported by the results of the log-rank test in the Kaplan–Meier curve analysis when constipation was stratified by level of severity (Figure 2). Similar findings were noted after being controlled for propensity scores. Overall, the correlation between study sets and development of autism could be observed not only in the constipation group, but also in the severity analysis of constipation (Table 2; Figure 2).

The Cox proportional hazard model revealed that regardless of the use of propensity score matching, patients with constipation who received any prescriptions for laxatives had significantly higher risk of autism compared to patients without constipation [age- and sex-matched subset (constipation with 1–2 laxatives: aHR = 1.517, 95% CI = 1.082–2.218, p = 0.0157; constipation with equal or more than 3 laxatives: aHR = 2.379, 95% CI = 1.419–3.990, p = 0.0010), PSM subset (constipation with 1–2 laxatives: aHR = 2.002, 95% CI = 1.341–2.989, p = 0.0007; constipation with equal or more than 3 laxatives: aHR = 2.932, 95% CI = 1.660–5.180, p = 0.0002)] (Table 3) [age- and sex-matched subset (constipation with 1–2 laxatives: aHR = 1.517, 95% CI = 1.082–2.218, p = 0.0157; constipation with equal or more than 3 laxatives: aHR = 2.379, 95% CI = 1.419–3.990, p = 0.0010), PSM subset (constipation with 1–2 laxatives: aHR = 2.002, 95% CI = 1.341–2.989, p = 0.0007; constipation with equal or more than 3 laxatives: aHR = 2.932, 95% CI = 1.660–5.180, p = 0.0002)] (Table 3). Male participants had a significantly higher risk of ASD compared to females (aHR = 3.700, 95% CI = 2.486–5.506, p < 0.0001) with or without PSM (aHR = 4.682, 95% CI = 3.351–6.541, p < 0.0001) (Table 3). Concerning the risk of ASD among all enrolled children with underlying comorbidities, congenital malformations (aHR = 1.418, 95% CI = 1.001–2.011, p = 0.0496), allergic rhinitis (aHR = 1.444, 95% CI = 1.043–1.999, p = 0.0269), and urticaria (aHR = 1.416, 95% CI = 1.029–1.949, p = 0.0326) were associated with higher risks of autism. After PSM, atopic dermatitis (aHR = 1.568, 95% CI = 1.109–2.218, p = 0.0110) and allergic rhinitis (aHR = 1.689, 95% CI = 1.160–2.458, p = 0.0062) were associated with higher risks of autism (Table 3). Aside from the comorbidities above, several other risk factors, namely, rural urbanization (aHR = 0.449, 95% CI = 0.221–0.912, p = 0.0268) and prescriptions for antihistamines (aHR = 0.648, 95% CI = 0.481–0.873, p = 0.0043) showed associations with a lower risk for ASD among the general population (Table 3).

Subgroup analysis of age, gender, and time from constipation to autism onset

The subgroup analysis of the relationship between the risk of developing autism and constipation revealed a significant correlation in males (aHR = 1.386, 95% CI = 1.014–1.895), although this elevated risk was not significant in females (aHR = 1.522, 95% CI = 0.817–2.835). Likewise, the risk of ASD in the constipation group was significantly higher among patients who had constipation during infancy (aged 0–1 years; aHR = 1.704, 95% CI = 1.082–2.686).

TABLE 2 Incidence of autism in study group.

	Person-months	New autism case	Incidence rate*(95% C.I.)	Crude Relative risk (95% C.I.)	Adjusted HR (95% C.I.)
Age-sex matched cohort					
Non-constipation (n = 25,844)	2,026,818	159	7.84 (6.72–9.16)	Reference	Reference
Constipation (n = 12,922)	1,011,294	125	12.36 (10.37–14.73)	1.458 (1.116–1.904)	1.431 (1.083–1.891)
Constipation subgroups					
Without laxatives at baseline (n = 4,813)	389,812	37	9.49 (6.88–13.10)	1.110 (0.733–1.682)	1.066 (0.699–1.626)
Laxatives with 1–2 prescriptions (n = 6,553)	505,838	65	12.85 (10.08–16.38)	1.528 (1.099–2.123)	1.517 (1.082–2.128)
Laxatives with ≥3 prescriptions (n = 1,556)	115,644	23	19.89 (13.22–29.93)	2.300 (1.387–3.815)	2.379 (1.419–3.990)
Propensity score-matched cohort					
Non-constipation (n = 12,469)	977,594	48	4.91 (3.70–6.52)	Reference	Reference
Constipation (n = 12,469)	977,794	91	9.31 (7.58–11.43)	1.896 (1.337–2.690)	1.891 (1.333–2.684)
Constipation subgroups					
Without laxatives at baseline (n = 4,643)	376,621	27	7.17 (4.92–10.45)	1.481 (0.924–2.373)	1.446 (0.902–2.318)
Laxatives with 1–2 prescriptions (n = 6,337)	490,016	48	9.80 (7.38–13.00)	1.984 (1.330–2.960)	2.002 (1.341–2.989)
Laxatives with ≥3 prescriptions (n = 1,489)	111,157	16	14.39 (8.82–23.50)	2.879 (1.635–5.070)	2.932 (1.660–5.180)

Adjusted HR (aHR), hazard ratio, estimated by the multiple Cox proportional hazard regression, and the covariates included sex, age, insured unit type, urbanization, geographic area, hospitalized stay, co-morbidities, and co-medication.*Incidence rate, per 100,000 person-months.

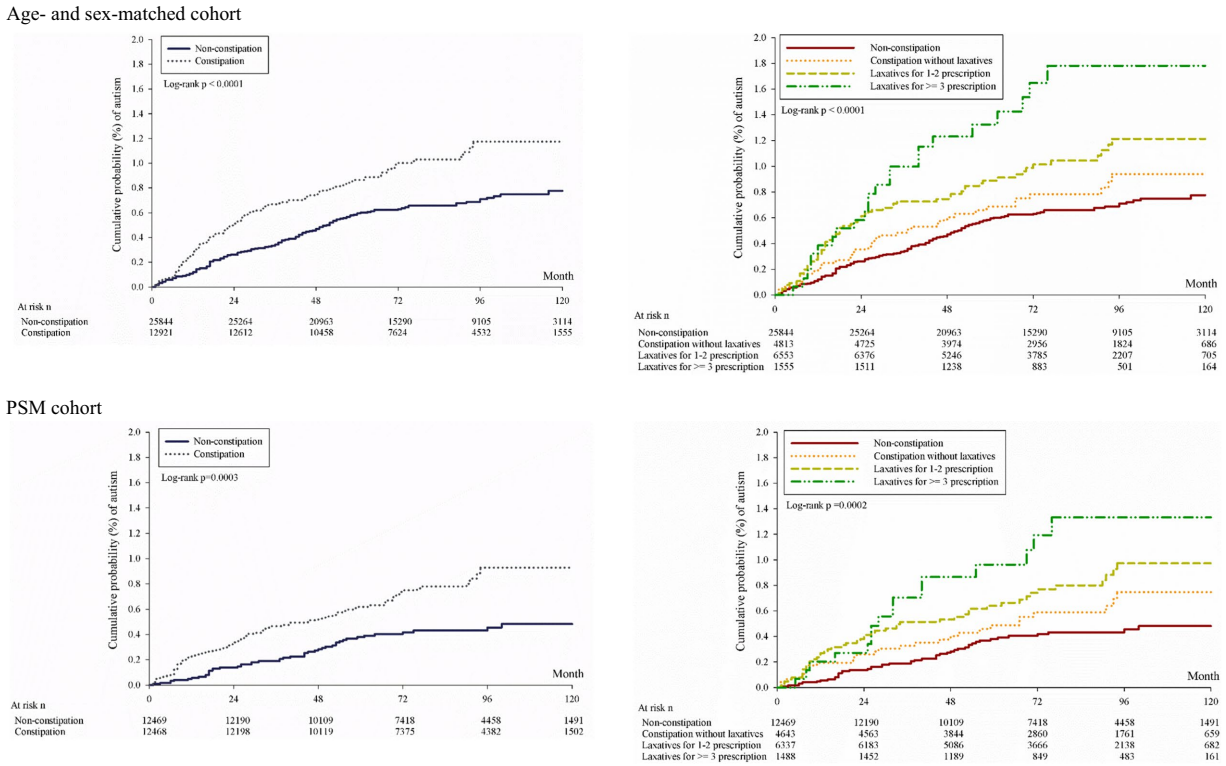


FIGURE 2
Kaplan–Meier curves of cumulative probability of development of autism. Age- and sex-matched cohort PSM cohort.

However, the risk of autism in the constipation group was not significantly higher for patients aged 1–2 years (aHR = 1.166, 95% CI = 0.745–1.823) or 2–3 years (aHR = 1.625, 95% CI = 0.896–2.946; Table 4).

For patients with constipation, those with atopic dermatitis (aHR = 1.770, 95% CI = 1.192–2.626), allergic rhinitis (aHR = 2.424, 95% CI = 1.412–4.161), and noninfectious enteritis and colitis (aHR = 1.450, 95% CI = 1.046–2.011) were at higher risk of developing autism. Moreover, patients with constipation who used antihistamines were at high risk of developing ASD (aHR = 1.579, 95% CI = 1.113–2.240; Table 4). After testing to determine the effects of interactions among sex, age, comorbidities, antihistamines, antibiotics, and constipation on autism risk, none showed any significant interactive effect.

Sensitivity analysis for exclusion of antibiotics exposure

As antibiotics are considered to have a notable impact on microbiome composition, subjects with antibiotics exposure prior to the index date were excluded from the analyses. The adjusted hazard ratio was estimated by multiple Cox proportional hazard regression, and the covariates included sex, age, insured unit type, urbanization, geographic area, hospitalized stay, comorbidities, and co-medication (Table 5). Even though the exclusion of some subjects reduced the number of cases, the result still showed that constipated early-aged children had significantly higher risk of autism (aHR = 1.486, 95% CI = 1.095–2.016) compared to the non-constipation group. Those receiving one or two laxative prescriptions (adjusted HR = 1.525, 95% CI = 1.048–2.220) or over three laxative prescriptions (adjusted HR = 2.355, 95% CI = 1.310–4.234) had a significantly higher severity-related risk of developing autism compared with the non-constipation group.

Discussion

To date and to our best knowledge, this investigation is the first, large, population-based, nationwide cohort study to evaluate the risk of autistic disorder in young pediatric patients with constipation. Higher risk of ASD was observed in constipated children, especially in more severely constipated children who received more laxative prescriptions. Other risk factors were male gender, constipation during infancy, congenital malformations, atopic dermatitis, allergic rhinitis, urticaria, and antihistamine usage. Clinicians should bear in mind that in severely constipated children, gut patency is vitally important and there is a higher risk of subsequent neurodevelopmental problems such as ASD.

It has been established over the past few decades that multiple genetic and environmental factors have a deleterious impact on gut flora, which in turn has been shown to be related to an increased risk of autism (31, 32, 34–48). Previous studies have indicated that the gut microbiome in children is still developing and is relatively unstable. The establishment of the microbiome begins from birth, with various life events causing disruptive changes in the gut microbiome, which is highly variable and develops continuously before the child reaches adulthood. Moreover, a previous study stated that there is an increased ASD risk in children with abnormal early brain structural development (39). In addition, structural and functional brain development happens

TABLE 3 Multiple Cox regression for estimation of hazard ratio of autism among the age- and sex-matched and PSM cohort.

	Age-sex matched		PSM	
	aHR (95% C.I.)	*p*	aHR (95% C.I.)	*p*
Study group				
Non-constipation	**Reference**		**Reference**	
Constipation without laxative	1.066 (0.699–1.626)	0.7675	1.446 (0.902–2.318)	0.1260
Constipation with 1–2 laxatives	1.517 (1.082–2.128)	0.0157	2.002 (1.341–2.989)	0.0007
Constipation with ≥3 laxatives	2.379 (1.419–3.990)	0.0010	2.932 (1.660–5.180)	0.0002
Sex				
Female	**Reference**		**Reference**	
Male	4.682 (3.351–6.541)	<0.0001	3.700 (2.486–5.506)	<0.0001
Age at diagnosis (years)				
0–1	1.310 (0.965–1.779)	0.0837	1.406 (0.956–2.070)	0.0837
1–2	**Reference**		**Reference**	
2–3	0.929 (0.649–1.330)	0.6879	0.942 (0.601–1.477)	0.7939
Insured unit type				
Government	0.954 (0.538–1.691)	0.8713	0.708 (0.309–1.625)	0.4153
Privately held company	**Reference**		**Reference**	
Agricultural organizations	0.737 (0.380–1.431)	0.3672	0.566 (0.246–1.303)	0.1807
Low-income	1.443 (0.199–10.461)	0.7168	1.976 (0.267–14.613)	0.5048
Non-labor force	0.977 (0.616–1.548)	0.9201	0.609 (0.303–1.224)	0.1637
Others	1.031 (0.731–1.453)	0.8635	0.993 (0.648–1.522)	0.9755
Urbanization				
Urban	**Reference**		**Reference**	
Suburban	0.840 (0.610–1.157)	0.2861	0.958 (0.647–1.419)	0.8304
Rural	0.449 (0.221–0.912)	0.0268	0.664 (0.293–1.502)	0.3250
Geographic area				
Taipei	**Reference**		**Reference**	
North	0.973 (0.652–1.450)	0.8915	1.174 (0.712–1.934)	0.5304
Central	1.066 (0.716–1.588)	0.7540	1.051 (0.632–1.747)	0.8475
South	1.154 (0.742–1.794)	0.5262	1.175 (0.674–2.050)	0.5695
Kaohsiung/Pingtung	0.731 (0.452–1.182)	0.2017	0.857 (0.479–1.534)	0.6036
East	1.295 (0.512–3.276)	0.5850	1.323 (0.396–4.416)	0.6494
Baseline hospitalized stays				
0 days	**Reference**		**Reference**	
1–6 days	0.847 (0.557–1.290)	0.4404	0.866 (0.535–1.400)	0.5564
≥7 days	1.084 (0.610–1.924)	0.7834	1.073 (0.554–2.076)	0.8353
Comorbidities				
Asthma	0.707 (0.450–1.113)	0.1348	0.593 (0.341–1.030)	0.0636
Atopic dermatitis	1.307 (0.991–1.724)	0.0584	1.568 (1.109–2.218)	0.0110
Preterm or low birth weight	1.548 (0.822–2.913)	0.1759	1.626 (0.772–3.425)	0.2006
Neonatal infections	1.161 (0.707–1.905)	0.5558	1.205 (0.672–2.162)	0.5319
Congenital malformations	1.418 (1.001–2.011)	0.0496	1.451 (0.954–2.207)	0.0821
Allergic rhinitis	1.444 (1.043–1.999)	0.0269	1.689 (1.160–2.458)	0.0062
Urticaria	1.416 (1.029–1.949)	0.0326	1.223 (0.821–1.822)	0.3225
Intestinal infectious diseases	1.016 (0.766–1.348)	0.9110	1.047 (0.740–1.480)	0.7958
Noninfective enteritis and colitis	1.040 (0.766–1.412)	0.8022	1.120 (0.740–1.695)	0.5929
Metabolic conditions	1.181 (0.852–1.638)	0.3176	1.247 (0.847–1.836)	0.2633
Co-medication				
Antihistamine	0.648 (0.481–0.873)	0.0043	0.727 (0.489–1.080)	0.1143
Antibiotics	1.033 (0.682–1.564)	0.8778	1.010 (0.623–1.636)	0.9689

TABLE 4 Subgroup analysis among the age- and sex-matched cohort.

	Non- constipation	Constipation	p for interaction
Sex			0.5072
Female ($n = 20,607$)	Reference	1.522 (0.817–2.835)	
Male ($n = 18,159$)	Reference	1.386 (1.014–1.895)	
Age at diagnosis			0.2437
0–1 ($n = 11,226$)	Reference	1.704 (1.082–2.686)	
1–2 ($n = 17,337$)	Reference	1.166 (0.745–1.823)	
2–3 ($n = 10,203$)	Reference	1.625 (0.896–2.946)	
Urbanization			0.7987
Urban ($n = 22,764$)	Reference	1.443 (1.021–2.041)	
Suburban ($n = 12,283$)	Reference	1.459 (0.882–2.414)	
Rural ($n = 3,719$)	Reference	1.098 (0.293–4.112)	
Baseline hospitalized stays			0.3004
0 day ($n = 31,822$)	Reference	1.631 (1.194–2.229)	
1–6 days ($n = 4,920$)	Reference	1.010 (0.471–2.164)	
≥7 days ($n = 2024$)	Reference	0.669 (0.235–1.906)	
Baseline co-morbidities			
Asthma			0.5811
Without ($n = 34,184$)	Reference	1.382 (1.026–1.860)	
With ($n = 4,582$)	Reference	1.816 (0.789–4.176)	
Atopic dermatitis			0.1245
Without ($n = 22,987$)	Reference	1.134 (0.753–1.707)	
With ($n = 15,779$)	Reference	1.770 (1.192–2.626)	
Preterm or low birth weight			0.8653
Without ($n = 37,757$)	Reference	1.405 (1.056–1.871)	
With ($n = 1,009$)	Reference	2.165 (0.567–8.270)	
Neonatal infections			0.9094
Without ($n = 36,559$)	Reference	1.426 (1.066–1.907)	
With ($n = 2,207$)	Reference	1.755 (0.668–4.612)	
Congenital malformations			0.4017
Without ($n = 34,028$)	Reference	1.347 (0.985–1.840)	
With ($n = 4,738$)	Reference	1.771 (0.940–3.336)	
Allergic rhinitis			0.0500
Without ($n = 30,578$)	Reference	1.170 (0.835–1.641)	
With ($n = 8,188$)	Reference	2.424 (1.412–4.161)	
Urticaria			0.3747
Without ($n = 31,789$)	Reference	1.504 (1.094–2.067)	
With ($n = 6,977$)	Reference	1.188 (0.667–2.114)	
Intestinal infectious diseases			0.2257
Without ($n = 22,954$)	Reference	1.715 (1.187–2.476)	
With ($n = 15,812$)	Reference	1.153 (0.755–1.760)	
Noninfective enteritis and colitis			0.7009
Without ($n = 12,826$)	Reference	1.393 (0.813–2.386)	
With ($n = 25,940$)	Reference	1.450 (1.046–2.011)	0.4085
Metabolic conditions			
Without ($n = 30,060$)	Reference	1.573 (1.132–2.187)	
With ($n = 8,706$)	Reference	1.110 (0.663–1.858)	
Baseline co-medication			
Antihistamine			0.3022
Without ($n = 14,484$)	Reference	1.234 (0.759–2.006)	
With ($n = 24,282$)	Reference	1.579 (1.113–2.240)	
Antibiotics			0.6598
Without ($n = 33,635$)	Reference	1.486 (1.095–2.016)	
With ($n = 5,131$)	Reference	1.066 (0.540–2.105)	

TABLE 5 Sensitivity analysis for exclusion of antibiotics exposure.

Exclusion of antibiotics exposure prior to index date	Adjusted HR (95% C.I.)
Non-constipation ($n = 22,969$)	Reference
Constipation ($n = 10,666$)	1.486 (1.095–2.016)
Constipation subgroups	
Without laxatives at baseline ($n = 3,996$)	1.204 (0.768–1.888)
Laxatives with 1–2 prescriptions ($n = 5,417$)	1.525 (1.048–2.220)
Laxatives with ≥3 prescriptions ($n = 1,253$)	2.355 (1.310–4.234)

Adjusted HR, hazard ratio, estimated by the multiple Cox proportional hazard regression, and the covariates included sex, age, insured unit type, urbanization, geographic area, hospitalized stay, co-morbidities, and co-medication at baseline.

mostly during the period from term birth to about 2 or 3 years old (49). This overlapping period between gut microbiota establishment and main brain volume development suggests a potential relationship between constipation and autism, which prompted the present investigation of autism in constipated children in this age group. In our study, compared with older children, constipated children younger than 1 year of age had the highest significant risk of developing ASD. We speculate that this finding may be explained, at least in part, by the immaturity of the gut microbiome and immune system in infants. It is conceivable that the earlier the development of constipation, the greater is the impact on dysbiosis and future ASD risk.

Constipation in infants and toddlers can lead to alterations of the gut microbiota, changed level of SCFA, abnormal levels of neurotransmitters, poor gut motility, and increased intestinal permeability, which through the gut-immune axis and gut-brain axis influence immune function, inflammatory processes, allergies, and metabolic conditions, potentially leading to changes in brain development with increased risk of ASD (22, 50–52). For example, Vuong and Hsiao noted that constipation can cause abnormal SCFA level, which was found to be closely related to proliferation and differentiation of immune cells and nerves, and these findings are consistent with findings reported by Stakenborg et al. and some animal models (53–55). In addition, abnormal levels of some gut microbiome-related neurotransmitters, such as serotonin and GABA, have been observed in constipated and autistic children in studies by Marler et al. and by Dinan and Cryan (56, 57). Poor motility and increased intestinal permeability were observed in constipated ASD patients (51), based on measurements of blood level of laxative agents after oral administration (58), *Lactobacillus* quantity in feces (27), and proteins of the tight junction in intestinal mucosa (40). As intestinal permeability increases, resulting in leaky gut, gut bacterial metabolites, such as lipopolysaccharide (LPS), brain-derived neurotrophic factor (BDNF), indole, may pass the mucosal barrier, causing altered cytokine levels, which then affect the brain *via* the gut-brain axis in young children (8–12, 52). Furthermore, Kang et al. achieved some success with microbiota transplant therapy in autistic children (59). In a recent study, constipation showed worsening pre-existing dysbiosis in patients with atopic diseases, such as atopic dermatitis and allergic rhinitis (14, 18). Consistent with these findings, in our study, we also found that constipated children with atopic dermatitis, allergic rhinitis, and urticaria seemed to be predisposed to subsequent ASD. Children with noninfective enteritis and colitis also had higher risk of autism. The findings suggest possible evidence of a shared or similar biophysical pathway in dysbiosis, involving a cofactor, bystander, or a crucial cause playing a role in the mechanism of autism. Hence, children with allergic diseases or an inflammatory condition should be followed up to monitor their gut microbiome composition and their developmental milestones.

It is known that exposure to antibiotics may have a serious deleterious effect on the microbiome composition, which might have markedly confounded our primary outcome, and therefore sensitivity analysis was performed to exclude subjects with antibiotics exposure lasting more than 28 days within 1 year prior to the index date. While this reduced the number of patients in the analysis, the result still showed that constipated early-aged children had significantly higher risk of future autism. Those receiving laxative prescriptions had a significantly higher severity-related risk of developing autism compared with the non-constipation group.

Our study findings imply that early childhood constipation leads to intestinal dysbiosis and the results of our analyses showed an association of constipation with future risk of ASD. During the important period when the microbiota is established, toddlers often have difficulty expressing physical discomfort. Abnormal behaviors are easily overlooked or may be incorrectly attributed to infantile colic, milk protein allergy, infantile anorexia, or failure to try soft food. Therefore, early detection of risks of dysbiosis, close monitoring of constipation, and precise evaluation of the condition of the bowels, may help to restore an imbalanced microbiome, and achieve patency and proper permeability of the guts. Education of the family and children, surveillance of developmental milestones, and referrals to a pediatric neurologist or psychiatrist should be considered when there is evidence of prodromal symptoms of ASD in young children with constipation (50).

The major advantages of this study include its large scale, with use of nationwide data analysis, detailed medical records, and a long follow-up period for both cohorts. Hence, our results can be considered reliable and selection biases were minimized. Nevertheless, there were some limitations that should be mentioned. First, the NHIRD does not contain information regarding diet exposure, schooling performance, neurological examinations, family history, personal lifestyle, genetic sequencing, or gut microbiota analysis, which may have confounded the analyses of ASD risk. Although we considered and adjusted for medications and comorbidities and used propensity scores matching to decrease the effects of possible confounding factors, these residual variables might have biased our results. Second, the diagnoses of ASD, constipation, and comorbidities were based on ICD-9 codes in this database. Thus, the validity of diagnoses could not be confirmed by reviewing personal medical documents and misclassifications may have occurred. Fortunately, these misclassifications were possibly random, and the association was often underestimated. Furthermore, an *ad hoc* committee in Taiwan has been set up to monitor claims data in Taiwan's NHI administration to prevent any violations. Additionally, we only

considered repeatedly-coded patients to improve the accuracy of these diagnoses. Finally, nearly all of the children in this study were born in Taiwan and therefore our findings might not be generalizable to other countries or ethnic groups. The authors hope that the epidemiological observations presented in this research can raise physicians' awareness of the relationship between autism and constipation in young pediatric patients.

Conclusion

Children with constipation in early childhood had a significantly greater risk of ASD compared with those without constipation. Clinicians should look out for prodromal symptoms of ASD in young children with constipation and be aware of the possibility of neurodevelopmental problems in these patients. Furthermore, pediatricians should assess the bowel condition, including the patency and gut microbiota, in children with ASD. Further research is needed to determine the precise pathophysiological mechanisms underlying the association between constipation and ASD.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Funding

This work was supported by a grant from Taichung Veterans General Hospital Research Foundation TCVGH-1116502C and 1118701B.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Lord C, Brugha TS, Charman T, Cusack J, Dumas G, Frazier T, et al. Autism spectrum disorder. *Nat Rev Dis Primers*. (2020) 6:5. doi: 10.1038/s41572-019-0138-4

2. Loomes R, Hull L, Mandy WPL. What is the male-to-female ratio in autism spectrum disorder? A systematic review and meta-analysis. *J Am Acad Child Adolesc Psychiatry*. (2017) 56:466–74. doi: 10.1016/j.jaac.2017.03.013

3. Liu YW, Liong MT, Chung YE, Huang HY, Peng WS, Cheng YF, et al. Effects of *Lactobacillus plantarum* Ps128 on children with autism spectrum disorder in Taiwan: a randomized, double-blind, placebo-controlled trial. *Nutrients*. (2019) 11:820. doi: 10.3390/nu11040820

4. Kotajima-Murakami H, Hagihara H, Sato A, Hagino Y, Tanaka M, Katoh Y, et al. Exposure to Gaba(a) receptor antagonist picrotoxin in pregnant mice causes autism-like behaviors and aberrant gene expression in offspring. *Front Psychol*. (2022) 13:821354. doi: 10.3389/fpsyt.2022.821354

5. Lin YH, Lin CH, Lin MC, Hsu YC, Hsu CT. Antenatal corticosteroid exposure is associated with childhood mental disorders in late preterm and term infants. *J Pediatr*. (2022) 4:S0022-3476(22)00872-1. doi: 10.1016/j.jpeds.2022.09.050

6. Sato A, Kotajima-Murakami H, Tanaka M, Katoh Y, Ikeda K. Influence of prenatal drug exposure, maternal inflammation, and parental aging on the development of autism spectrum disorder. *Front Psychol*. (2022) 13:821455. doi: 10.3389/fpsyt.2022.821455

7. Abelson N, Meiri G, Solomon S, Flusser H, Michaelovski A, Dinstein I, et al. Association between antenatal antimicrobial therapy and autism spectrum disorder-a nested case-control study. *Front Psychol*. (2021) 12:771232. doi: 10.3389/fpsyt.2021.771232

8. Mirzaei R, Bouzari B, Hosseini-Fard SR, Mazaheri M, Ahmadyousefi Y, Abdi M, et al. Role of microbiota-derived short-chain fatty acids in nervous system disorders. *Biomed Pharmacother*. (2021) 139:111661. doi: 10.1016/j.biopha.2021.111661

9. Barbosa AG, Pratesi R, Paz GSC, Dos Santos M, Uenishi RH, Nakano EY, et al. Assessment of Bdnf serum levels as a diagnostic marker in children with autism spectrum disorder. *Sci Rep*. (2020) 10:17348. doi: 10.1038/s41598-020-74239-x

10. Han YMY, Yau SY, Chan MMY, Wong CK, Chan AS. Altered cytokine and Bdnf levels in individuals with autism spectrum disorders. *Brain Sci*. (2022) 12:460. doi: 10.3390/brainsci12040460

11. Li F, Ke H, Wang S, Mao W, Fu C, Chen X, et al. Leaky gut plays a critical role in the pathophysiology of autism in mice by activating the lipopolysaccharide-mediated toll-like receptor 4-myeloid differentiation factor 88-nuclear factor kappa B signaling pathway. *Neurosci Bull*. (2022):1–18. doi: 10.1007/s12264-022-00993-9

12. Mehra A, Arora G, Sahni G, Kaur M, Singh H, Singh B, et al. Gut microbiota and autism spectrum disorder: from pathogenesis to potential therapeutic perspectives. *J Tradit Complement Med*. (2022). doi: 10.1016/j.jtcme.2022.03.001

13. Belsey J, Greenfield S, Candy D, Geraint M. Systematic review: impact of constipation on quality of life in adults and children. *Aliment Pharmacol Ther*. (2010) 31:938–49. doi: 10.1111/j.1365-2036.2010.04273.x

14. Huang YC, Wu MC, Wang YH, Wei JC. Influence of constipation on atopic dermatitis: a nationwide population-based cohort study in Taiwan. *Int J Clin Pract*. (2020) 75:e13691. doi: 10.1111/ijcp.13691

15. Lu CY, Chen YC, Lu YW, Muo CH, Chang RE. Association of constipation with risk of end-stage renal disease in patients with chronic kidney disease. *BMC Nephrol*. (2019) 20:304. doi: 10.1186/s12882-019-1481-0

16. Hsiao YC, Wang JH, Chang CL, Hsieh CJ, Chen MC. Association between constipation and childhood nocturnal enuresis in Taiwan: a population-based matched case-control study. *BMC Pediatr*. (2020) 20:35. doi: 10.1186/s12887-020-1939-z

17. Lin CH, Lin JW, Liu YC, Chang CH, Wu RM. Risk of Parkinson's disease following severe constipation: a nationwide population-based cohort study. *Parkinsonism Relat Disord*. (2014) 20:1371–5. doi: 10.1016/j.parkreldis.2014.09.026

18. Wu MC, Jan MS, Chiou JY, Wang YH, Wei JC. Constipation might be associated with risk of allergic rhinitis: a nationwide population-based cohort study. *PLoS One*. (2020) 15:e0239723. doi: 10.1371/journal.pone.0239723

19. Ohkusa T, Koido S, Nishikawa Y, Sato N. Gut microbiota and chronic constipation: a review and update. *Front Med*. (2019) 6:19. doi: 10.3389/fmed.2019.00019

20. Dimidi E, Christodoulides S, Scott SM, Whelan K. Mechanisms of action of probiotics and the gastrointestinal microbiota on gut motility and constipation. *Adv Nutr*. (2017) 8:484–94. doi: 10.3945/an.116.014407

21. Mou Y, Du Y, Zhou L, Yue J, Hu X, Liu Y, et al. Gut microbiota interact with the brain through systemic chronic inflammation: implications on neuroinflammation, neurodegeneration, and aging. *Front Immunol*. (2022) 13:796288. doi: 10.3389/fimmu.2022.796288

22. Rooks MG, Garrett WS. Gut microbiota, metabolites and host immunity. *Nat Rev Immunol*. (2016) 16:341–52. doi: 10.1038/nri.2016.42

23. Brookes SJ, Spencer NJ, Costa M, Zagorodnyuk VP. Extrinsic primary afferent signalling in the gut. *Nat Rev Gastroenterol Hepatol*. (2013) 10:286–96. doi: 10.1038/nrgastro.2013.29

24. Srikantha P, Mohajeri MH. The possible role of the microbiota-gut-brain-axis in autism spectrum disorder. *Int J Mol Sci*. (2019) 20. doi: 10.3390/ijms20092115

25. Fulceri F, Morelli M, Santocchi E, Cena H, Del Bianco T, Narzisi A, et al. Gastrointestinal symptoms and behavioral problems in preschoolers with autism spectrum disorder. *Dig Liver Dis*. (2016) 48:248–54. doi: 10.1016/j.dld.2015.11.026

26. Marler S, Ferguson BJ, Lee EB, Peters B, Williams KC, McDonnell E, et al. Association of rigid-compulsive behavior with functional constipation in autism spectrum disorder. *J Autism Dev Disord*. (2017) 47:1673–81. doi: 10.1007/s10803-017-3084-6

27. Iovene MR, Bombace F, Maresca R, Sapone A, Iardino P, Picardi A, et al. Intestinal dysbiosis and yeast isolation in stool of subjects with autism spectrum disorders. *Mycopathologia*. (2017) 182:349–63. doi: 10.1007/s11046-016-0068-6

28. Liu S, Li E, Sun Z, Fu D, Duan G, Jiang M, et al. Altered gut microbiota and short chain fatty acids in Chinese children with autism spectrum disorder. *Sci Rep*. (2019) 9:287. doi: 10.1038/s41598-018-36430-z

29. De Angelis M, Piccolo M, Vannini L, Siragusa S, De Giacomo A, Serrazzanetti DI, et al. Fecal microbiota and metabolome of children with autism and pervasive developmental disorder not otherwise specified. *PLoS One*. (2013) 8:e76993. doi: 10.1371/journal.pone.0076993

30. Kang DW, Park JG, Ilhan ZE, Wallstrom G, Labaer J, Adams JB, et al. Reduced incidence of prevotella and other fermenters in intestinal microflora of autistic children. *PLoS One*. (2013) 8:e68322. doi: 10.1371/journal.pone.0068322

31. Nadeem MS, Al-Abbasi FA, Kazmi I, Murtaza BN, Zamzami MA, Kamal MA, et al. Multiple risk factors: a challenge in the management of Autism. *Curr Pharm Des*. (2020) 26:743–54. doi: 10.2174/1381612826666200226101218

32. Lee I-C, Wang Y-H, Chiou J-Y, Wei JC-C. Perinatal factors in newborn are insidious risk factors for childhood autism spectrum disorders: a population-based study. *J Autism Dev Disord*. (2021) 52:52–60. doi: 10.1007/s10803-021-04921-0

33. Hsieh CY, Su CC, Shao SC, Sung SF, Lin SJ, Kao Yang YH, et al. Taiwan's national health insurance research database: past and future. *Clin Epidemiol*. (2019) 11:349–58. doi: 10.2147/CLEP.S196293

34. 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) 135:29–41. doi: 10.1111/acps.12666

35. Christensen J, Gronborg TK, Sorensen MJ, Schendel D, Parner ET, Pedersen LH, et al. Prenatal valproate exposure and risk of autism spectrum disorders and childhood autism. *JAMA*. (2013) 309:1696–703. doi: 10.1001/jama.2013.2270

36. Guy A, Seaton SE, Boyle EM, Draper ES, Field DJ, Manktelow BN, et al. Infants born late/moderately preterm are at increased risk for a positive autism screen at 2 years of age. *J Pediatr*. (2015) 166:269–275.e3. doi: 10.1016/j.jpeds.2014.10.053

37. Modabbernia A, Velthorst E, Reichenberg A. Environmental risk factors for autism: an evidence-based review of systematic reviews and meta-analyses. *Mol Autism*. (2017) 8:13. doi: 10.1186/s13229-017-0121-4

38. Tierney AL, Gabard-Durnam L, Vogel-Farley V, Tager-Flusberg H, Nelson CA. Developmental trajectories of resting Eeg power: an endophenotype of autism spectrum disorder. *PLoS One*. (2012) 7:e39127. doi: 10.1371/journal.pone.0039127

39. Hazlett HC, Gu H, Munsell BC, Kim SH, Styner M, Wolff JJ, et al. Early brain development in infants at high risk for autism spectrum disorder. *Nature*. (2017) 542:348–51. doi: 10.1038/nature21369

40. Fiorentino M, Sapone A, Senger S, Camhi SS, Kadzielski SM, Buie TM, et al. Blood-brain barrier and intestinal epithelial barrier alterations in autism Spectrum disorders. *Mol Autism*. (2016) 7:49. doi: 10.1186/s13229-016-0110-z

41. Satterstrom FK, Kosmicki JA, Wang J, Breen MS, De Rubeis S, An JY, et al. Large-scale exome sequencing study implicates both developmental and functional changes in the neurobiology of autism. *Cells*. (2020) 180:568–584.e23 e23. doi: 10.1016/j.cell.2019.12.036

42. Groer MW, Gregory KE, Louis-Jacques A, Thibeau S, Walker WA. The very low birth weight infant microbiome and childhood health. *Birth Defects Res C Embryo Today*. (2015) 105:252–64. doi: 10.1002/bdrc.21115

43. Sm OM, Stilling RM, Dinan TG, Cryan JF. The microbiome and childhood diseases: focus on brain-gut axis. *Birth Defects Res C Embryo Today*. (2015) 105:296–313. doi: 10.1002/bdrc.21118

44. Tanaka M, Nakayama J. Development of the gut microbiota in infancy and its impact on health in later life. *Allergol Int*. (2017) 66:515–22. doi: 10.1016/j.alit.2017.07.010

45. Francino MP. Early development of the gut microbiota and immune health. *Pathogens*. (2014) 3:769–90. doi: 10.3390/pathogens3030769

46. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography. *Nature*. (2012) 486:222–7. doi: 10.1038/nature11053

47. Buie T. Potential etiologic factors of microbiome disruption in autism. *Clin Ther*. (2015) 37:976–83. doi: 10.1016/j.clinthera.2015.04.001

48. Diaz HR. Fetal, neonatal, and infant microbiome: perturbations and subsequent effects on brain development and behavior. *Semin Fetal Neonatal Med*. (2016) 21:410–7. doi: 10.1016/j.siny.2016.04.012

49. Gilmore JH, Knickmeyer RC, Gao W. Imaging structural and functional brain development in early childhood. *Nat Rev Neurosci*. (2018) 19:123–37. doi: 10.1038/nrn.2018.1

50. Shi Y, Chen Q, Huang Y, Ni L, Liu J, Jiang J, et al. Function and clinical implications of short-chain fatty acids in patients with mixed refractory constipation. *Color Dis*. (2016) 18:803–10. doi: 10.1111/codi.13314

51. de Magistris L, Familiari V, Pascotto A, Sapone A, Frolli A, Iardino P, et al. Alterations of the intestinal barrier in patients with autism spectrum disorders and in their first-degree relatives. *J Pediatr Gastroenterol Nutr*. (2010) 51:418–24. doi: 10.1097/MPG.0b013e3181dcc4a5

52. Santocchi E, Guiducci L, Fulceri F, Billeci L, Buzzigoli E, Apicella F, et al. Gut to brain interaction in autism spectrum disorders: a randomized controlled trial on the role of probiotics on clinical, biochemical and neurophysiological parameters. *BMC Psychiatry*. (2016) 16:183. doi: 10.1186/s12888-016-0887-5

53. Stakenborg N, Viola MF, Boeckxstaens GE. Intestinal neuro-immune interactions: focus on macrophages, mast cells and innate lymphoid cells. *Curr Opin Neurobiol*. (2020) 62:68–75. doi: 10.1016/j.conb.2019.11.020

54. Vuong HE, Hsiao EY. Emerging roles for the gut microbiome in autism spectrum disorder. *Biol Psychiatry*. (2017) 81:411–23. doi: 10.1016/j.biopsych.2016.08.024

55. Wang G, Yang S, Sun S, Si Q, Wang L, Zhang Q, et al. *Lactobacillus rhamnosus* strains relieve loperamide-induced constipation via different pathways independent of short-chain fatty acids. *Front Cell Infect Microbiol*. (2020) 10:423. doi: 10.3389/fcimb.2020.00423

56. Marler S, Ferguson BJ, Lee EB, Peters B, Williams KC, McDonnell E, et al. Brief report: whole blood serotonin levels and gastrointestinal symptoms in autism spectrum disorder. *J Autism Dev Disord*. (2016) 46:1124–30. doi: 10.1007/s10803-015-2646-8

57. Dinan TG, Cryan JF. Gut instincts: microbiota as a key regulator of brain development, ageing and neurodegeneration. *J Physiol*. (2017) 595:489–503. doi: 10.1113/JP273106

58. D'Eufemia P, Celli M, Finocchiaro R, Pacifico L, Viozzi L, Zaccagnini M, et al. Abnormal intestinal permeability in children with autism. *Acta Paediatr*. (1996) 85:1076–9. doi: 10.1111/j.1651-2227.1996.tb14220.x

59. Kang DW, Adams JB, Gregory AC, Borody T, Chittick L, Fasano A, et al. Microbiota transfer therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. *Microbiome*. (2017) 5:10. doi: 10.1186/s40168-016-0225-7

frontiers | Frontiers in Psychiatry

TYPE Original Research
PUBLISHED 26 April 2023
DOI 10.3389/fpsyt.2023.1181466

OPEN ACCESS

EDITED BY
Martina Micai,
National Institute of Health (ISS), Italy

REVIEWED BY
Magdalena Budisteanu,
Prof. Dr. Alexandru Obregia Psychiatry Hospital, Romania
Lucia Marzulli,
University of Bari Aldo Moro, Italy

*CORRESPONDENCE
Sara Calderoni
✉ sara.calderoni@fsm.unipi.it

RECEIVED 07 March 2023
ACCEPTED 06 April 2023
PUBLISHED 26 April 2023

CITATION
Distefano G, Calderoni S, Apicella F, Cosenza A, Igliozzi R, Palermo G, Tancredi R, Tritto G, Craig F, Muratori F and Turi M (2023) Impact of sleep disorders on behavioral issues in preschoolers with autism spectrum disorder. *Front. Psychiatry* 14:1181466. doi: 10.3389/fpsyt.2023.1181466

Impact of sleep disorders on behavioral issues in preschoolers with autism spectrum disorder

Giacomo Distefano[1], Sara Calderoni[1,2]*, Fabio Apicella[2], Angela Cosenza[2], Roberta Igliozzi[2], Giuseppina Palermo[3], Raffaella Tancredi[2], Giovanna Tritto[3], Francesco Craig[4], Filippo Muratori[2] and Marco Turi[5]

[1]Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy, [2]IRCCS Fondazione Stella Maris, Pisa, Italy, [3]Stella Maris Mediterraneo Foundation, Chiaromonte, Italy, [4]Department of Cultures, Education and Society, University of Calabria, Cosenza, Italy, [5]Department of Human and Social Studies, University of Salento, Lecce, Italy

Background: Sleep disorders are one of the most common problems in children with Autism Spectrum Disorder (ASD). However, they often tend to be underdiagnosed and incorrectly treated in clinical practice. This study aims to identify sleep disorders in preschool children with ASD and to explore their relationship with the core symptoms of autism, the child's developmental and cognitive level as well as the psychiatric comorbidities.

Methods: We recruited 163 preschool children with a diagnosis of ASD. The Children's Sleep Habits Questionnaire (CSHQ) assessed sleep conditions. Multiple standardized tests were used to evaluate intellectual abilities, the presence of repetitive behaviors (through the Repetitive Behavior Scale-Revised), as well as the emotional-behavioral problems and the psychiatric comorbidities (through the Child Behavior Checklist -CBCL $1^{1/2}$-5).

Results: The results showed that poor disorders had consistently higher scores in all areas assessed by the CSHQ and on the CBCL across all domains. The correlational analysis showed that severe sleep disorders were associated with higher scores in internalizing, externalizing, and total problems at the CBCL syndromic scales, and in all DSM-oriented CBCL subscales. Moreover, we found that the association between sleep disorders and restricted and repetitive behaviors (RRBs) is explained by the anxiety-related symptoms.

Conclusion: Based on these findings, the study recommends that screening for sleep problems followed by early intervention should constitute a routine part of clinical practice for children with ASD.

KEYWORDS

sleep disorders, autism spectrum disorders, preschoolers, psychiatric comorbidities, repetitive behaviors, behavioral problems

Introduction

Autism spectrum disorders (ASD) refer to a diverse set of neurodevelopmental conditions that are marked by noticeable deficits in social interaction and communication, as well as unusual sensori-motor behaviors or interests that may be repetitive or restrictive in nature. The symptoms caused by ASD appear from early childhood and negatively impact the child's daily functioning (1).

Sleep disorders (SD) are certainly among the most reported problems in individuals with ASD of all ages and have a negative impact on daily functioning, learning, and behavior, not only of the person with ASD but also of the entire family (2). Indeed, an up-to-date review that used the Diagnostic and Statistical Manual of Mental Disorders Fifth Edition (DSM-5)'s most stringent criteria to identify sleep disorders emphasized that the ASD population had a prevalence rate of 13%, which is significantly higher than the 3.7% rate observed in the general population (3). Studies specifically focusing on pediatric samples report that one-half up to two-thirds of children with ASD might have SD (4–7) and underline the importance of early diagnosis and treatment to avoid the tendency of these disorders to become chronic (6, 8). A different comprehensive assessment of sleep disturbances using both subjective and objective measures discovered notable sleep difficulties in children with ASD in comparison to their typically developing counterparts. These issues included significantly reduced total sleep duration, prolonged time taken to fall asleep, and lower quality of sleep (9).

While there is substantial literature on sleep disorders in individuals with ASD, only a few studies have specifically focused on the preschool population. A study by Krakowiak and colleagues investigated the sleep patterns of children between the ages of 2 and 5 with ASD, as compared to children with other types of developmental delays (DD) and typical development (TD). The authors used the Children's Sleep Habits Questionnaire (CSHQ), which was administered to parents to collect data on their children's sleep patterns. They observed that at least one sleep issue occurred frequently in 53% of children with ASD, which is higher than the 46% observed in children with DD and the 32% noted in TD peers. The most common sleep difficulties reported in the ASD group were trouble falling asleep and waking up during the night (10). In a similar vein, a more recent study using a larger cohort of patients aged 2 to 5 years found similar results using the CSHQ, comparing different groups (ASD, DD with ASD symptoms, DD without ASD, and TD). Caregiver reports of sleep issues in children with ASD were 47%, DD with ASD were 57%, DD without ASD were 29%, and TD were 25% (11). Both studies emphasized the significance of early detection of sleep issues due to the effects of inadequate sleep on a child's behavior throughout the day and on their quality of life (10, 11).

Sleep questionnaires are one of the most commonly used tools to investigate subjective sleep characteristics and problems in children. These questionnaires are also important in highlighting the impact of sleep problems on the quality of life of families with children who have ASD and SD. One of the most frequently used questionnaires is the Children's Sleep Habits Questionnaire (CSHQ), which was designed specifically for the evaluation of children by Owens et al. in 2000 (12). This questionnaire was later validated in toddlers and preschool children with TD, ASD, and other DD by Goodlin-Jones et al. (13). Eventually, it was specifically modified for the ASD population by Katz et al. (14). The validity and usefulness of the CSHQ in the ASD population and in particular in preschool children have been confirmed by several studies (9, 15, 16).

Other similar questionnaires, such as the Sleep Disturbance Scale for Children (SDSC), are used to evaluate sleep disturbances in infants and toddlers (17). The SDSC was recently used by Romeo et al. to assess sleep disorders in preschoolers with ASD, and revealed that 46% of children with ASD have at least one SD, compared to 15% in the control group. Difficulty in initiating and maintaining sleep, excessive daily somnolence, and sleep hyperhidrosis showed higher subscale scores (18).

Overall, screening for sleep problems in all children with ASD should be recommended, and a sleep specialist should be consulted when comorbid sleep disorders are suspected (19). Parental questionnaires have the benefit of saving time and costs, can be used as a follow-up instrument, and can measure a broad range of sleep parameters.

Recent literature reviews have analyzed the relationship between sleep disturbance and daytime behavior in the ASD population (4, 20–22). A large cross-sectional study on 1,193 children with ASD showed that children with ASD and SD demonstrated higher internalizing and externalizing behavior problems identified by the Child Behavior Check-List (CBCL) (23). Malow et al. investigated the correlation between parentally reported sleep problems in ASD, objective polysomnographic findings, and measures of daytime behavior. The authors divided ASD children into two groups: poor sleepers and good sleepers, based on the CSHQ total score, and observed higher T-scores on all scales of the CBCL in the poor sleeper group, with more clinical scores in affective, attention, and anxious/depressed subscales (24).

The vast majority of studies investigating the correlation between SD and daytime behavior in ASD are conducted with school-aged samples, while only a few have focused on the pre-school population. Among these, a recent study using the CSHQ and the CBCL found a remarkable difference in the effect of parasomnias on internalizing, externalizing, and total problems in children with ASD (25).

In the current study, we focus on an ASD population of infants and toddlers, with an age range between 21 and 66 months. Sleep problems in this age range are certainly less investigated in literature compared to older ages. We used the CSHQ filled in by parents to identify good and poor sleepers in our sample of children with ASD, with the aim of (a) assessing the prevalence of SD in our population sample, and (b) identifying possible correlations between sleep problems and autistic core symptoms, cognitive functioning, restricted and repetitive behaviors, as well as emotional-behavioral problems through standardized tools and questionnaires used in daily clinical practice.

Participants

Between January 2020 and August 2022, we conducted a cross-sectional study (refer to Table 1) on 163 preschoolers with a diagnosis of ASD. These children, aged between 21 and 66 months (with a mean age of 43.37 months and SD of 12.56 months) were recruited from two different Italian child care centers. Of the 163 children, 127 were boys and 36 were girls, with 50 children from IRCCS Fondazione Stella Maris in Pisa and 113 children from Stella Maris Mediterraneo Foundation in Matera. In the current study, we enrolled participants who had received a diagnosis of ASD in accordance with DSM-5 criteria (1). A multidisciplinary team, consisting of a senior child psychiatrist and an experienced research child psychologist, was responsible for performing the diagnosis. To confirm the clinical diagnosis, we used the Autism Diagnostic Observation Schedule, Second Edition (ADOS-2) (26), which is considered the gold-standard semi-structured instrument for observing and assessing communication abilities, social interaction, play quality, and imagination in children.

10.3389/fpsyt.2023.1181466

TABLE 1 Demographic, clinical characteristics in the total sample (n=163), and in each sleep group.

	Whole sample (n=163)	Good sleeper (n=89)	Poor sleeper (n=74)	t-test or X^2	Value of p	Effect size
Age (months)						
M (SD)	43.37 (12.56)	43.56 (13.1)	43.14 (11.96)	$t_{(161)} = 0.21$	$p = 0.83$	–
Range	21–66	25–66	21–66			
Gender (F:M)	36 (22%): 127 (78%)	19 (22%): 70 (78%)	17 (23%): 57 (77%)	$X^2 = 0.80$	$p = 0.85$	–
Performance IQ						
M (SD)	65.59 (22.90)	66.94 (22.04)	63.96 (23.94)	$t_{(161)} = 0.82$	$p = 0.40$	–
Range	13–133	23–122	13–133			
Social affect ADOS						
M (SD) range	14.03 (4) 4–20	13.88 (3.82) 4–20	14.20 (4.22) 4–20	$t_{(161)} = -0.48$	$p = 0.91$	–
RRB ADOS						
M (SD) range	3.76 (1.89) 0–8	3.85 (1.93) 0–8	3.65 (1.86) 0–8	$t_{(161)} = 0.66$	$p = 0.62$	–
ADOS total score						
M (SD) range	17.80 (4.88) 5–28	17.76 (4.65) 7–28	17.85 (5.18) 5–27	$t_{(161)} = -0.14$	$p = 0.51$	–

Significant comparisons are highlighted in bold ($p < 0.05$).

We excluded any cases of syndromic autism or recognized causes of ASD, as well as children who had used psychotropic medications in the 2 months before the evaluation. All participants were residents of Italy. We conducted the study in accordance with the ethical standards for good practice and the guidelines outlined in the Declaration of Helsinki. Written informed consent was acquired from the parent or caregiver of each participant.

Cognitive assessment

Given the variations in verbal skills and functioning levels in children with ASD, a variety of standardized tests have been used to measure their intellectual abilities. These tests include the Leiter International Performance Scale-Revised (LIPS-R) (27), the Griffiths Mental Developmental Scales-Extended-Revised (GMDS-ER) (28), and the Italian version of the Wechsler Preschool and Primary Scale of Intelligence (WPPSI) (29).

We selected these tests to ensure a comprehensive assessment of intellectual abilities in children with ASD, considering the diverse range of skills and abilities that may be present in this population. In cases where a mental age (MA) was provided by the test, we estimated the child's IQ by dividing their MA by their chronological age (CA) and multiplying the result by 100: MA/CA × 100. For this study, we focused on non-verbal IQ scores (performance IQ or pIQ).

Children's Sleep Habits Questionnaire

The Children's Sleep Habits Questionnaire (CSHQ) is a widely accepted and scientifically validated questionnaire used to assess sleep-related problems in children aged 3 to 10 years old (12). It has been used with even younger children, as young as 2 years old (13), and has also been applied in studies with autistic adolescents (30). The Children's Sleep Habits Questionnaire (CSHQ) covers various aspects of sleep, including sleep onset, sleep maintenance, parasomnias, and daytime sleepiness. This questionnaire is widely used in research to evaluate the prevalence and severity of sleep disturbances in children and to assess the effectiveness of interventions aimed at improving sleep. In clinical settings, the CSHQ is used to identify children with sleep problems and track changes in their sleep patterns over time. The CSHQ provides both a total score and subscale scores across different sleep problems. A total score of 41 is suggested to be the cut-off for identifying sleep problems (12). The psychometric properties of the CSHQ are provided alongside reference values for subscales, which are useful for comparing clinical and community groups (12). In the present investigation, based on the approach of previous studies (11, 23), CSHQ total scores of 48 and above were defined as a more conservative cut-off to identify children having SD. The reason for using a cut-off of 48 was first of all to avoid an overestimation of sleep disturbances in children of such a young age as in our sample, who are very often subject to sleep disturbances that are not necessarily pathological and because the CSQH is calibrated on a larger reference population in terms of age (12). This methodological choice is also supported by literature: indeed, there are several different works that have highlighted how the cut-off of 48 had a higher sensitivity in different populations both with TD (31, 32), and with neurodevelopmental disorders, such as DD and young children with ASD in very large samples (11, 23).

Based on the clinical cut-off of the CSHQ, we divided the whole sample into good sleepers (CSQH Total Score < 48) and poor sleepers (CSQH Total Score ≥ 48), as shown in Table 1.

Child Behavior Checklist 1½–5

The Child Behavior Checklist (CBCL 1½–5) (33), which is widely used to evaluate children's behavior, was used in this study. The questionnaire involves parents rating a child's behaviors using a three-point scale, with 0 meaning "not true," 1 meaning "somewhat or sometimes true," and 2 meaning "very true or often true." The CBCL generates scores for seven syndrome scales, three summary scales, and

five DSM-Oriented scales (DOS). Clinical significance is defined as a T-score of 64 or higher for summary scales and a T-score of 70 or higher for syndrome and DOS. A borderline clinical range is indicated by values between 60 and 63 for summary scales or between 65 and 69 for syndrome and DOS. Clinically relevant values are those above 65 for the other scales and below 60 for the summary scales. According to other research on screening (34–36) and psychiatric comorbidity (37–39) in young children with ASD, we adopted the borderline cut-off score (T score 60 for summary scales and T score 65 for DOS) in this investigation. Recently, it has been suggested that the adoption of lower clinical CBCL thresholds for preschoolers is being considered, as using identical thresholds for both preschoolers and school-age children is being questioned (40). Moreover, research has indicated that parents and teachers evaluate this age group of children using less strict standards than they do older children, both in terms of internalizing (41), and externalizing behaviors (42).

Repetitive Behavior Scale-Revised

The Italian version of the Repetitive Behavior Scale-Revised (RBS-R) (43, 44) was used to measure repetitive behaviors in people with ASD. The RBS-R is a 43-item questionnaire completed by caregivers, which assesses a wide range of restricted and repetitive behaviors observed in the individual over the past month. It is divided into six subcategories that cover different types of behaviors, such as compulsive, self-injurious, ritualistic, restricted, insistence on sameness, and stereotypical behaviors. Studies on the RBS-R have found that it can be divided into either five or three factors (45, 46). The questionnaire produces both a count score and a severity score, with the count score reflecting the number of items endorsed by the parents or caregivers and the severity score reflecting the parents or caregivers' assessment of the severity of the behavior. Previous studies have recommended using the count score in analysis to reduce bias and increase accuracy (47).

Data analysis

We examined the normality of continuous variables through both skewness tests and Kolgomorov-Smirnov testing. For categorical and continuous independent variables, we utilized descriptive analysis, chi-square analysis, and t-tests, respectively. To assess whether there were differences in age, PIQ, CSHQ scores, and CBCL scales across all groups, we performed an independent sample t-test. We conducted both simple and partial correlation analyses to investigate the relationships between sleep and clinical variables, and used logistic regression to examine any associations between sleep and behavioral problems. Appropriate effect size was evaluated in accordance with the statistical method chosen for the analysis (e.g., Cohen's *d* for independent sample *t*-tests).

Results

In our study, we recruited a sample of 163 children and used a cut-off point from the CHSQ to divide them into two groups: those who were considered to be good sleepers -GS- (CHSQ total score < 48), referred to as children without a clinical total score on the CHSQ, and those who were considered to be poor sleepers -PS- (CHSQ total score ≥ 48), referred to as children with a clinical total score on the CHSQ. After applying this cut-off, it was found that 54% of the sample, or 89 children, were classified as GS, and 46% of the sample, or 74 children, were classified as PS. The mean CSHQ subscale scores for the whole sample were compared with the standard values for a community group (12), showing that ASD children had clinically higher scores on the following CSHQ subscales: bedtime resistance (54%), sleep onset delay (23%), sleep duration (16%), sleep anxiety (26%), night wakings (25%), parasomnias (11%), sleep disordered breathing (6%), daytime sleepiness (1%).

Table 1 demonstrates that both groups were similar in terms of age and PIQ (age: value of $p = 0.083$: PIQ: value of $p = 0.40$), and there were no statistically significant differences in the male to female ratio between the groups (value of $p = 0.85$). Additionally, we found that there were no statistically significant differences in autism symptoms between the sleep groups as evaluated by the ADOS-2, which includes the ADOS Total Score, Social Affect (SA), and Repetitive Restricted Behaviors (RRB) (ADOS Total Score: value of $p = 0.51$; SA: value of $p = 0.91$; RRB: value of $p = 0.62$). Table 2 compares the CSHQ scores among the two sleep groups. As expected, the group with poor sleep had consistently higher scores in all areas assessed by the CSHQ (all value of $p < 0.05$), indicating an overall worsening of all aspects of sleep investigated by the CSHQ.

Regarding the association between emotional-behavioral problems and type of sleeper, Table 3 shows the distribution of the CBCL scores on the summary scales (Total, Internalizing and Externalizing problems), and on DOS scales among the two sleep groups. Simple group comparison on the CBCL scales showed higher T-scores on all domains for the PS when compared with GS (all value of ps <0.05).

Considering the whole sample, a bivariate Pearson correlation also revealed a statistically significant association between the occurrence of sleep disorders (CSHQ Total Score) and the presence of psychiatric comorbidities, i.e., higher sleep problems were associated with higher T-Score on DOS scales (Table 4). These results are consistent also controlling for age, gender and PIQ (all value of $p<0.05$).

Considering CBCL-DOS (Table 3), 64.9% (48/74) of the PS and 30% (27/89) of GS had a score over the borderline cut-off on one or more of the DOS, excluding the PDP scale given their clinical diagnosis of ASD. A logistic regression was performed to investigate the effects of the presence of sleep problems (GS or PS classification), age, gender and PIQ on the likelihood that children had at least one psychiatric comorbidity in addition to ASD. The logistic regression model was statistically significant, $\chi 2(4) = 21.79$, $p < 0.0001$. The model explained 16% (Nagelkerke R^2) of the variance in the presence of comorbidity and correctly classified 67% of cases. As shown in Table 5, analysis reveals that being a PS increases the odds 4.3 times of having at least one psychiatric comorbidity in association with ASD, with a 95% CI of 2.20 to 8.39. No statistically significant association was found with the other variables included in the model.

Finally, as shown in Table 6, we found differences between the sleep groups on the RBS-R subscales. The PS group showed higher scores on all subscale, except for the Self Injurious (value of $p = 0.25$), and on the Total Score when compared to the GS group (all value of $p<0.05$).

In order to assess the relationship between sleep and restricted and repetitive behaviors (RRBs) in the whole sample, we employed a

TABLE 2 Differences in CSHQ scales between poor and good sleepers.

	Good sleeper (*n*=89)	Poor sleeper (*n*=74)	*t*-test	Value of *p*	Effect size
Bedtime resistance *M* (SD)	9.33 (2.04)	12.75 (1.98)	$t_{(161)} = -10.60$	**$p < 0.0001$**	$d = 1.66$
Sleep onset delay *M* (SD)	1.53 (0.67)	1.98 (0.86)	$t_{(161)} = -4.05$	**$p = 0.001$**	$d = 0.73$
Sleep duration *M* (SD)	3.40 (0.90)	4.49 (1.57)	$t_{(161)} = -5.84$	**$p < 0.0001$**	$d = 0.89$
Sleep anxiety *M* (SD)	5.33 (1.13)	7.80 (1.67)	$t_{(161)} = -9.91$	**$p < 0.0001$**	$d = 1.65$
Night wakings *M* (SD)	4.13 (1.16)	5.21 (1.48)	$t_{(161)} = -5.57$	**$p < 0.0001$**	$d = 0.73$
Parasomnias *M* (SD)	7.69 (0.91)	9.36 (1.87)	$t_{(161)} = -7.43$	**$p < 0.0001$**	$d = 1.13$
Sleep disorder breathing *M* (SD)	3.13 (0.65)	3.46 (0.92)	$t_{(161)} = -2.98$	**$p = 0.003$**	$d = 0.47$
Daytime sleepiness *M* (SD)	7.84 (1.57)	10.54 (2.61)	$t_{(161)} = -7.57$	**$p < 0.0001$**	$d = 1.17$
Total score M(SD)	42.46 (3.25)	55.64 (6.16)	$t_{(161)} = -16.94$	**$p < 0.0001$**	$d = 2.59$

Significant comparisons are highlighted in bold ($p < 0.05$).

TABLE 3 Differences in DOS and Summary Scale of CBCL between poor and good sleepers.

	Good sleeper (*n*=89)	Poor sleeper (*n*=74)	*t*-test	Value of *p*	Effect size
Internalizing problems					
M (SD)	56.16 (10.11)	65.38 (8.82)	$t_{(161)} = -5.07$	**$p < 0.0001$**	$d = 0.79$
Externalizing problems					
M (SD)	53.75 (9.49)	61.05 (10.05)	$t_{(161)} = -3.89$	**$p < 0.0001$**	$d = 0.90$
Total problems					
M (SD)	55.13 (9.87)	65.42 (10.25)	$t_{(161)} = -5.26$	**$p < 0.0001$**	$d = 0.82$
Affective problems					
M (SD)	56.28 (7.68)	65.71 (9.20)			
Number case	11 (12.4)	35 (47.3)	$t_{(161)} = -7.22$	**$p < 0.0001$**	$d = 1.12$
Anxiety problems					
M (SD)	53.66 (5.08)	61.44 (8.86)	$t_{(161)} = -5.92$	**$p < 0.0001$**	$d = 0.91$
Number case	4 (4.5)	23 (31.1)			
PDP					
M (SD)	66.68 (8.63)	73.71 (9.52)	$t_{(161)} = -3.35$	**$p = 0.001$**	$d = 0.52$
ADHD					
M (SD)	57.69 (6.88)	61.96 (7.62)	$t_{(161)} = 3.47$	**$p = 0.001$**	$d = 0.46$
Number case	24 (27)	34 (45.9)			
Oppositional problems					
M (SD)	54.37 (5.88)	58.44 (7.93)	$t_{(161)} = -3.68$	**$p = 0.001$**	$d = 0.57$
Number case	11 (12.5)	22 (29.7)			

Significant comparisons are highlighted in bold ($p < 0.05$). N Case [Percent of Case (%) above borderline cut-off for CBCL DOS Scales].

simple bivariate Pearson correlation. We found a significant correlation between sleep problems and all subscales of the RBS-R (Table 7). This finding indicates that there is a strong association between sleep problems and RRBs in the sample studied, with all value of *p*s being less than 0.05. However, this association did not remain statistically significant after controlling for the degree of anxiety-related symptoms (Table 7), in line with previous research that showed anxiety could be a potential confounding variable (48) in the relationship between RRBs and sleep problems.

Discussion

The current study specifically investigated the presence and types of sleep problems in preschool children with ASD, as well as their relationship with clinical symptoms. We used the CSHQ parent questionnaire to assess the prevalence and severity of sleep problems through a quantitative measure. The use of parent-filled questionnaires such as the CSHQ has been shown to be a quick and easy-to-use tool for the early identification of sleep problems in both

10.3389/fpsyt.2023.1181466

TABLE 4 Pearson correlations among the CSHQ total score and the CBCL DOS Scales.

	CSHQ-total score	Affective problems	Anxiety problems	PDP	ADHD	Oppositional problems
CSHQ-total score		0.594(**0.000**)	0.53(**0.000**)	0.308(**0. 000**)	0.345(**0.000**)	0.361(**0.000**)
Affective problems	0.606(**0.000**)		0.652(**0.000**)	0.572(**0.000**)	0.525(**0.000**)	0.578(**0.000**)
Anxiety problems	0.525(**0.000**)	0.662(**0.000**)		0.558(**0.000**)	0.509(**0.000**)	0.578(**0.000**)
PDP	0.295(**0.000**)	0.571(**0.000**)	0.550(**0.000**)		0.466(**0.000**)	0.547(**0.000**)
ADHD	0.357(**0.000**)	0.533(**0.000**)	0.518(**0.000**)	0.480(**0.000**)		0.628(**0.000**)
Oppositional problems	0.366(**0.000**)	0.576(**0.000**)	0.580(**0.000**)	0.545(**0.000**)	0.623(**0.000**)	

Above the diagonal simple bivariate correlations, below the diagonal partial Pearson correlations, after controlling for age, gender and PIQ among the CSHQ total score and the CBCL DOS Scales. Significant correlations are highlighted in bold (value of $p < 0.05$).

TABLE 5 Logistic regression model of having one or more psychiatric comorbidities.

Variables	*B*	SE B	Wald χ^2	*p*	OR	95% CI OR
Comorbidity						
Gender	−0.08	0.41	0.45	0.83	0.91	[0.41, 2.04]
Age	0.02	0.01	2.36	0.12	1.02	[0.99, 1.05]
PIQ	−0.00	0.00	0.02	0.88	0.99	[0.98, 1.01]
Group	1.45	0.34	18.27	**<0.0001**	4.29	[2.20, 8.39]

Significant comparisons are highlighted in bold ($p < 0.05$).

TABLE 6 Differences in RBS-R scales between poor and good sleepers.

	Good sleeper (n=89)	Poor sleeper (n=74)	*t*-test	Value of *p*	Effect size
Stereotypic behavior					
M (SD)	6.22 (4.34)	8.15 (5.97)	$t_{(161)} = -2.37$	$\boldsymbol{p = 0.02}$	$d = 0.36$
Self-injurious					
M (SD)	1.40 (2.31)	1.89 (3.08)	$t_{(161)} = -1.15$	$p = 0.25$	–
Compulsive					
M (SD)	2.02 (2.50)	3.19 (2.62)	$t_{(161)} = -2.89$	$\boldsymbol{p = 0.004}$	$d = 0.45$
Ritualistic					
M (SD)	4.36 (4.51)	6.22 (5.60)	$t_{(161)} = -2.34$	$\boldsymbol{p = 0.02}$	$d = 0.36$
Restricted					
M (SD)	2.01 (1.94)	2.77 (2.15)	$t_{(161)} = -2.32$	$\boldsymbol{p = 0.02}$	$d = 0.36$
Total score					
M (SD)	16.06 (12.21)	22.31 (16.10)	$t_{(161)} = -2.81$	$\boldsymbol{p = 0.005}$	$d = 0.43$

Significant comparisons are highlighted in bold ($p < 0.05$).

ASD and TD children (12, 13, 16, 25). According to previous investigations (11, 23), the established cut-off of 41 on the CSHQ could overestimate SD. Therefore, we decided to apply the more conservative cut-off of 48 to split our sample into two sub-groups based on the total score: good sleepers (GS) and poor sleepers (PS). As previously mentioned, the prevalence of sleep disturbance in ASD is extremely high, estimated from 15% up to 75% depending on the criteria used to identify sleep problems. However, only a few studies have focused on the preschool age group, identifying an even higher prevalence of SD in this population, ranging between 53 and 81% (10, 11, 49). In our sample, a significant proportion (46%) of children with ASD were found to have sleep problems as early as preschool age, even when applying a more stringent cut-off in the CSHQ. All CHSQ subscales were significantly higher in the PS group compared to the GS group. Our study confirms the high prevalence of sleep disturbance in the preschool population of children with ASD, which is significantly higher than that in preschool children with TD, as reported in previous studies (50–52). Focusing on this age group is important in terms of early identification because sleep problems tend to appear at a young age in ASD (53) and persist over time (54). Detecting SD in the ASD population before 6 years of age is fundamental for early intervention and to prevent behavioral issues related to sleep problems.

Our data also confirm recently reported findings in the literature indicating a strong association between SD and both internalizing problems (including anxiety, withdrawal, depression) and externalizing problems (including aggression, tantrums, and inattention) in ASD (21, 55). Specifically, looking at the number of psychiatric comorbidities, we found that more severe sleep problems increase the risk (four times) of having at least one psychiatric comorbidity in association with ASD, as measured through the CBCL. Sleep disruption may exacerbate associated psychiatric symptoms, or associated psychiatric comorbidities may worsen sleep problems already present in individuals with ASD (22). In the current study, the PS group had a higher score on CBCL across all domains with clinical relevance in internalizing, externalizing, and total problems in the syndromic scales. It is also noteworthy that unlike when we examine the ADOS scores, where the analysis did not find a difference in the severity of autism evaluated by clinicians between the two groups, parents reported on CBCL-PDP more problems related to the severity of autism in the PS group.

In particular, the correlational analysis we performed indicates that the total score on the CSHQ is associated with higher scores on anxiety problems, affective problems, pervasive developmental problems, attention deficit/hyperactivity problems, and oppositional deficiency problems on the DSM-Oriented subscales of CBCL, even when corrected for IQ, gender, and age.

TABLE 7 Correlations between RBS-R subscale and CSHQ-total score, and partial correlations controlling for anxiety (DOS-CBCL anxiety problems).

RBS-R scores	Correlation		Partial correlation	
	r	*p*	*r*	*p*
Stereotypic behavior	0.26	**0.001**	0.09	0.21
Self-injurious	0.21	**0.006**	0.13	0.07
Compulsive	0.29	**0.000**	0.11	0.13
Ritualistic	0.29	**0.000**	0.07	0.35
Restricted	0.22	**0.004**	0.17	0.82
Total score	0.32	**0.000**	0.11	0.14

Significant correlations are highlighted in bold (value of $p < 0.05$).

These findings are in line with previous literature on this topic. For example, Sikora et al. reported in a sample of 1193 children with ASD aged 4–10 years that individuals with SD had significantly higher scores on the internalizing and externalizing behavior problems scales of the CBCL (23). Similar results were found by Park et al. in a study comparing 166 ASD children with their 111 unaffected siblings, demonstrating that children with ASD and sleep disturbances were more likely to exhibit aggressive behaviors, internalizing and externalizing behavior problems, and overall behavioral issues than peers without sleep issues (56). Focusing on younger ages, Roussis and colleagues recently reported that behavior problems and attention deficit were significantly greater in a group of ASD preschoolers with severe sleep problems than in peers with ASD but without sleep disorders (57).

Overall, evidence suggests that children with ASD and sleep difficulties have higher rates of Attention and Hyperactivity Disorder and Defiant Oppositional Disorder compared to children without sleep problems (7, 25, 57, 58). Generally, emotional or behavioral issues, including hyperactivity, aggression, anxiety, or mood disorders, may be the cause or consequence of sleep issues in children with ASD. An interesting finding that emerges from our data is that the correlation between SD and internalizing problems (specifically anxiety problems and affective problems) appears to be even stronger than that with externalizing disorders, in contrast to several studies in the literature (23, 51, 58–60). This finding is significant and worthy of further investigation. Future studies may attempt to characterize patients with externalizing and internalizing problems in more detail, trying to identify different subpopulations with specific clinical features.

Several studies suggest that anxiety and mood problems are also linked to SD in individuals with ASD (22, 47, 58, 61). Previous studies have found that children with higher levels of anxiety tend to exhibit increased repetitive and restrictive behaviors (RRBs) (62, 63), and have more difficulty sleeping (64). This suggests that anxiety may be a potential confounding variable in the relationship between sleep and RRBs in individuals with ASD (48). Although in our study, we found that the PS group displayed an increase in RRBs compared to the GS group, this difference disappears after controlling for the degree of anxiety-related symptoms, in line with previous research that showed anxiety could be a potential confounding variable (48). One possible explanation for this finding could be that RRBs might constitute a way to reduce perceived anxiety levels and to exert greater environmental control in patients with ASD (62, 63). According to this view, a higher number of RRBs would be an indicator of a higher level of anxiety, and higher levels of anxiety would correspond to greater difficulties in sleeping.

Furthermore, in our study, no significant association emerged between sleep disturbances and the extent of autistic symptoms measured through ADOS Score and level of cognitive development. Research has found a correlation between the severity of autistic symptoms and the presence of sleep problems in children with ASD (30, 65). It is not entirely clear what the relationship between sleep problems and autistic symptoms is in children with ASD. Some researchers have suggested that sleep problems may contribute to the severity of autistic symptoms, while others have proposed that the reverse may be true, with the severity of autistic symptoms contributing to the presence of sleep problems. In our study, we found no correlation between sleep problems and the severity of autistic symptoms, in agreement with other studies (61, 66).

Overall, while some evidence suggests a correlation between SD and the severity of autistic symptoms in children with ASD, more research is necessary to fully comprehend the connection between these two elements. Some studies have found that children with ASD and intellectual disability are more likely to experience SD than children with ASD alone (4, 5). However, we found no association between SD and developmental quotient, supporting the results of other studies (61, 65). These differences could be explained by the heterogeneity of the ASD populations analyzed by the different studies, particularly in terms of age groups and the tests used to assess cognitive development.

Sleep problems could be an early, although non-specific, symptom of ASD and are among parents' first concerns (67), suggesting the presence of higher vulnerability during the early stages of life. Sleep disruption during developmental ages might be directly associated with abnormal brain development and could be an additional risk factor for cognitive and behavioral dysfunction (7). In this context, a recent study reported that sleep issues in the first year of life often occur before ASD diagnosis and are associated with atypical patterns of brain development in the hippocampal region (68). Several hypotheses have been formulated regarding the etiopathogenetic mechanisms of sleep alteration in ASD. According to some studies, the secretion of neurotransmitters, including serotonin, GABA, and melatonin, which are essential for establishing a regular sleep–wake cycle, may be altered (69, 70). Other studies suggest the possible involvement of genes implicated in the control of circadian rhythm (71, 72) or the presence of general disrupted sleep architecture in ASD (50, 70). Assuming that an intrinsic cause of disrupted sleep in ASD may be related to variations in brain wave organization and maturational development, further studies involving objective measurements of sleep, such as sleep EEG, polysomnography, or actigraphy, may be beneficial in investigating the underlying pathophysiological mechanisms (73).

Major limitations to our study are the use of predominantly subjective instruments such as parent-completed CSHQ, CBCL and RBS-R questionnaires. Such instruments may obviously be affected by parents' personal perception of the issues. Future studies using objective parameters such as polysomnography-EEG and actigraphs will be needed. Another limitation is the possible presence of medical conditions underlying sleep disorders such as the presence of enlarged tonsils and / or adenoids causing obstructive sleep

apnea: indeed, in the absence of specialized medical evaluations, we cannot exclude *a priori* the presence of underlying organic causes. Further medical investigations should be performed to rule out the presence of any underlying diseases. Moreover, a comparison of sleep problems and their impact on global functioning and emotional-behavioral problems between ASD and TD preschoolers, as well as between ASD and preschoolers with other neurodevelopmental disorders could be addressed in future studies.

To sum up, sleep problems are among the most frequently represented comorbid symptoms in children with ASD as early as preschool age. A wide number of literature reviews demonstrate that sleep disruption is link to emotional and behavioral issues in ASD, thus influencing children's health, cognition, executive functions and consequently school performance besides having an important impact on the quality of life of families (3, 16, 21, 74, 75). Despite this, they are often not properly identified and treated in clinical practice (8, 21), as treatment guidelines to help parents manage challenging behaviors in children with ASD frequently fail to include sleep at all or only briefly address the topic. Identifying and providing correct treatment for sleep problems in ASD is crucial not only to improve sleep, but also for better daytime conducts and family functioning in this population. Practice guidelines and recommendations for the treatment of disrupted sleep behavior and insomnia in children and adolescents with ASD updated to 2017 have been provided by the American Academy of Neurology (76), but continuous upgrading of expert consensus statements is needed to deal with this disabling symptom.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by IRCCS Stella Maris committee. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

GD, MT, SC, and FM were involved in designing the study and drafting the initial manuscript. MT performed the data analysis. GD, MT, SC, GT, GP, FA, RI, and AC assessed the patients and collected the data. GD, MT, SC, GT, GP, FA, RI, AC, FM, FC, and RT contributed to editing the manuscript, providing critical feedback, and performing a thorough review. All authors have reviewed and approved the current version of the manuscript and take full responsibility for its contents.

Funding

MT received funding from the GenPercept grant agreement (no. 832813). This study was partially funded by grants from the IRCCS Fondazione Stella Maris, including the Ricerca Corrente and the 5 × 1000 voluntary contributions from the Italian Ministry of Health, as well as by AIMS-2-Trials.

Acknowledgments

We thank the families who were involved in this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. American Psychiatric Association. *Diagnostic and statistical manual of mental disorders. 5th* ed. Virginia: American Psychiatric Association (2013).

2. Lord C, Charman T, Havdahl A, Carbone P, Anagnostou E, Boyd B, et al. The Lancet Commission on the future of care and clinical research in autism. *Lancet*. (2022) 399:271–334. doi: 10.1016/S0140-6736(21)01541-5

3. Lai MC, Kassee C, Besney R, Bonato S, Hull L, Mandy W, et al. Prevalence of co-occurring mental health diagnoses in the autism population: a systematic review and meta-analysis. *Lancet Psychiatry*. (2019) 6:819–29. doi: 10.1016/S2215-0366(19)30289-5

4. Liu X, Hubbard JA, Fabes RA, Adam JB. Sleep disturbances and correlates of children with autism spectrum disorders. *Child Psychiatry Hum Dev*. (2006) 37:179–91. doi: 10.1007/s10578-006-0028-3

5. Richdale AL, Schreck KA. Sleep problems in autism spectrum disorders: prevalence, nature, & possible biopsychosocial aetiologies. *Sleep Med Rev*. (2009) 13:403–11. doi: 10.1016/j.smrv.2009.02.003

6. Herrmann S. Counting sheep: sleep disorders in children with autism spectrum disorders. *J Pediatr Heal Care*. (2016) 30:143–54. doi: 10.1016/j.pedhc.2015.07.003

7. Halstead EJ, Joyce A, Sullivan E, Tywyn C, Davies K, Jones A, et al. Sleep disturbances and patterns in children with neurodevelopmental conditions. *Fron Pediatr*. (2021) 9:637770. doi: 10.3389/fped.2021.637770

8. Cortese S, Wang F, Angriman M, Masi G, Bruni O. Sleep disorders in children and adolescents with autism spectrum disorder: diagnosis, epidemiology, and management. *CNS Drugs*. (2020) 34:415–23. doi: 10.1007/s40263-020-00710-y

9. Díaz-Román A, Zhang J, Delorme R, Beggiato A, Cortese S. Sleep in youth with autism spectrum disorders: systematic review and meta-analysis of subjective and objective studies. *Evid Based Ment Health*. (2018) 21:146–54. doi: 10.1136/ebmental-2018-300037

10. Krakowiak P, Goodlin-jones B. Sleep in autism sleep problems in children with autism spectrum disorders, developmental delays, and typical development: a population-based study. *J Sleep Res*. (2008) 17:197–206. doi: 10.1111/j.1365-2869.2008.00650.x

11. Reynolds AM, Soke GN, Sabourin KR, Hepburn S, Katz T, Wiggins LD, et al. Sleep problems in 2- to 5-year-olds with autism spectrum disorder and other developmental delays. *Pediatrics*. (2019) 143:e20180492. doi: 10.1542/peds.2018-0492

12. Owens JA, Spirito A, McGuinn M. The Children's sleep habits questionnaire (CSHQ): psychometric properties of a survey instrument for school-aged children. *Sleep*. (2000) 23:1043–51. doi: 10.1093/sleep/23.8.1d

13. Goodlin-Jones BL, Sitnick SL, Tang K, Liu J, Anders TF. The Children's Sleep Habits Questionnaire in toddlers and preschool children. *J of Dev Behav Pediatr*. (2008) 29:82–8. doi: 10.1097/DBP.0b013e318163c39a

14. Katz T, Shui AM, Johnson CR, Richdale AL, Reynolds AM, Scahill L, et al. Modification of the children's sleep habits questionnaire for children with autism spectrum disorder. *J Autism Dev Disord*. (2018) 48:2629–41. doi: 10.1007/s10803-018-3520-2

15. Johnson CR, DeMand A, Lecavalier L, Smith T, Aman M, Foldes E, et al. Psychometric properties of the children's sleep habits questionnaire in children with autism spectrum disorder. *Sleep Med*. (2016) 20:5–11. doi: 10.1016/j.sleep.2015.12.005

16. Zaidman-Zait A, Zwaigenbaum L, Duku E, Bennett T, Szatmari P, Mirenda P, et al. Factor analysis of the children's sleep habits questionnaire among preschool children with autism spectrum disorder. *Res Dev Disabil*. (2020) 97:103548. doi: 10.1016/j.ridd.2019.103548

17. Romeo DM, Cordaro G, Macchione E, Venezia I, Brogna C, Mercuri E, et al. Application of the Sleep Disturbance Scale for Children (SDSC) in infants and toddlers (6–36 months). *Sleep Med*. (2021) 81:62–8. doi: 10.1016/j.sleep.2021.02.001

18. Romeo DM, Brogna C, Belli A, Lucibello S, Cutrona C, Apicella M, et al. Sleep disorders in autism spectrum disorder pre-school children: an evaluation using the sleep disturbance scale for children. *Medicina*. (2021) 57:1–10. doi: 10.3390/medicina57020095

19. Banaschewski T, Bruni O, Fuentes J, Hill CM, Hvolby A, Posserud MB, et al. Practice tools for screening and monitoring insomnia in children and adolescents with autism spectrum disorder. *J Autism Dev Disord*. (2021) 52:3758–68. doi: 10.1007/s10803-021-05236-w

20. Anders T, Iosif AM, Schwichtenberg AJ, Tang K, Goodlin-Jones B. Sleep and daytime functioning: a short-term longitudinal study of three preschool-age comparison groups. *Am J Intellect Dev Disabil*. (2012) 117:275–90. doi: 10.1352/1944-7558-117.4.275

21. Cohen S, Conduit R, Lockley SW, Rajaratnam SM, Cornish KM. The relationship between sleep and behavior in autism spectrum disorder (ASD): a review. *J Neurodev Disord*. (2014) 6:44. doi: 10.1186/1866-1955-6-44

22. Mazzone L, Postorino V, Siracusano M, Riccioni A, Curatolo P. The relationship between sleep problems, neurobiological alterations, core symptoms of autism spectrum disorder, and psychiatric comorbidities. *J Clin Med*. (2018) 7:7(5). doi: 10.3390/jcm7050102

23. Sikora DM, Johnson K, Clemons T, Katz T. The relationship between sleep problems and daytime behavior in children of different ages with autism spectrum disorders. *Pediatrics*. (2012) 130:S83–90. doi: 10.1542/peds.2012-0900F

24. Malow BA, Marzec ML, McGrew SG, Wang L, Henderson LM, Stone WL. Characterizing sleep in children with autism spectrum disorders: a multidimensional approach. *Sleep*. (2006) 29:1563–71. doi: 10.1093/sleep/29.12.1563

25. Wang Y, Lin J, Zeng Y, Liu Y, Li Y, Xia K, et al. Effects of sleep disturbances on behavioral problems in preschool children with autism spectrum disorder. *Front Psych*. (2021) 11:559694. doi: 10.3389/fpsyt.2020.559694

26. Lord C, Rutter M, DiLavore PC, Risi S, Gotham K, Bishop SL. *Autism diagnostic observation schedule, Second Edition (ADOS-2)*. Torrence, CA: Western Psychological Services (2012).

27. Roid GM, Miller LJ. *Leiter international performance scale–revised: examiners manual*. (1997)

28. Griffiths R. *The Griffiths mental developmental scales, extended revised*. UK: Association for Research in Infant and Child Development, the Test Agency (2006).

29. Wechsler D. *Wechsler preschool and primary scale of intelligence – revised*. San Antonio, TX: Psychological Corporation (1989).

30. Goldman SE, Richdale AL, Clemons T, Malow BA. Parental sleep concerns in autism spectrum disorders: variations from childhood to adolescence. *J Autism Dev Disord*. (2012) 42:531–8. doi: 10.1007/s10803-011-1270-5

31. Ishii R, Obara H, Nagamitsu S, Matsuoka M, Suda M, Yuge K, et al. The Japanese version of the children's sleep habits questionnaire (CSHQ-J): a validation study and influencing factors. *Brain and Development*. (2022) 44:595–604. doi: 10.1016/j.braindev.2022.06.003

32. Silva FG, Silva CR, Braga LB, Serrão Neto A. Portuguese Children's Sleep Habits Questionnaire-validation and cross-cultural comparison. *J Pediatr*. (2014) 90:78–84. doi: 10.1016/j.jped.2013.06.009

33. Achenbach TM, Rescorla LA. *Manual for the ASEBA preschool forms and profiles*. Burlington, VT: University of Vermont, Research Center for Children, Youth, & Families (2000).

34. Muratori F, Narzisi A, Tancredi R, Cosenza A, Calugi S, Saviozzi I, et al. The CBCL 1.5-5 and the identification of preschoolers with autism in Italy. *Epidemiol Psychiatr Sci*. (2011) 20:329–38. doi: 10.1017/S204579601100045X

35. Narzisi A, Calderoni S, Maestro S, Calugi S, Mottes E, Muratori F. Child Behavior Check List 1½-5 as a tool to identify toddlers with autism spectrum disorders: a case-control study. *Res Dev Disabil*. (2013) 34:1179–89. doi: 10.1016/j.ridd.2012.12.020

36. Rescorla L, Kim YA, Oh KJ. Screening for ASD with the Korean CBCL/1(1/2)-5. *J Autism Dev Disord*. (2015) 45:4039–50. doi: 10.1007/s10803-014-2255-y

37. Llanes E, Blacher J, Stavropoulos K, Eisenhower A. Parent and teacher reports of comorbid anxiety and ADHD symptoms in children with ASD. *J Autism Dev Disord*. (2018) 50:1520–31. doi: 10.1007/s10803-018-3701-z

38. Muratori F, Turi M, Prosperi M, Narzisi A, Valeri G, Guerrera S, et al. Parental perspectives on psychiatric comorbidity in preschoolers with autism spectrum disorders receiving publicly funded mental health services. *Front Psych*. (2019) 10:107. doi: 10.3389/fpsyt.2019.00107

39. Prosperi M, Turi M, Guerrera S, Napoli E, Tancredi R, Igliozzi R, et al. Sex differences in autism spectrum disorder: an investigation on core symptoms and psychiatric comorbidity in preschoolers. *Front Integr Neurosci*. (2021) 14:594082. doi: 10.3389/fnint.2020.594082

40. Melegari MG, Sacco R, Manzi B, Vittori E, Persico AM. Deficient emotional self-regulation in preschoolers with ADHD: identification, comorbidity, and interpersonal functioning. *J Atten Disord*. (2016):23, 887–899. doi: 10.1177/10870547156.22015

41. Luby J, Belden A, Sullivan J, Hayen R, McCadney A, Spitznagel E. Shame and guilt in preschool depression: evidence for elevations in self-conscious emotions in depression as early as age 3. *J Child Psychol Psychiatry*. (2009) 50:1156–66. doi: 10.1111/j.1469-7610.2009.02077.x

42. Studts CR, van Zyl MA. Identification of developmentally appropriate screening items for disruptive behavior problems in preschoolers. *J Abnorm Child Psychol*. (2013) 41:851–63. doi: 10.1007/s10802-013-9738-8

43. Bodfish JW, Symons FJ, Parker DE, Lewis MH. Varieties of repetitive behavior in autism: comparisons to mental retardation. *J Autism Dev Disord*. (2000) 30:237–43. doi: 10.1023/a:1005596502855

44. Fulceri F, Narzisi A, Apicella F, Balboni G, Baldini S, Brocchini J, et al. Application of the Repetitive Behavior Scale-Revised--Italian version--in preschoolers with autism spectrum disorder. *Res Dev Disabil*. (2016) 48:43–52. doi: 10.1016/j.ridd.2015.10.015

45. Lam KS, Aman MG. The Repetitive Behavior Scale-Revised: independent validation in individuals with autism spectrum disorders. *J Autism Dev Disord*. (2007) 37:855–66. doi: 10.1007/s10803-006-0213-z

46. Mirenda P, Smith IM, Vaillancourt T, Georgiades S, Duku E, Szatmari P, et al. Validating the Repetitive Behavior Scale-Revised in young children with autism spectrum disorder. *J Autism Dev Disord*. (2010) 40:1521–30. doi: 10.1007/s10803-010-1012-0

47. Wolff JJ, Botteron KN, Dager SR, Elison JT, Estes AM, Gu H, et al. Longitudinal patterns of repetitive behavior in toddlers with autism. *J Child Psychol Psychiatry*. (2014) 55:945–53. doi: 10.1111/jcpp.12207

48. Hundley RJ, Shui A, Malow BA. Relationship between subtypes of restricted and repetitive behaviors and sleep disturbance in autism spectrum disorder. *J Autism Dev Disord*. (2016) 46:3448–57. doi: 10.1007/s10803-016-2884-4

49. Kang YQ, Song XR, Wang GF, Su YY, Li PY, Zhang X. Sleep problems influence emotional/behavioral symptoms and repetitive behavior in preschool-aged children with autism spectrum disorder in the unique social context of China. *Front Psych*. (2020) 11:273. doi: 10.3389/fpsyt.2020.00273

50. Chen H, Yang T, Chen J, Chen L, Dai Y, Zhang J, et al. Sleep problems in children with autism spectrum disorder: a multicenter survey. *BMC Psychiatry*. (2021) 21:406. doi: 10.1186/s12888-021-03405-w

51. DeVincent CJ, Gadow KD, Delosh D, Geller L. Sleep disturbance and its relation to DSM-IV psychiatric symptoms in preschool-age children with pervasive developmental disorder and community controls. *J Child Neurol*. (2007) 22:161–9. doi: 10.1177/0883073807300310

52. Goodlin-Jones BL, Tang K, Liu J, Anders TF. Sleep patterns in preschool-age children with autism, developmental delay, and typical development. *J Am Acad Child Adolesc Psychiatry*. (2008) 47:930–8. doi: 10.1097/CHI.ObO13e3181799f7c

53. Humphreys JS, Gringras P, Blair PS, Scott N, Henderson J, Fleming PJ, et al. Sleep patterns in children with autistic spectrum disorders: a prospective cohort study. *Arch Dis Child*. (2014) 99:114–8. doi: 10.1136/archdischild-2013-304083

54. Morgan B, Nageye F, Masi G, Cortese S. Sleep in adults with Autism Spectrum Disorder: a systematic review and meta-analysis of subjective and objective studies. *Sleep Med*. (2020) 65:113–20. doi: 10.1016/j.sleep.2019.07.019

55. Mazurek MO, Dovgan K, Neumeyer AM, Malow BA. Course and predictors of sleep co-occurring problems in children with autism spectrum disorder. *J Autism Dev Disord*. (2019) 49:2101–15. doi: 10.1007/s10803-019-03894-5

56. Park S, Cho S, Hee I, Kim B, Kim J, Shin M, et al. Research in Autism Spectrum Disorders Sleep problems and their correlates and comorbid psychopathology of children with autism spectrum disorders. *Res. Autism Spect. Disor.* (2012) 6:1068–72. doi: 10.1016/j.rasd.2012.02.004

57. Roussis S, Richdale AL, Katz T, Malow BA, Barbaro J, Sadka N. Research in autism spectrum disorders behaviour, cognition, and autism symptoms and their relationship with sleep problem severity in young children with autism spectrum disorder. *Res Autism Spectr Disord*. (2021) 83:101743. doi: 10.1016/j.rasd.2021.101743

58. Mayes SD, Calhoun SL. Variables related to sleep problems in children with autism. *Res Autism Spectr Disord*. (2009) 3:931–41. doi: 10.1016/j.rasd.2009.04.002

59. Goldman SE, McGrew S, Johnson KP, Richdale AL, Clemons T, Malow BA. Sleep is associated with problem behaviors in children and adolescents with autism spectrum disorders. *Res Autism Spectr Disord*. (2011) 5:1223–9. doi: 10.1016/j.rasd.2011.01.010

60. Henderson JA, Barry TD, Bader SH, Jordan SS. The relation among sleep, routines, and externalizing behavior in children with an autism spectrum disorder. *Res Autism Spectr Disord*. (2011) 5:758–67. doi: 10.1016/j.rasd.2010.09.003

61. Van Steensel FJ, Bögels SM, Perrin S. Anxiety disorders in children and adolescents with autistic spectrum disorders: a meta-analysis. *Clin Child Fam Psychol Rev*. (2011) 14:302–17. doi: 10.1007/s10567-011-0097-0

62. Lidstone J, Uljarević M, Sullivan J, Rodgers J, McConachie H, Freeston M, et al. Relations among restricted and repetitive behaviors, anxiety and sensory features in children with autism spectrum disorders. *Res Autism Spectr Disord*. (2014) 8:82–92. doi: 10.1016/j.rasd.2013.10.001

63. Rodgers J, Glod M, Connolly B, McConachie H. The relationship between anxiety and repetitive behaviours in autism spectrum disorder. *J Autism Dev Disord*. (2012) 42:2404–9. doi: 10.1007/s10803-012-1531-y

64. Alfano CA, Ginsburg GS, Kingery JN. Sleep-related problems among children and adolescents with anxiety disorders. *J Am Acad Child Adolesc Psychiatry*. (2007) 46:224–32. doi: 10.1097/01.chi.0000242233.06011.8e

65. Mazurek MO, Sohl K. Sleep and behavioral problems in children with autism spectrum disorder. *J Autism Dev Disord*. (2016) 46:1906–15. doi: 10.1007/s10803-016-2723-7

66. Souders MC, Mason TB, Valladares O, Bucan M, Levy SE, Mandell DS, et al. Sleep behaviors and sleep quality in children with autism spectrum disorders. *Sleep*. (2009) 32:1566–78. doi: 10.1093/sleep/32.12.1566

67. Herlihy L, Knoch K, Vibert B, Fein D. Parents' first concerns about toddlers with autism spectrum disorder: effect of sibling status. *Autism*. (2015) 19:20–8. doi: 10.1177/1362361313509731

68. MacDuffie KE, Shen MD, Dager SR, Styner MA, Kim SH, Paterson S, et al. Sleep onset problems and subcortical development in infants later diagnosed with autism spectrum disorder. *Am J Psychiatry*. (2020) 177:518–25. doi: 10.1176/appi.ajp.2019.19060666

69. Tordjman S, Najjar I, Bellissant E, Anderson GM, Barburoth M, Cohen D, et al. Advances in the research of melatonin in autism spectrum disorders: literature review and new perspectives. *Int J Mol Sci*. (2013) 14:20508–42. doi: 10.3390/ijms141020508

70. Rana M, Kothare S, DeBassio W. The assessment and treatment of sleep abnormalities in children and adolescents with autism spectrum disorder: a review. *J Can Acad Child Adolesc Psychiatry* (2021);30:25–35.

71. Geoffray MM, Falissard B, Green J, Kerr B, Evans DG, Huson S, et al. Autism spectrum disorder symptom profile across the RASopathies. *Front Psych*. (2021) 11:585700. doi: 10.3389/fpsyt.2020.585700

72. Carmassi C, Palagini L, Caruso D, Masci I, Nobili L, Vita A, et al. Systematic review of sleep disturbances and circadian sleep desynchronization in autism Spectrum disorder: toward an integrative model of a self-reinforcing loop. *Front Psych*. (2019) 10:366. doi: 10.3389/fpsyt.2019.00366

73. Petruzzelli MG, Matera E, Giambersio D, Marzulli L, Gabellone A, Legrottaglie AR, et al. Subjective and electroencephalographic sleep parameters in children and adolescents with autism spectrum disorder: a systematic review. *J Clin Med*. (2021) 10:3893. doi: 10.3390/jcm10173893

74. Lindor E, Sivaratnam C, May T, Stefanac N, Howells K, Rinehart N. Problem behavior in autism spectrum disorder: considering core symptom severity and accompanying sleep disturbance. *Front Psych*. (2019) 10:487. doi: 10.3389/fpsyt.2019.00487

75. Masi A, Moni MA, Azim SI, Choi B, Heussler H, Lin PI, et al. Clinical and behavioral attributes leading to sleep disorders in children on the autism spectrum. *Autism Res*. (2022) 15:1274–87. doi: 10.1002/aur.2745

76. Williams Buckley A, Hirtz D, Oskoui M, Armstrong MJ, Batra A, Bridgemohan C, et al. Practice guideline: treatment for insomnia and disrupted sleep behavior in children and adolescents with autism spectrum disorder: Report of the Guideline Development, Dissemination, and Implementation Subcommittee of the American Academy of Neurology. *Neurology*. (2020) 94:392–404. doi: 10.1212/WNL.0000000000009033

frontiers | Frontiers in Psychiatry

TYPE Review
PUBLISHED 29 June 2023
DOI 10.3389/fpsyt.2023.1147327

OPEN ACCESS

EDITED BY
Antonio M. Persico,
University of Modena and Reggio Emilia, Italy

REVIEWED BY
Youlin Long,
Sichuan University, China
Lisa Asta,
University of Modena and Reggio Emilia, Italy
Diego Centonze,
University of Rome Tor Vergata, Italy

*CORRESPONDENCE
Zhimei Jiang
✉ mynard93@163.com

RECEIVED 18 January 2023
ACCEPTED 08 June 2023
PUBLISHED 29 June 2023

CITATION
Liu A, Gong C, Wang B, Sun J and Jiang Z (2023) Non-invasive brain stimulation for patient with autism: a systematic review and meta-analysis.
Front. Psychiatry 14:1147327.
doi: 10.3389/fpsyt.2023.1147327

Non-invasive brain stimulation for patient with autism: a systematic review and meta-analysis

Annan Liu[1], Chao Gong[2], Bobo Wang[2], Jiaxing Sun[2] and Zhimei Jiang[3]*

[1]Jiamusi University Affiliated No.3 Hospital, Jiamusi, China, [2]Jiamusi Medical College, Jiamusi, Heilongjiang, China, [3]Jiamusi University College of Rehabilitation Medicine, Jiamusi, Heilongjiang, China

Objective: To comprehensively evaluate the efficacy of non-invasive brain stimulation (NIBS) in patients with autism spectrum disorder (ASD) in randomized controlled trials (RCT), providing a reference for future research on the same topic.

Methods: Five databases were searched (Pubmed, Web of Science, Medline, Embase, and Cochrane library) and tracked relevant references, Meta-analysis was performed using RevMan 5.3 software.

Results: Twenty-two references (829 participants) were included. The results of the meta-analysis showed that NIBS had positive effects on repetitive and stereotypical behaviors, cognitive function, and executive function in autistic patients. Most of the included studies had a moderate to high risk of bias, Mainly because of the lack of blinding of subjects and assessors to treatment assignment, as well as the lack of continuous observation of treatment effects.

Conclusion: Available evidence supports an improvement in some aspects of NIBS in patients with ASD. However, due to the quality of the original studies and significant publication bias, this evidence must be treated with caution. Further large multicenter randomized double-blind controlled trials and appropriate follow-up observations are needed to further evaluate the specific efficacy of NIBS in patients with ASD.

KEYWORDS

autism, non-invasive neurostimulation, transcranial direct current stimulation, transcranial magnetic stimulation, meta-analysis

1. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that is typically characterized by social communication and interaction impairments, restricted and repetitive behavior or interests, often accompanied by a range of psychiatric problems such as attention deficit hyperactivity disorder and sleep disorders. Clinically usually presents with cognitive, behavioral, emotional, and expressive language impairments (1–3). In recent years, the incidence of the disease is increasing, the global prevalence rate is about 7.6‰ (4). According to 2020, Recent data from the Centers for Disease Control and Prevention show that The overall ASD prevalence aged 8 years rises to 1 in 44, which is already more than the sum of the world's three major diseases (AIDS, cancer, diabetes) (5). ASD starts in early childhood and the relevant symptoms can last for a lifetime, causing a heavy emotional, financial, and medical burden on

patients, their families, and society, which has become a serious public health issue (6–8). At present, the origin of ASD is not clear, and it is usually caused by multiple factors alone or together, among which genetic and environmental factors play an important role in its occurrence and development (9).

Regarding the pathology of ASD, structural and connectivity differences have been identified in the brains of ASD patients. Scholars in imaging have proposed many theories of abnormal brain connectivity in ASD patients to try to explain the pathological mechanisms of the brains of ASD and their abnormal social behaviors. There are three examples, Cohen et al. proposed the amygdala theory of autism, the evidence of this theory shows that there is a significant correlation between the amygdala and social behavior. Abnormalities of the amygdala (including damage, volume abnormalities, microscopic lesions, etc.) can lead to socio-intellectual deficits, and social dysfunction of ASD is closely related to it (10). Rubenstein et al. proposed the hypothesis of abnormal brain activation or inhibition of ASD, This hypothesis believes that the key nervous system excitation/inhibition ratio increases under genetic factors or environmental factors, resulting in cortical "noise" is the key to the occurrence of autism, thus providing a new way to treat ASD by inhibiting neural excitability (11). In recent years, Just et al. proposed the hypothesis, of disconnection of the cerebral cortex in ASD, that is the functional connections of various cerebral regions in the cortex were weakened, which revealed that the brain information coordination and integration ability of ASD was weakened (12). In particular, high cell counts in the prefrontal cortex, enlarged amygdala volume, and reduced functional connectivity between the anterior cingulate cortex (ACC) and the dorsolateral prefrontal cortex (DLPFC) are considered to be specific neurostructural features in children with ASD (13).

In order to change and improve the developmental trajectory of ASD, numerous scholars have conducted extensive research and discussion on various aspects of autism rehabilitation treatment. Most of these studies are based on behavioral interventions accompanied by related medications, but the results are often unsatisfactory (14). Therefore, it is necessary to explore a new therapeutic approach to complement the behavioral intervention-based treatment model in order to improve the intervention effect. NIBS is a kind of emerging therapy, and that is increasingly being used in adult and pediatric neurological rehabilitation (15). NIBS interventions refer to non-invasive and painless transcranial stimulation neuromodulation techniques, including transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (TDCS) (16). TMS works by converting coil currents into intermittent, localized pulsed magnetic fields that act on the cerebral cortex, causing local depolarization and firing of neurons. TMS mainly includes single-pulse TMS (sTMS), paired TMS (pTMS), and repetitive TMS (rTMS). Theta-Burst Stimulation (TBS) also known as theta-burst transcranial magnetic stimulation, a variation of TMS, consists of a 50-Hz triplet pulse burst with a 200-ms interburst interval typically at 80% of the Active Motor Threshold (AMT) (17). iTBS, a type of TBS (18), is similar to the Long-term Potentiation (LTP) of rTMS, which achieves excitability by high-frequency stimulation (> 1 Hz), and could induce excitability in the primary motor cortex (19). Among them, the use of rTMS in the treatment of pediatric and adolescent diseases is more applied, which refers to the repeated stimulation of specific areas of the scalp with the same pattern and time intervals to achieve the treatment effect (20–22). rTMS includes high-frequency stimulation (≥5 Hz) and low-frequency stimulation≤ (1 Hz), high-frequency rTMS produces long-duration enhancement, which increases cortical excitability, and low-frequency rTMS produces long-duration inhibition, which decreases cortical excitability (23). In recent years, rTMS has been tried as an adjunctive therapy for ASD and can improve some of the core symptoms of ASD (24).

tDCS can cause hyperpolarization of resting membrane potential and regulate the activity of neural networks by using direct electrical currents to stimulate a targeted cortical area, to achieve the therapeutic purpose (25). Compared with TMS, tDCS does not directly induce brain activity but changes the excitability of spontaneous brain activity by subliminal regulation of neuronal membrane potential (26). In general, the anode of tDCS increases neuronal excitability and the cathode decreases it. Continuous regulation of neural flexibility induced by tDCS may be the basis for its treatment of psychiatric disorders (27). At present, there are many studies and applications of tDCS on speech impairment, social impairment, stereotyped behavior, and emotional changes in ASD patients of different ages (28, 29). tDCS provides appropriate stimulation to the patient based on the accurate positioning given by the pathological analysis of ASDs to achieve relief of symptoms.

Clinical studies have shown that the NIBS techniques for the treatment of patients with ASD were effective. Luckhardt et al. (30) systematically searched for the database before 2020, and six eligible randomized, sham-controlled clinical trials of tDCS in patients of ASD were included. The analysis indicated that tDCS improved significantly ASD patients' cognitive and social-communication skills. Recently, 10 studies were rated as low risk of bias in a systematic review by Zhang et al. (31). Four investigated the efficacy of tDCS on ASD while six focused on TMS, the results showed that tDCS significantly improved empathy quotient (EQ) and facial emotion recognition and processing (FERP) scores for emotions that conveyed a threat, and social and health/behavioral domains of autism treatment evaluation checklist (ATEC) also improved significantly. Active deep rTMS significantly reduced social relating impairments as measured by the Ritvo Autism Asperger Diagnostic Scale (RAADS) and decreased self-oriented anxiety in difficult social environments as measured by the interpersonal reactivity index (IRI), as compared to sham stimulation. However, the problems at this stage are that the sample size of clinical research is small and the types of trials are different, and the quality of literature is uneven, resulting in the inconsistent interpretation of research results. Therefore, it's necessary to comprehensively and systematically evaluate the curative effect of the NIBS method on ASD patients. We conduct a rigorous systematic review and meta-analysis of related clinical randomized controlled trials (RCT). Looking for Evidence-Based Medicine Evidence of Objective Science, to provide decision-making and basis for the clinical rehabilitation treatment of NIBS method.

2. Methods

This systematic review and meta-analysis strictly followed the protocol developed by Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (32) and is registered with PROSPERO (reference number: CRD42022366000).

2.1. Inclusion and exclusion criteria

Inclusion Criteria: ① Subjects: Clinically diagnosed autistic patients, regardless of race or gender; ② Intervention method: The intervention group was treated with TMS or tDCS; ③ Control group: sham stimulation, conventional treatment or blank control; ④ Study type: Randomized controlled trial. Include placebo (sham) control, baseline control, or candidate control; ⑤ Published in English.

Exclusion Criteria: ① Self before and after-control studies, cohort and case–control studies, cross-sectional studies, and other non-RCT; ② Literatures with no comparable baseline or no baseline reported; ③ Literature with imprecise design or inappropriate statistical methods; ④ Literatures with incomplete data, whose original data and the full text cannot be obtained after contacting the author; ⑤ Literatures with no corresponding outcome indicators; ⑥ Literatures with unclear diagnostic criteria, intervention time, and intervention programs; ⑦ Duplicate publications; ⑧ Conference abstracts, animal experiments, experimental protocols, expert experience summaries, case reports, meta-analysis, and review literature, etc.

2.2. Search strategy

Combination of computer and manual retrieval, from the establishment of the database to October 2022, the database includes PubMed, Web of Science, Medline, Embase, and Cochrane Library. Collect all RCTs of NIBS improving ASD, and supplement the literature by reading relevant reviews and references, etc. According to the way the combination of medical subject terms and free words, the retrieval time is from the establishment of the database to October 2022. Taking PubMed as an example, the retrieval strategy:#1: "autism spectrum disorder" [MeSH] OR autism spectrum disorders OR autism OR autistic spectrum disorder OR autistic spectrum disorders ; #2: "transcranial direct current stimulation" [Mesh] OR "transcranial magnetic stimulation" [Mesh] OR repetitive transcranial magnetic stimulation OR noninvasive brain stimulation OR non-invasive brain stimulation OR transcranial electrical stimulation OR rTMS OR tDCS OR TMS OR NIBS ; #3: "randomized controlled trial" [MeSH] OR random OR random allocation OR RCT ; #4:#1 and #2 and #3.

2.3. Study selection and data extraction

The literature was collected, read, screened, and extracted according to the principle of independent extraction by two persons, Extract contents include ① Basic characteristics of the included literature: author, year, sample size, intervention measures, time, stimulus parameters, and outcome indicators, etc.; ② Key points related to biased risk assessment of literature; ③ The specific data of the outcome indicators.

2.4. Outcomes

Rehabilitation outcome indicators for patients with autism are mainly assessed by using graded scales or clinically set scales, Using continuous variables (mean and standard deviation) as the basis for symptom classification, including the following 4 parts: ① Autism Behavioral Checklist (ABC): The ABC is a behavior questionnaire which is completed by child's parents or caregivers. The questionnaire marks five aspects of the child on a 4-point scale (including sensory, relating, body concept and object use, language, social, and self-care), ranging from 0 (no problem) to 3 (severe problem). The higher the score, the more serious the problem. The cutoff score was 49, and a score above 49 points indicated a high probability of ASD (33, 34). ② Autism Treatment Evaluation Checklist (ATEC): The ATEC is a self-administered questionnaire completed by the patient's parents, teachers, or caregivers and consists of 77 items divided into 4 subtests. The first assesses speech or language communication with comprises 14 items. The second assesses sociability with 20 items. The third assesses sensory or cognitive awareness with 18 items. The fourth assesses the health/physical/behavior with 25 items. Score range, 0–179. A higher score indicates more serious symptoms of ASD (35). ③ Childhood Autism Rating Scale (CARS): The CARS is a tool that incorporates information from the caregiver's report and direct observation from Clinicians, completed by clinicians, A score of ≥30 points indicates a possible diagnosis of ASD (36). ④ Repetitive Behavior Scale-Revised (RBS-R): The RBS-R was intended for use in evaluating repetitive behaviors observed in ASD primarily. It is a comprehensive 44-item parent/caregiver report questionnaire. The scale measures stereotyped behavior, self-injurious behavior, compulsive behavior, routine behavior, sameness behavior, and restricted behavior. Each item score range 0–4.The higher the score, the more frequently the behavior occurs (37).

2.5. Assessment of the risk of bias in the included studies

Two researchers assessed the bias risk of the included RCT according to the RCT bias risk assessment tool recommended by the Cochrane system and cross-checked the results (38). The content of the evaluation includes the following six aspects: ① Random allocation method; ② schemes of Allocation concealment; ③ Blinding of participants, personnel, and outcome assessment; ④ Completeness of outcome data; ⑤ Selective reporting; ⑥ Other bias. The risk of inclusion in the literature is divided into three levels: low bias risk, bias ambiguity risk, and high bias risk. If there are differences, resolve them through discussion. If no agreement can be reached, consult the third author. In addition, funnel charts of major outcome indicators were evaluated to assess publication bias.

2.6. Statistical analyses

RevMan 5.3 software (computer program, version 5.3, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) was used for statistical analysis. Since the included studies are different in terms of intervention measures, measurement methods, and outcome assessment, SMD and its 95% CI were selected for the heterogeneity test for the combined effect value. If $p > 0.1$, $I^2 < 50\%$, it can be considered that the included studies are homogeneous, and the fixed effect model should be used for meta-analysis; If $p > 0.1$, $I^2 < 50\%$, it can be considered that the included studies are homogeneous, and the fixed effect model should

be used for meta-analysis; If the data with research results cannot be meta-analyzed, only descriptive analysis will be performed. Because the results of the random effect model are more conservative, to ensure that the data results are more credible, this study adopts the random effect model, and all analyses calculate 95% confidence intervals.

3. Results

3.1. Literature search results

Figure 1 shows the flow diagram for the selection of the included studies. 1,226 related literature were obtained in the preliminary examination. After removing duplicate publications by EndnoteX9, there are 464 articles. After reading titles and abstracts, 382 articles were removed. After reading the full text of the remaining 82 articles, 22 articles were finally included. A total of 25 RCTs were included in 22 articles.

3.2. Study characteristics

Tables 1–3 show the characteristics, technical parameters, and results of the included studies. In this study, one of the 22 studies included three RCTS (39), so in the end we selected 25 RCTs studies using TMS or tDCS to treat ASD patients through standard and rigorous screening procedures. Ten studies used tDCS (40–49) and 15 studies used TMS (19, 39, 50–59) as a treatment tool. Among the included articles, 19 controlled studies used a sham stimulation group as a control group, and the remaining 5 (50–54) compared patients with autism with patients in a waiting group. Among included studies, a total of 829 ASD patients were treated with NIBS (305 patients received tDCS intervention and 521 patients received TMS intervention). Four studies (19, 55–57) recruited adults with ASD and all focused on TMS intervention studies, the remaining studies mainly recruited children and adolescents with ASD. Subjects spanned virtually the entire autism spectrum, including high-functioning (101 patients), low-functioning (35 patients), and Asperger's with and without language and cognitive impairments (35 patients). There are

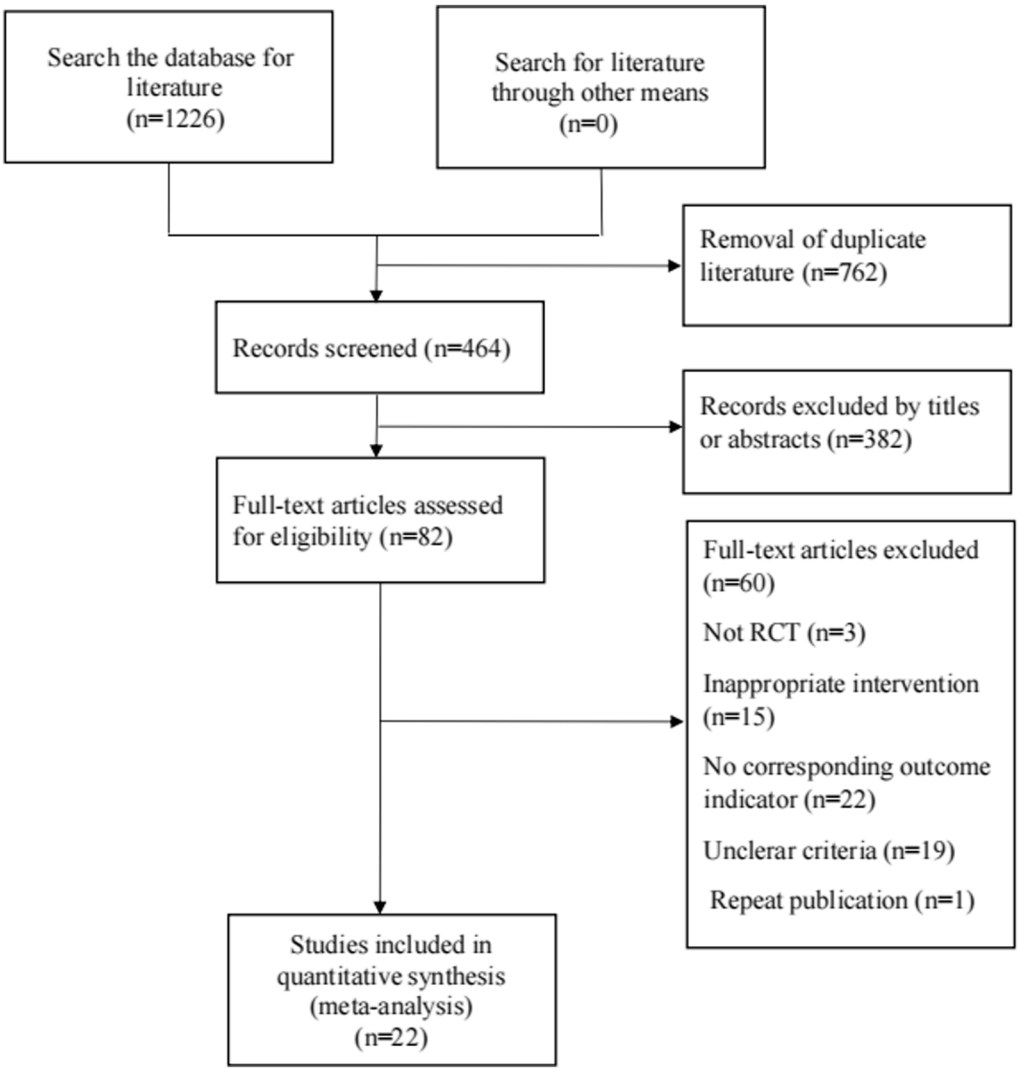


FIGURE 1
Literature selection process.

TABLE 1 Study and sample characteristics for included studies.

Author	Country	Study design	Experimental group				Control group				
			Number	Male	Female	Age (years)	Number	Male	Female	Age (years)	Diagnosis
Qiu (2021)	China	RCT (sham controlled)	20	16	4	-	20	14	6	-	ASD
Hadoush (2020)	Jordan	RCT (sham controlled)	25	19	6	7.6 ± 2.2	25	22	3	8.0 ± 2.8	ASD
Salehinejad (2021)	Iran	RCT (sham controlled)	7	-	-	10.7 ± 1.9	7	-	-	10.7 ± 1.9	ASD
Zemestani (2022)	Iran	RCT (sham controlled)	17	-	-	-	15	-	-	-	ASD
Amatachaya (2014)	Thailand	RCT (sham controlled)	10	10	0	6.4 ± 1.1	10	10	0	6.4 ± 1.1	ASD
Amatachaya (2015)	Thailand	RCT (sham controlled)	10	10	0	6.4 ± 1.1	10	10	0	6.4 ± 1.1	ASD
Sun (2022)	China	RCT (sham controlled)	19	15	4	8.0 ± 1.9	18	15	3	8.0 ± 1.9	ASD
Mahmoodifar (2020)	Iran	RCT (sham controlled)	9	-	-	11.04 ± 2.80	9	-	-	9.31 ± 2.70	ASD
Han (2021)	China	RCT (sham controlled)	20	18	2	17.03 ± 2.55	21	20	1	17.10 ± 2.30	ASD
Hadoush (2022)	Iran	RCT (sham controlled)	18	15	3	8.1 ± 2.9	18	16	2	7.6 ± 2.6	ASD
Joshua (2010)	America	RCT (waitlist controlled)	16	-	-	13.9 ± 5.3	9	-	-	13.5 ± 2.0	ASD
Panerai (2014) study I	Italy	RCT (sham controlled)	9	-	-	13.56 ± 1.83	-	-	-	13.56 ± 1.83	Low-function ASD
Panerai (2014) study II	Italy	RCT (sham controlled)	12	-	-	13.56 ± 1.88	5	-	-	13.24 ± 2.95	Low-function ASD
Panerai (2014) study III	Italy	RCT (sham controlled)	6	-	-	16.13 ± 3.11	-	-	-	16.13 ± 3.11	Low-function ASD
Panerai (2014) study IV	Italy	RCT (training controlled)	8	-	-	13.27 ± 4.03	5	-	-	14.17 ± 4.24	Low-function ASD
Ni (2021)	China	RCT (sham controlled)	40	35	5	13.0 ± 2.8	35	30	5	12.5 ± 2.9	ASD
Kang (2021)	China	RCT (sham controlled)	16	13	3	7.8 ± 2.1	16	13	3	7.2 ± 1.6	ASD
Ni (2017)	China	RCT (sham controlled)	19	-	-	20.8 ± 1.4	19	-	-	20.8 ± 1.4	ASD
Iska (2021)	Canada	RCT (sham controlled)	16	13	3	23.1 ± 4.66	12	8	4	23.4 ± 4.93	ASD
Peter (2014)	Israel	RCT (sham controlled)	15	13	2	33.87 ± 13.07	13	10	3	30.54 ± 9.83	High-function ASD, Asperger
Stephanie (2020)	Canada	RCT (sham controlled)	20	14	6	23.50 ± 4.2	20	14	6	21.65 ± 4.6	ASD
Sokhadze (2009)	America	RCT (waitlist controlled)	8	8	0	18.3 ± 4.8	5	5	0	16.2 ± 5.7	ASD
Casanova (2012)	America	RCT (waitlist controlled)	25	-	-	12.9 ± 3.1	20	-	-	13.1 ± 2.2	ASD
Sokhadze (2012)	America	RCT (waitlist controlled)	20	16	4	13.5 ± 2.5	20	16	4	14.1 ± 2.4	ASD, Asperger
Sokhadze (2018)	America	RCT (waitlist controlled)	86	71	15	13.1 ± 1.78	26	22	4	13.3 ± 1.78	High-function ASD

10.3389/fpsyt.2023.1147327

TABLE 2 Stimulation parameters for included studies.

Study	Interventions		tDCS procedure				TMS procedure				Treatment duration
	E	C	Polarity	Anodal location	Cathodal location	Intensity (mA)	Target location	Frequency (Hz)	MT (%)	Pulses	
Qiu (2021)	tDCS	Sham	Anodal	Left DLPFC	Right shoulder	1					15 × 20 min (five times a week)
Hadoush (2020)	tDCS	Sham	Bilateral anodal	Left and right frontocentral (FC1-FC2)	Left and right supraorbital (Fp1-Fp2)	1					10 × 20 min (five times a week)
Salehinejad (2021)	tDCS	Sham	Anodal	1.Right temporoparietal junction (CP6) 2.vmPFC (Fpz)	Left shoulder	1					20 min (3 single sessions)
Zemestani (2022)	tDCS	Sham	Anodal	Left DLPFC	right DLPFC	1.5					10 × 15 min (two times a week)
Amatachaya (2014)	tDCS	Sham	Anodal	Left DLPFC	Right shoulder	1					10 × 20 min (last 8 weeks)
Amatachaya (2015)	tDCS	Sham	Anodal	Left DLPFC	Right shoulder	1					20 min (last 3 weeks)
Sun (2022)	tDCS + rehabilitation	Sham + rehabilitation	Anodal	Left DLPFC	Right supraorbital	1.5					12 × 20 min (three times a week)
Mahmoodifar (2020)	tDCS	Sham	Anodal	Left motor cortex (M1)	Right supraorbital	1.5					10 × 20 min
Han (2021)	tDCS + cognitive training	Sham + cognitive training	Anodal	Left DLPFC	Right supraorbital	1					10 × 20 min (last 2 weeks)
Hadoush (2022)	tDCS	Sham	Bilateral anodal	Left and right cerebellar hemispheres	left and right supra-orbital area	1					10 × 20 min (five times a week)
Joshua (2010)	rTMS	Waitlist					Left and right DLPFC	1	90	150 (15 × 10)	per week (last 12 weeks)
Panerai (2014) study I	rTMS	Sham					Left and right premotor cortex,2.5 cm rostral to primary motor cortex	LFrTMS: 1; HFrTMS: 8	90	LFrTMS: 900; HFrTMS: 30 trains of 30 stimuli each trial lasting 3.6 s	Every 2 weeks
Panerai (2014) study II	rTMS	Sham					Left premotor cortex, 2.5 cm rostral to primary motor cortex	LFrTMS: 1; HFrTMS: 8	90	LFrTMS: 900; HFrTMS: 30 trains of 30 stimuli each trial lasting 3.6 s	Every weekday (10 days), over 2 weeks
Panerai (2014) study III	rTMS	Sham					Left premotor cortex, 2.5 cm rostral to primary motor cortex	LFrTMS: 1; HFrTMS: 8	90	LFrTMS: 900; HFrTMS: 30 trains of 30 stimuli each trial lasting 3.6 s	Daily (last 5 days)
Panerai (2014) study IV	rTMS	Eye–hand integration training					Left premotor cortex, 2.5 cm rostral to primary motor cortex	8	90	LFrTMS: 900; HFrTMS: 30 trains of 30 stimuli each trial lasting 3.6 s	Every weekday (10 days), over 2 weeks
Ni (2021)	iTBS	Sham					Bilateral pSTS	50	80	38,400	twice a week (last 4 weeks)
Kang (2021)	rTMS	Sham					Left, right and bilateral DLPFC	1	90	180 (18 × 10)	twice a week (last 9 weeks)
Ni (2017)	iTBS	Sham					Left and right DLPFC; posterior superior temporal sulcus	50	80	Two courses of 600 on each hemisphere, left first, 5 min apart	1 week interval between sessions
Iska (2021)	rTMS	Sham					Bilateral DLPFC	20	90	1,500	five times a week (last four weeks)
Peter (2014)	rTMS	Sham					Bilateral DMPFC, coil centered and 7 cm anterior to M1, 3–4 cm from nasion	5	100	1,500	Every weekday (10 days)
Stephanie (2020)	rTMS	Sham					Left and right DLPFC	20	90	1,500	20-session (last 4 weeks)
Sokhadze (2009)	rTMS	Waitlist					Left DLPFC, 5 cm anterior to maximal FDI response	0.5	90	150 (15 × 10)	twice a week (last 3 weeks)
Casanova (2012)	rTMS	Waitlist					Left and right DLPFC	1	90	150 (15 × 10)	once a week (last 12 weeks)
Sokhadze (2012)	rTMS	Waitlist					Left and right DLPFC	1	90	150 (15 × 10)	once a week (last 12 weeks)
Sokhadze (2018)	rTMS	Waitlist					Left and right DLPFC	1	90	180 (9 × 20)	once a week (last 18 weeks)

E, experimental group; C, control group; MT, Motor threshold; tDCS, transcranial direct current stimulation; HFrTMS, High-frequency repetitive transcranial magnetic stimulation; iTBS, Intermittent Theta-burst stimulation; LFrTMS, Low-frequency repetitive transcranial magnetic stimulation; rTMS, Repetitive transcranial magnetic stimulation; RCT, randomized controlled trial; DLPFC, dorsolateral prefrontal cortex; DMPFC, dorsomedial prefrontal cortex; FDI, First Dorsal Interosseous; vmPFC/Fpz, ventromedial prefrontal cortex; Fp1, left supraorbital area; Fp2, right supraorbital area; M1, left primary motor cortex; CP6/rTPJ, right temporoparietal junction; FC1/FC2, left and right frontocentral regions; pSTS, posterior superior temporal sulcus.

10.3389/fpsyt.2023.1147327

large differences between the various treatment options. In tDCS treatment studies, the common stimulation area is the DLPFC. In most cases, the left hemisphere is preferred for anodal or cathodal stimulation. The anodal is the most commonly selected stimulation method in the study, and the intensity is often selected for 1 to 1.5 mA. The treatment lasted 20 min and the frequency of treatment varied from once a day to twice a week. Of these, 8 studies (40–42, 45–49) looked at behavioral improvements in patients with autism as the primary outcome indicator, The Autism Treatment Evaluation Checklist (ATEC) and the Aberrant Behavior Checklist (ABC) are the most commonly used tools for post-treatment evaluation. In addition, 4 studies (40–43) used more objective neurobiological markers as the outcome of the measurement, focusing on patients' neuropsychological function, brain connectivity, and spontaneous, rhythmic electrical activity of brain cell groups. At present, we can see that patients showed significant improvement after active tDCS stimulation. Of all the included studies, only one study (44) reported mild side effects, which disappeared soon after discontinuation of treatment.

In the rTMS study, 2 studies (55, 58) used iTBS for clinical intervention. The remaining 13 studies used rTMS traditionally. Most studies have applied unilateral or bilateral stimulation to DLPFC using low-frequency stimulation (0.5–1 Hz). An article (44) delivered multisegmental stimulation to the bilateral premotor cortex (PrMC) at a stimulation frequency of 1–8 Hz. 1 study (47) implemented rTMS targeting the dorsal medial prefrontal cortex at a stimulation frequency of 5 Hz. It is worth noting that Ni et al. (58) chose the posterior superior temporal sulcus (posts) (60), one of the three target stimulation areas currently recognized by the academic community as promising to improve the core symptoms of ASD, as the target for treatment with 50 Hz iTBS. The results showed that cognitive improvement was not significant in a patient with autism, which may be related to individual signs and the length of treatment.

Treatment schedules also varied widely, with most of the included studies receiving daily or twice-weekly treatments for a minimum of 5 days and a maximum of 12 weeks. Only two studies used neuronavigation to guide stimulation of the intended cortical region, other common alternatives are EEG positioning or recommended by the developers of the coil. The most common side effects were mild headache and skin irritation, but the symptoms were mild.

3.3. Risk of bias of included studies

Table 3 and Figures 2, 3 shows a summary of the quality assessment of the selected studies. Details of bias risk in all included studies are shown in Figures 2, 3. The included studies were evaluated using the Cochrane Bias Risk Assessment Scale. All 18 studies reported subjects at baseline and were comparable; The literature refers to "randomization" or "randomized controlled trials," 14 described specific random methods, such as random number table and computer random, and 6 carried out Allocation concealment. Thirteen mentioned signing informed consent. In allocation concealment, only 2 studies were high risk and 8 studies were low risk. For the blind approach to outcome assessment, only 1 study had a high risk and 11 had an undefined risk of bias. Due to the nature of the NIBS intervention, all studies are not free from implementation bias. Analysis of funnel plot using the ABC scale score as the outcome indicator (Figure 4). It shows that the distribution of scattered points on both sides of the midline is basically symmetrical, showing an inverted funnel shape, the possibility of bias in the included literature is small, and the meta-analysis results are more reliable. Because the number of included literature using ATEC, CARS, and RBS-R as indicators is small, it is not suitable for funnel plots.

3.4. Meta-analysis results

Of the 22 included articles, 11 had extractable data for a meta-analysis of behavioral or cognitive outcomes in patients treated with NIBS. In the process of data extraction, we found that the evaluation indicators used in some literature are different, which makes it impossible to combine the data for analysis. So we chose ABC, ATEC, CARS, and RBS-R which are commonly used internationally, as indicators for evaluating the behavior and cognitive abilities of autistic patients for analysis.

3.4.1. ABC

A total of 5 studies (41, 45, 50, 51, 54) were included, and 4 literature (17, 34, 35, 38) reported the score of the hyperactivity subscale, Meta-analysis results of the random effects model showed that the score of the experimental group was lower than that of the control group, there was a significant difference (SMD = −0.6, 95%CI [−0.93, −0.28], $p < 0.01$). The heterogeneity test showed no significant heterogeneity among different studies ($\chi^2 = 0.55$, $p = 0.91$, $I^2 = 0\% < 50$).

Four articles (45, 50, 51, 54) reported irritability subscale scores and meta-analysis of the random-effects model showed no significant difference between the test group and the control group (SMD = −0.61, 95%CI [−1.26, 0.04], $p = 0.06$). The heterogeneity mainly comes from the study of Qiu et al. (39), after removing outliers, I2 drops to 0. We found that Qiu et al. (45) studied autistic children aged 2–6 years, whereas the other three studies studied autistic adolescents aged 13–18. Therefore, the discrepancy may be due to different study subjects. Notably, although the difference between the test and control groups was not significant, there was a trend in favor of NIBS treatment.

Two literature (45, 54) reported the scores of the Social Withdrawal subscale, and the meta-analysis results of the random effect model showed that there was no significant difference between the scores of the test group and the control group (SMD = −0.71, 95%CI [−1.40, 1.06], $p = 0.78$). Two literature (41, 45) reported the total score of ABC, the meta-analysis results of the random effect model showed that there was no significant difference between the scores of the test group and the control group (SMD = −0.32, 95%CI [−0.98, 0.33], $p = 0.33$). But, due to the small number of studies included, the results should be interpreted with caution (Figure 5).

3.4.2. ATEC

A total of 3 studies (46, 47, 54) were included, and meta-analysis was performed on the five dimensions of ATEC. The results showed that test groups are significant improvements in the three dimensions of ATEC: social (SMD = −0.62, 95% CI [−1.05, −0.20], $p = 0.004$), health and behavioral problems (SMD = −0.65, 95% CI [−1.08, −0.23], $p = 0.003$), and ATEC total score (SMD = −0.75, 95% CI [−1.19, −0.32], $p < 0.001$). The heterogeneity test showed no significant heterogeneity among studies (Figure 6).

TABLE 3 Outcome measures and results for included studies.

Study	Cognitive measures	Behavioral measures	Biological measures	Cognitive outcomes	Behavioral outcomes	Biological outcomes	Follow up	Side effects
Qiu (2021)		Key symptoms (CARS, ABC, RBS-R), sleep condition (CSHQ)			Real tDCS significantly reduced CARS and sleep habit scores, while sham tDCS significantly reduced ABC scores			None
Hadoush (2020)		Symptoms (ATEC)			Significant potential therapeutic effects on children with ASD in terms of improvements in sociability, behavior, health, and physical conditions			None
Salehinejad (2021)	Theory of mind test (TOM)			Compared with rTPJ tDCS and sham stimulation, anodal vmPFC tDCS significantly improved ToM in children with ASD				Mild adverse effects
Zemestani (2022)	Theory of Mind (ToM)	Gilliam Autism Rating Scale-second edition (GARS-2),Emotion Regulation Checklist (ERC)			A significant improvement of autism symptom severity, theory of mind, and emotion regulation strategies was observed for the active as compared to the sham stimulation group			None
Amatachaya (2014)	CGI-I	CARS, ATEC, CGAS			Anodal F3 tDCS Improved social functioning, behavior, sensory or cognition, ATEC scores compared to sham tDCS			None
Amatachaya (2015)		ATEC	EEG record		Improvement in social behavior and behavioral ATEC scores after receiving the tDCS intervention	PAF significantly increased at the stimulation site		None
Sun (2022)		ABC	Electroencephalography		Behavioral abilities improved significantly in both groups after receiving the intervention. The active tDCS group was significantly better than the control group.	MMN amplitude was elevated between both groups, but there was no significant difference		None
Mahmoodifar (2020)		Movement Assessment Battery for Children-2 (MNBC-2)			sham tDCS combined with motor training improved balance. Active tDCS + training showed a significantly higher improvement compared to sham + training			None
Han (2021)		Social functioning (SRS-2)	Measured prefrontal resting-state functional connectivity (rsFC)		improvement in overall social functioning in the active and sham tDCS groups differed significantly	Greater interindividual variability among participants in rsFC raw change in the right medial PFC		None
Hadoush (2022)			Record and calculate the approximate entropy (ApxEnt) values of the resting-state electroencephalograph (EEG) data obtained from a 64-channel EEG system			Bilateral cerebellar anodal tDCS modulated and increased the brain complexity in children with ASD		None
Joshua (2010)	Reaction time and error rates in an oddball- type task	ABC, RBS-R, SRS	Gamma activity	No significant differences	Significant decrease in irritability and repetitive behavior subscales of ABC, and in repetitive behavior subscale of RBS-R after treatment	Increased gamma power to targets and decreased gamma power to non-targets after treatment		Itching sensation at nose, mild headache

(Continued)

TABLE 3 (Continued)

Study	Cognitive measures	Behavioral measures	Biological measures	Cognitive outcomes	Behavioral outcomes	Biological outcomes	Follow up	Side effects
Panerai (2014) study I	Degree of completion of hand-eye combination tasks from PER-P			HFrTMS: increase in eye–hand integration after TMS to left premotor cortex. LFrTMS and sham: no differences in eye–hand integration.				None
Panerai (2014) study II	Degree of completion of hand-eye combination tasks from PER-P			LFrTMS, HFrTMS, and sham: highest increase in mean performance with HFrTMS, followed by LFrTMS and sham. Pre-post comparisons showed difference only for HFrTMS				None
Panerai (2014) study III	Degree of completion of hand-eye combination tasks from PER-P			Significant increase in eye-hand integration after TMS, in comparison to sham			2,5 days follow up; HfrTMS showed no difference from sham TMS or from baseline assessment	None
Panerai (2014) study IV	Degree of completion of hand-eye combination tasks from PER-P			Pairwise comparisons showed a statistical difference between HFrTMS + Eye–hand integration training and both treatments alone			1 month follow up; TMS + training significantly better than either intervention alone after 1 week; at 2 weeks TMS + training superior to training alone; at 4 weeks no differences between groups	None
Ni (2021)	Reading the Mind in the Eyes test (RMET), Frith–Happe animations task	SRS, RBS-R		no significant group-by-time interaction	no significant group-by-time interaction			Slight headache, dizziness, tinnitus, and anxiety
Kang (2021)		ABC	Recurrence quantification analysis (RQA) was employed to quantify the nonlinear features of electroencephalogram (EEG) signals recorded during the resting state. Three RQA measures, including recursive rate (RR), deterministic (DET) and mean diagonal length (L) were extracted from the EEG signals to characterize the deterministic features of cortical activity.		Significant improvements in ABC scores in social relating behaviors for the experimental group	Significant differences in RR and DET were observed between the experimental group and the control group.		None

(Continued)

TABLE 3 (Continued)

Study	Cognitive measures	Behavioral measures	Biological measures	Cognitive outcomes	Behavioral outcomes	Biological outcomes	Follow up	Side effects
Ni (2017)	CCPT, WCST	Y-BOCS, SRS		Significant decrease in reaction time in the CCPT after DLPFC stimulation compared to sham	Comparison to sham, significant reduction in compulsive behaviors subscale of Y-BOCS after pSTS stimulation, and improvement in social communication subscale of SRS after DLPFC stimulation			Transient muscle twitches around the eyes
Iska (2021)			Examined glutamatergic (Glx) or γ-aminobutyric acid (GABA) metabolite levels			Active rTMS can modulate Glx levels in individuals with ASD, and that the direction of change is associated with baseline Glx levels.		None
Peter (2014)	Reading the mind in the eyes test and mentalizing test	RAADS, AQ, IRI		No significant differences in mentalizing measures	Significant decrease in social relatedness subscale of RAADS, and in personal distress subscale of IRI compared to sham			light headedness and facial discomfort during stimulation
Stephanie (2020)	Cambridge Neuropsychological Test Automated Battery (CANTAB) and BRIEF Metacognition Index (BRIEF-MCI)			No significant difference between active vs. sham rTMS on executive functions performance				Mild and transient discomfort
Sokhadze (2009)	Reaction time and error rates in an oddball- type task	ABC; RBS-R; SRS; CGI	Gamma activity; ERPs	No significant differences in reaction time and error rates after treatment	Significant decrease in repetitive behavior of RBS-R	Decrease in gamma power, amplitude of the frontal P3a and latency of the centro-parietal P3b to non-targets after treatment		None
Casanova (2012)	Reaction time and error rates in an oddball- type task	ABC; RBS-R; SRS	ERPs	Significant decrease in total error and omission error rates after treatment	Significant decrease in irritability subscale of ABC, and in repetitive behavior subscale of RBS-R after treatment	Increased amplitude of the frontal and parietal N200 and frontal P3a and reduced latency of the frontal N200 to targets after treatment		None
Sokhadze (2012)	Reaction time and error rates in an oddball- type task		ERPs	Slowing of post-error reaction time in TMS group compared to waiting list, and significant decrease in omission error rate after treatment		Increased amplitude and reduced latency of ERN component after treatment		None
Sokhadze (2018)	Reaction time and error rates in an oddball- type task	ABC, RBS-R	ERPs	Lower percentage of committed errors, slower latency of commission errors	Decreased of T-score of the RBS-R after 18 sessions of rTMS, along with decreased irritability, lethargy/social withdrawal and hyperactivity rating scores of the ABC questionnaire.	Restored normative post-error reaction time slowing in both early and later-stage ERP indices, enhanced magnitude of error-related negativity (ERN), improved error monitoring and post-error correction functions		None

CARS, Childhood Autism Rating Scale; ABC, Aberrant Behavior Checklist; RBS-R, Repetitive Behavior Scale-Revised; CSHQ, Children's Sleep Habits Questionnaire; ATEC, Autism Treatment Evaluation Checklist; TOM, theory of mind test; GARS-2, Gilliam Autism Rating Scale-second edition; ERC, Emotion Regulation Checklist; CGAS, Children's Global Assessment Scale; CGI-I, Clinical Global Impression-Improvement; F3, left dorsolateral prefrontal cortex; EEG, Electroencephalography; PAF, peak alpha frequency; MMN, mismatch negativity; MNBC-2, Movement Assessment Battery for Children-2; SRS-2, Social Responsiveness Scale-2nd; CCPT, Conner's Continuous Performance Test; WCST, Wisconsin Card Sorting Test; Y-BOCS, Yale-Brown Obsessive Compulsive Scale; RAADS, Ritvo Autism-Aspergers Diagnostic Scale; AQ, Autism Spectrum Quotient; IRI, Interpersonal Reactivity Index; ERP, event-related potential.

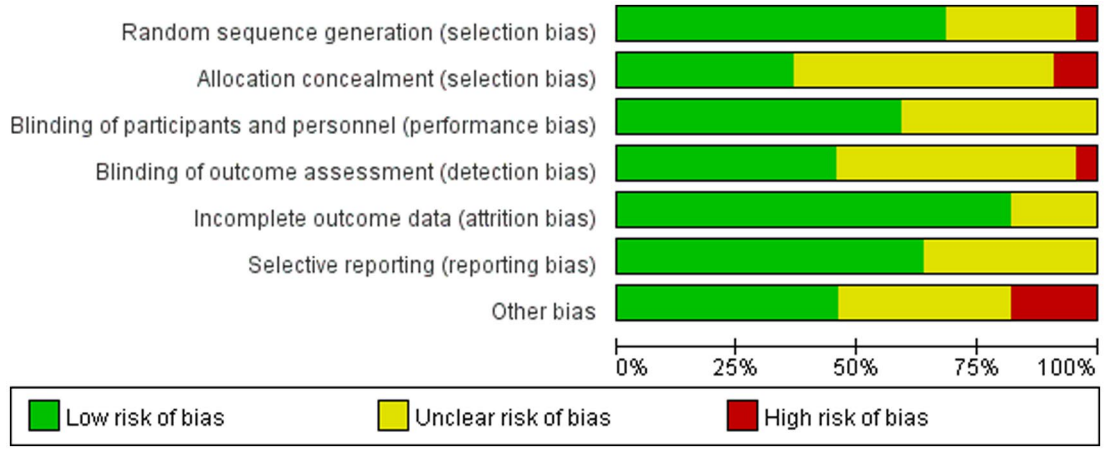


FIGURE 2
Risk of bias of the included studies.

3.4.3. CARS

A total of 2 studies (45, 46) were included, $\chi^2 = 1.77$, $p = 0.18$, $I^2 = 44\% < 50\%$. The results showed no significant heterogeneity. Analysis using fixed effects model, the total combined effect shows: Combined effect value SMD = −0.14 (95%CI = −0.65, 0.37), the overall effect test $Z = 0.54$, $p = 0.59$, As seen in the forest plot, the combined effect size (black diamond) intersects the invalid vertical lines, showing no significant difference in CARS scores between the two groups. In other words, the NIBS did not have a significant effect on patients with ASD. But given the small number of studies included, the results should be treated with caution (Figure 7).

3.4.4. RBS-R

A total of 4 studies (50, 51, 54, 58) were included, $\chi^2 = 5.05$, $p = 0.17$, $I^2 = 41\% < 50\%$. The results showed no significant heterogeneity. Analysis using fixed effects model, the total combined effect shows: SMD = −0.62 (95%CI = −0.91, −0.33), the overall effect test $Z = 4.16$, $p < 0.001$. As seen in the forest plot. The combined effect size (black diamond) was located on the left side of the vertical line, indicating a significant difference in RBS-R scores between the two groups, indicating that NIBS significantly improved repetitive behavior in ASD patients (Figure 8).

4. Discussion

The purpose of this study was to comprehensively and systematically evaluate the clinical efficacy of NIBS in patients with ASD. To our knowledge, this is the first meta-analysis of NIBS treatment in ASD populations. These studies point to NIBS as a potential intervention to reduce autism-related symptoms and improving neuropsychological function in people with autism. This meta-analysis showed that NIBS improved behavioral and cognitive abilities in people with autism compared to controls. This result is consistent with the conclusion of the latest meta-analysis (61, 62).

Behavioral and cognitive abnormalities of ASD are often characterized by severe social difficulties and stereotyped behaviors. Neuropsychology suggests that this inappropriate behavior of ASD is related to executive dysfunction, which may lead to the weakened ability of patients with ASD to regulate their own behaviors, unable to restrain unconscious behaviors, and unable to learn new behaviors, thus being subject to their own stereotyped behaviors. Qiu et al. (45) explored the effect of tDCS on children with ASD under 7 years old, by placing the anode on the left DLPFC and the cathode on the right upper arm, giving tDCS for 1 mA, 20 min, 15 times in total. CARS, ABC, and RBS-R were used to evaluate before and after treatment. The results showed that the social interaction ability of children with ASD was improved and well tolerated, That tDCS is a promising therapeutic method. Amatachaya et al. (46) used anode of tDCS to intervene in the left DLPFC of ASD patients, and found that the stereotyped behavior improved. In addition, Han et al. (42) performed 10 times tDCS interventions on 41 adult patients with ASD, which also showed a good rehabilitation effect in terms of executive function. This suggests that tDCS can indeed improve executive dysfunction in patients of ASD, for reasons closely related to the DLPFC site. Because in the process of participating in executive function, DLPFC combines with striatum and other structures to form a loop structure, which is involved in executive function, problem-solving, cognitive function, etc. (62). In addition, the DLPFC is also the highest cortical area involved in motor planning, organization, and regulation/inhibition, and is closely related to other areas, such as the orbitofrontal cortex, thalamus, parts of the basal ganglia (especially the dorsal caudate nucleus) (63). This function and association also link the DLPFC to behavioral abnormalities such as restrictive and repetitive behaviors, hypersensitivity (overreaction), and blunted response to various stimuli (under response). Therefore, DLPFC is also regarded as the main target brain area for the treatment of ASD (45). Amir (64) and Nelson et al. (65) confirmed that tDCS intervention in DLPFC could improve working memory, attention, and vigilance, which indicated that tDCS could produce neuroregulatory effects on DLPFC, thus improving the cognitive function of patients with ASD. Other areas of the brain, the ventromedial prefrontal cortex (vmPFC)associated is also with ASD. Salehinejad et al. (44) stimulated the vmPFC region of ASD patients by giving anode of tDCS,1 mA, and evaluated it by ToM before and after treatment, the results showed that vmPFCtDCS can effectively improve the patients' social ability and social cognitive

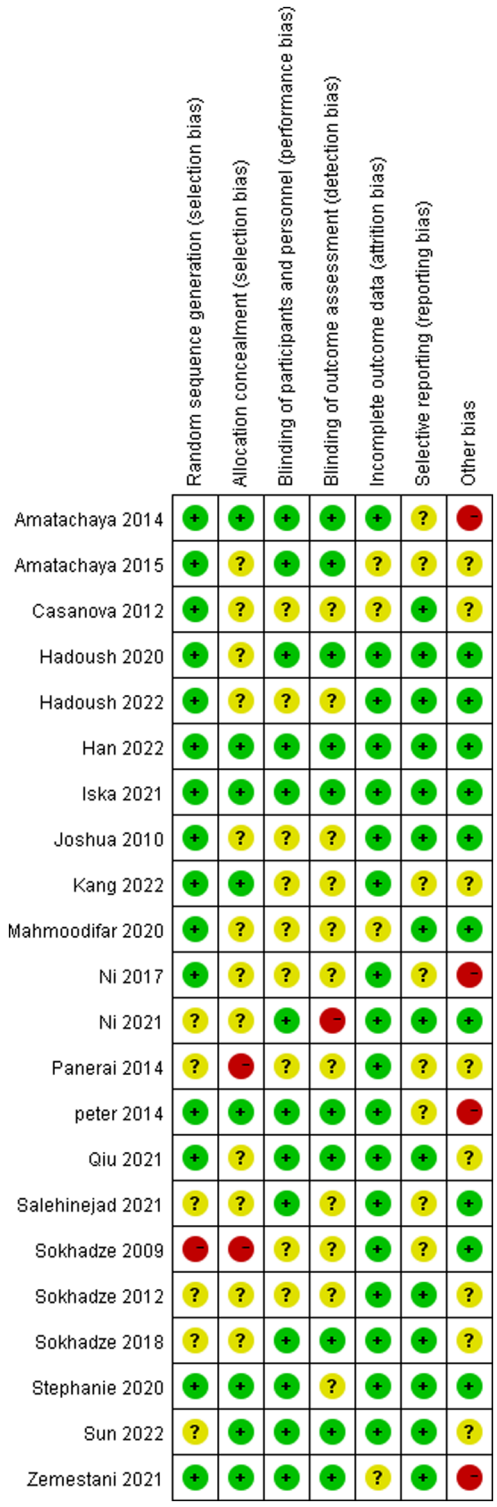


FIGURE 3
Risk of bias summary of the included studies.

function compared with the temporoparietal junction (TPJ) stimulation and false stimulation.

According to existing studies, TMS and tDCS have different mechanisms of action, but both have similar positive effects on patients with ASD. Sokhadze et al. (54) treated 112 high-functioning ASD whose IQ > 80 with low-frequency (1.0 Hz) rTMS and evaluated them with ABC and RBS-R before and after treatment. The motor accuracy and core symptoms such as stereotypical behavior and narrow interest of the experimental group were improved. Especially in the 18-week rTMS group, the patient's cognitive abilities such as attention, discrimination, and executive function improved. Ni et al. (55) applied high-frequency iTBS (50 Hz) on bilateral DLPFC of patients with ASD, the results showed that patients' anxiety, social disorder, stereotypical behavior, and cognitive function were improved. Of the 15 included studies on rTMS treatment, 9 of them (50–57, 59) selected the DLPFC as the target of stimulation, which appears to be the most favored site for rTMS treatment. This may be due to the fact that DLPFC mainly involves cognitive functions such as short-term memory, decision-making, and execution (66, 67). However, in children with ASD, due to the abnormal structure of the "microcolumn," the inhibition of the cortex is weakened, and the excitability of the cortex is increased, which leads to the weakened connection between the anterior cingulate cortex and the DLPFC, and the brain's processing ability to abnormal neural responses and wrong behaviors is decreased, and the brain is unable to make the real-time adjustment to the abnormal neural responses and wrong behaviors. Over time, it can lead to a decline in the executive ability of the child (68–70). By stimulating part of the brain and acting on the neural network around the "micro column," TMS temporarily improves its inhibitory effect on the cortex, restores the connection between the anterior cingulate cortex and the DLPFC, reestablishes the monitoring-feedback system of the brain for abnormal behaviors and reactions, and improves the problem behaviors of patients. Moreover, TMS not only affects the brain regions stimulated directly, but also related brain regions, and strengthens the functional connections between these brain regions by virtue of the interconnected characteristics of the brain neural network (71). Currently, it is encouraging to see a diversity of therapeutic targets for TMS. Panerai et al. (39) applied high-frequency rTMS (8 Hz) to the left premotor cortex (PrMC) of patients with low-functioning ASD. The results were revised using the psychoeducational profile-revised (PEP-R) scale for children with ASD. The results showed that hand-eye coordination training was significantly improved when combined with high-frequency rTMS on the left PrMC. Peter et al. (19) used high-frequency rTMS (5 Hz) in the medial prefrontal cortex (mPFC) of high-functioning ASD. After 10 days of treatment, the patient's social ability and communication skills were improved. Studies have shown that PrMC, especially left PrMC, is related to motor attention, tool use, hand-eye coordination, and other functions, mPFC is closely related to social and cognitive function (72–75).

It is worth noting that although most current studies use scale form to evaluate the therapeutic effect, the changes in scale data sometimes cannot objectively show the specific impact of NIBS on the brain function of patients with ASD. At present, it has become a novel measurement trend to observe the intervention effect of NIBS by biological means. Electrophysiological methods such as electroencephalography, event-related potential (ERP), and other measurements can objectively reflect the neuroregulatory effect of NIBS on patients with ASD, and more directly reflect the regulatory effect of NIBS on brain connectivity.

The above studies indicated that the application of tDCS in ASD intervention, patients with ASD who under 18 years of age were selected, and the majority were male. Choose more 1 mA or 1.5 mA

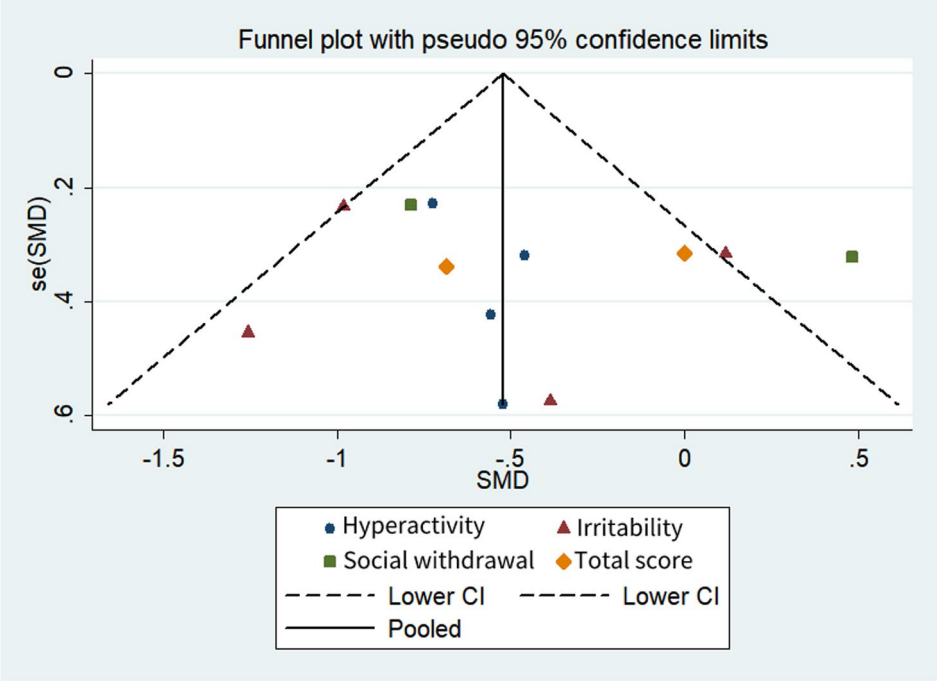


FIGURE 4
Funnel plot comparison of the ABC scores of the active and control groups. Asymmetries were not observed.

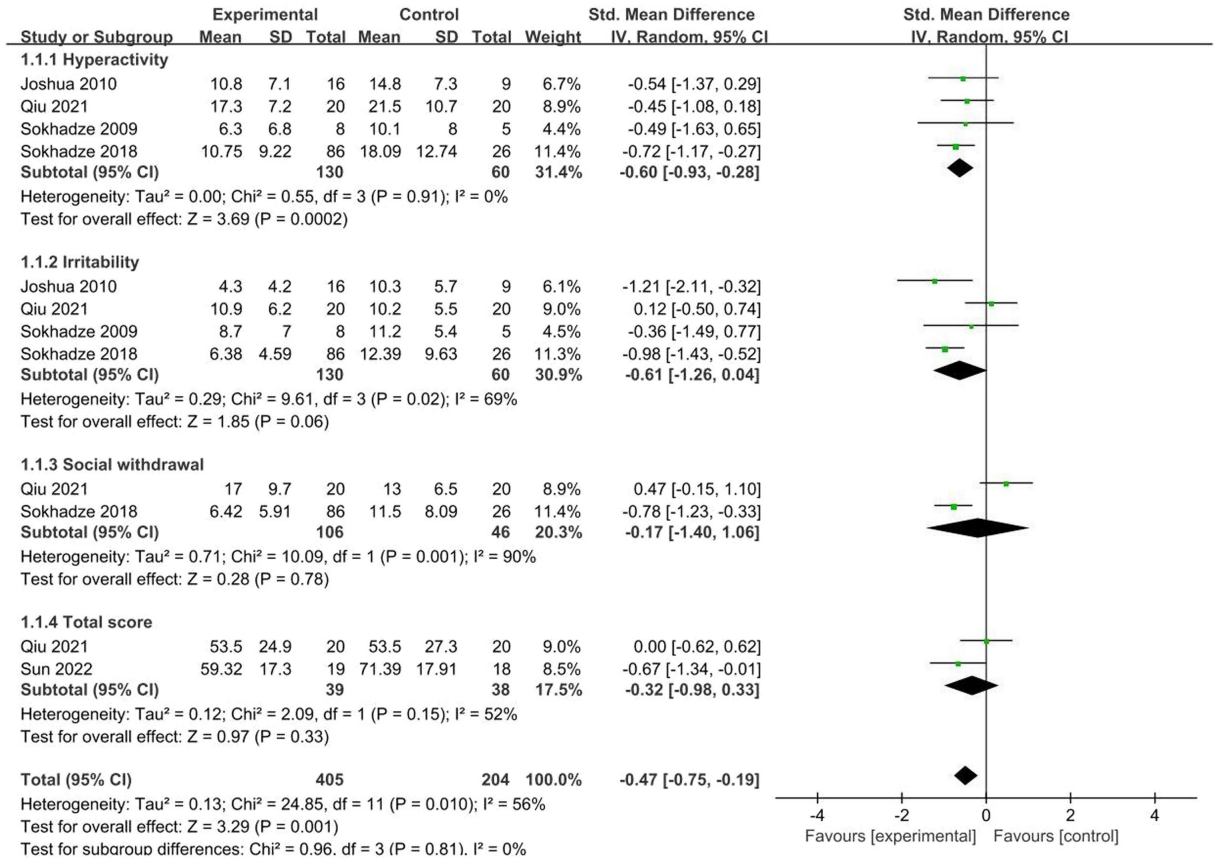


FIGURE 5
Forest plot of the effect of NIBS on ABC improvement.

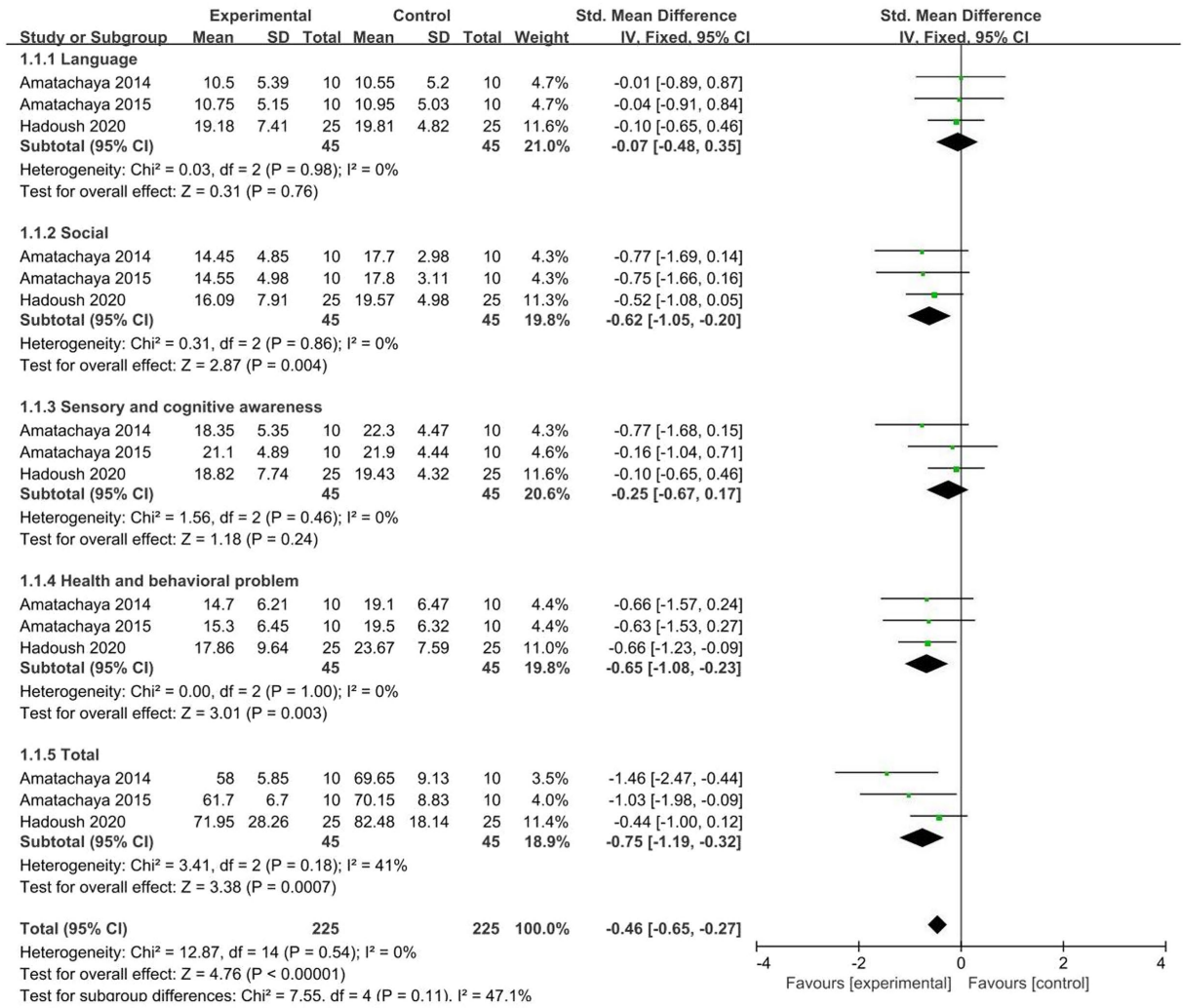


FIGURE 6
Forest plot of the effect of NIBS on ATEC improvement.

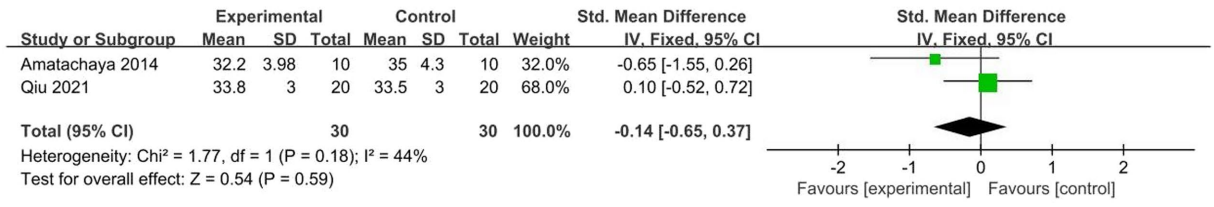


FIGURE 7
Forest plot of the effect of NIBS on CARS improvement.

anode stimulation to improve the core symptoms of ASD. However, 1.5 mA cathodic stimulation was used to improve the irritability and hyperactivity of patients with ASD, and 20 min was usually selected for each treatment. Selecting different cortical areas of the brain can also produce different therapeutic effects: applying to DLPFC can improve social impairment, abnormal behavior performance, short-term memory, etc. and applying to vmPFC can improve social impairment and cognitive level.

The patients selected for TMS were mostly male patients with high-functioning ASD, and most of them were adults or adolescents, which may be because TMS requires the cooperation of patients with higher cognitive function to complete the treatment, while adolescent or adult patients' Neurodevelopmental function is relatively complete, so it is suitable for TMS. As far as application parameters are concerned, low frequency (1 Hz or 0.5 Hz) is used more to improve core symptoms of ASD. The treatment time is also closely related to

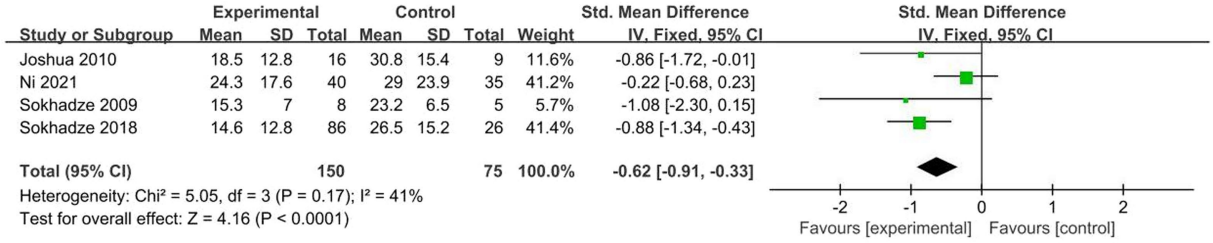


FIGURE 8
Forest plot of the effect of NIBS on RBS-R improvement.

the treatment effect. The treatment effect of 18 weeks is better than that of 6 weeks and 12 weeks, and the lasting effect is longer. High-frequency rTMS is mostly used to improve anxiety, promote social skills and communication skills, and improve the effectiveness of rehabilitation training, etc. Moreover, it is applied in different cortical areas of the brain, and different effects are obtained: application in DLPFC can improve stereotyped behavior, executive function, irritability, etc.; application in PrMC can improve language function and improve the effect of hand-eye coordination training; application in mPFC can improve social interaction ability, emotional state, etc.

5. Limitations

There are certain limitations in this study. The current clinical research on the treatment of ASD with NIBS is complex and diverse, and there are many difficulties, which makes the existing medical evidence difficult to meet the needs of reality in terms of quantity and quality, resulting in a greatly reduced accuracy of evaluation results. Meta-analysis is a retrospective observational study and cannot replace systematic, comprehensive, and in-depth clinical trials. In addition, high-strength medical evidence can improve the quality of meta-analysis. However, the literature included in this research has been published, and there is a lack of relevant gray literature, such as unpublished literature, special academic conference reports, etc. Among the 22 kinds of literature included, most of them were of low quality, and most of the studies did not adopt the double-blind method, thus reducing the strength of the conclusions of this study. Some of the included studies used a waiting group rather than a sham stimulus as a control, which may make the effect less objective. In addition, more than 80% of the studies were conducted by self-report or caregiver-report (mainly based on parent reports), therefore, results from behavioral measures may be limited by informant- vs. self-reporting. Close family members are often used as informants. However, many family members of individuals with ASD carry the diagnosis or demonstrate autistic traits without meeting the criteria of the disorder and often underreport symptoms in others that they experience. Additionally, due to interpersonal and social deficits observed in ASD, self-appraisal of social/emotional symptoms can be uniquely challenging.

Meta-analysis chooses scale scores such as ABC, ATEC, CARS, and RBS-R as evaluation indicators. Although they are simple and practical, they are greatly affected by the subjective factors of the evaluators, which may cause bias in the results and lead to unreliable conclusions. For example, the meta-analysis found that after NIBS treatment, there were no significant differences in CARS scores between the two groups. However, this result should be considered with caution due to the limited number of studies included, the small trial size, and inconsistent baseline patient characteristics. It is recommended that further large sample RCTS be performed to confirm the clinical efficacy of NIBS in patients with ASD, so as to better guide clinical decision-making.

6. Conclusion

Current studies have shown that rTMS and tDCS act on the same cerebral cortex region, producing highly overlapping therapeutic effects. It can be found from various studies that DLPFC stimulation can affect a wide range of areas and has certain effects on the core symptoms and cognitive functions of patients with ASD. Therefore, DLPFC is currently an important brain region where NIBS acts on patients with ASD. Encouragingly, stimulating other areas of the brain has also shown considerable therapeutic effects, but this needs to be seen in larger clinical studies. Meanwhile, the duration, intensity, interval, and other parameters of NIBS, as well as the accompanying treatment methods and individual characteristics, still need to be further discussed and studied. The dose, duration, and location of neuromodulation applied to different ASD children are different, and the research on the individual aspects of the treated children can become a new focus, especially in younger and lower-functioning patients with autism. In addition, it is strongly recommended that the medical history, current medication or psychotherapy, and risk–benefit ratio should be carefully assessed. Given that NIBS not only affects the stimulation site but also modulates other brain regions, future studies should carefully monitor the behavioral and physiological domains of patients in the long-term follow-up periods for any potential NIBS-induced negative effects. Although so far there are few reports on the side effects of NIBS, and the symptoms are relatively mild and easy to eliminate since most ASD patients affected by NIBS are children, long-term continuous observation and testing are needed in terms of safety and tolerance. In addition, some children with ASD will have epilepsy, attention deficit hyperactivity disorder, and other aspects of medication. How to achieve collaborative treatment under the condition of medication, or how to use NIBS treatment to relieve the side effects of drugs, to achieve the best therapeutic effect, will also become a new direction of research.

In summary, the existing results suggest that the therapeutic effect of NIBS on patients with ASD is limited, relevant clinical evidence is still insufficient and clear evidence of long-term efficacy is lacking. At this stage, NIBS cannot be recommended as a viable or evidence-based treatment for ASD. However, it is undeniable that the NIBS method is a promising treatment technique. In future clinical studies, it is necessary to conduct large-scale multi-center randomized

double-blind controlled trials to establish safe and effective stimulation parameters, select homogenous research objects, and specify treatment plans for specific symptoms, in order to bring more powerful clinical evidence for patients with ASD.

Author contributions

AL to conceived, designed, and wrote the articles. CG and BW carried out the literature retrieval and collection, literature content, data extraction, and quality evaluation. JS undertook data processing, applied RevMan software to draw relevant charts, and related statistics processing. ZJ was responsible for the quality control and proofreading of the articles. All authors contributed to the article and approved the submitted version.

Funding

This study was funded by Excellent discipline team project of Jiamusi University (JDXKTD—2019006).

Acknowledgments

The authors are grateful to all the participants who were involved in this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Cai RY, Richdale AL, Uljarević M, Dissanayake C, Samson AC. Emotion regulation in autism spectrum disorder: where we are and where we need to go. *Autism Res*. (2018) 11:962–78. doi: 10.1002/aur.1968

2. Lai M-C, Kassee C, Besney R, Bonato S, Hull L, Mandy W, et al. Prevalence of co-occurring mental health diagnoses in the autism population: a systematic review and meta-analysis. *Lancet Psychiatry*. (2019) 6:819–29. doi: 10.1016/S2215-0366(19)30289-5

3. Skuse D. Autism – 25 years on: a lot has changed! *Clin Child Psychol Psychiatry*. (2020) 25:721–5. doi: 10.1177/1359104520929729

4. Baxter AJ, Brugha TS, Erskine HE, Scheurer RW, Vos T, Scott JG. The epidemiology and global burden of autism spectrum disorders. *Psychol Med*. (2015) 45:601–13. doi: 10.1017/S003329171400172X

5. Maenner MJ, Shaw KA, Bakian AV, Bilder DA, Durkin MS, Esler A, 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) 70:1–16. doi: 10.15585/mmwr.ss7011a1

6. Yirmiya N, Seidman I, Koren-Karie N, Oppenheim D, Dolev S. Stability and change in resolution of diagnosis among parents of children with autism spectrum disorder: child and parental contributions. *Dev Psychopathol*. (2015) 27:1045–57. doi: 10.1017/S095457941500067X

7. Öz B, Yüksel T, Nasiroğlu S. Depression-anxiety symptoms and stigma perception in mothers of children with autism Spectrum disorder. *Noro Psikiyatr Ars*. (2020) 57:50–5. doi: 10.29399/npa.23655

8. Hajiabolhasani-Nargani Z, Najafi M, Mehrabi T. Effect of mobile parenting skills education on anxiety of the mothers with autistic children. *Iran J Nurs Midwifery Res*. (2016) 21:572–6. doi: 10.4103/1735-9066.197668

9. Sharma SR, Gonda X, Tarazi FI. Autism Spectrum disorder: classification, diagnosis and therapy. *Pharmacol Ther*. (2018) 190:91–104. doi: 10.1016/j.pharmthera.2018.05.007

10. Baron-Cohen S, Ring HA, Bullmore ET, Wheelwright S, Ashwin C, Williams SC. The amygdala theory of autism. *Neurosci Biobehav Rev*. (2000) 24:355–64. doi: 10.1016/S0149-7634(00)00011-7

11. Rubenstein JLR, Merzenich MM. Model of autism: increased ratio of excitation/inhibition in key neural systems. *Genes Brain Behav*. (2003) 2:255–67. doi: 10.1034/j.1601-183X.2003.00037.x

12. Just MA, Keller TA, Malave VL, Kana RK, Varma S. Autism as a neural systems disorder: a theory of frontal-posterior underconnectivity. *Neurosci Biobehav Rev*. (2012) 36:1292–313. doi: 10.1016/j.neubiorev.2012.02.007

13. Tsuchiyagaito A, Hirano Y, Asano K, Oshima F, Nagaoka S, Takebayashi Y, et al. Cognitive-behavioral therapy for obsessive-compulsive disorder with and without autism Spectrum disorder: gray matter differences associated with poor outcome. *Front Psych*. (2017) 8:143. doi: 10.3389/fpsyt.2017.00143

14. Howes OD, Rogdaki M, Findon JL, Wichers RH, Charman T, King BH, et al. Autism spectrum disorder: consensus guidelines on assessment, treatment and research from the British Association for Psychopharmacology. *J Psychopharmacol*. (2018) 32:3–29. doi: 10.1177/0269881117741766

15. Begemann MJ, Brand BA, Ćurčić-Blake B, Aleman A, Sommer IE. Efficacy of non-invasive brain stimulation on cognitive functioning in brain disorders: a meta-analysis. *Psychol Med*. (2020) 50:2465–86. doi: 10.1017/S0033291720003670

16. Kesikburun S. Non-invasive brain stimulation in rehabilitation. *Turk J Phys Med Rehabil*. (2022) 68:1–8. doi: 10.5606/tftrd.2022.10608

17. Chu H-T, Cheng C-M, Liang C-S, Chang W-H, Juan C-H, Huang Y-Z, et al. Efficacy and tolerability of theta-burst stimulation for major depression: a systematic review and meta-analysis. *Prog Neuro-Psychopharmacol Biol Psychiatry*. (2021) 106:110168. doi: 10.1016/j.pnpbp.2020.110168

18. Berlim MT, McGirr A, Rodrigues Dos Santos N, Tremblay S, Martins R. Efficacy of theta burst stimulation (TBS) for major depression: an exploratory meta-analysis of randomized and sham-controlled trials. *J Psychiatr Res*. (2017) 90:102–9. doi: 10.1016/j.jpsychires.2017.02.015

19. Enticott PG, Fitzgibbon BM, Kennedy HA, Arnold SL, Elliot D, Peachey A, et al. A double-blind, randomized trial of deep repetitive transcranial magnetic stimulation (rTMS) for autism spectrum disorder. *Brain Stimul*. (2014) 7:206–11. doi: 10.1016/j.brs.2013.10.004

20. Iglesias AH. Transcranial magnetic stimulation as treatment in multiple neurologic conditions. *Curr Neurol Neurosci Rep*. (2020) 20:1. doi: 10.1007/s11910-020-1021-0

21. Lee JC, Kenney-Jung DL, Blacker CJ, Doruk Camsari D, Lewis CP. Transcranial direct current stimulation in child and adolescent psychiatric disorders. *Child Adolesc Psychiatr Clin N Am*. (2019) 28:61–78. doi: 10.1016/j.chc.2018.07.009

22. Lefaucheur J-P, Aleman A, Baeken C, Benninger DH, Brunelin J, Di Lazzaro V, et al. Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS): an update (2014-2018). *Clin Neurophysiol*. (2020) 131:474–528. doi: 10.1016/j.clinph.2019.11.002

23. Kaur M, Michael JA, Hoy KE, Fitzgibbon BM, Ross MS, Iseger TA, et al. Investigating high- and low-frequency neuro-cardiac-guided TMS for probing the frontal vagal pathway. *Brain Stimul*. (2020) 13:931–8. doi: 10.1016/j.brs.2020.03.002

24. Doruk Camsari D, Kirkovski M, Croarkin PE. Therapeutic applications of invasive Neuromodulation in children and adolescents. *Psychiatr Clin North Am*. (2018) 41:479–83. doi: 10.1016/j.psc.2018.04.008

25. Chase HW, Boudewyn MA, Carter CS, Phillips ML. Transcranial direct current stimulation: a roadmap for research, from mechanism of action to clinical implementation. *Mol Psychiatry*. (2020) 25:397–407. doi: 10.1038/s41380-019-0499-9

26. Stagg CJ, Antal A, Nitsche MA. Physiology of Transcranial direct current stimulation. *J ECT*. (2018) 34:144–52. doi: 10.1097/YCT.0000000000000510

27. Lefaucheur J-P, Antal A, Ayache SS, Benninger DH, Brunelin J, Cogiamanian F, et al. Evidence-based guidelines on the therapeutic use of transcranial direct current stimulation (tDCS). *Clin Neurophysiol*. (2017) 128:56–92. doi: 10.1016/j.clinph.2016.10.087

28. Esse Wilson J, Quinn DK, Wilson JK, Garcia CM, Tesche CD. Transcranial direct current stimulation to the right Temporoparietal junction for social functioning in autism Spectrum disorder: a case report. *J ECT*. (2018) 34:e10–3. doi: 10.1097/YCT.0000000000000445

29. Sousa B, Martins J, Castelo-Branco M, Gonçalves J. Transcranial direct current stimulation as an approach to mitigate neurodevelopmental disorders affecting excitation/inhibition balance: focus on autism Spectrum disorder, schizophrenia, and attention deficit/hyperactivity disorder. *J Clin Med*. (2022) 11:2839. doi: 10.3390/jcm11102839

30. Luckhardt C, Boxhoorn S, Schütz M, Fann N, Freitag CM. Brain stimulation by tDCS as treatment option in autism Spectrum disorder-a systematic literature review. *Prog Brain Res*. (2021) 264:233–57. doi: 10.1016/bs.pbr.2021.03.002

31. Zhang J, Zhang H. Effects of non-invasive neurostimulation on autism spectrum disorder: a systematic review. *Front Psych*. (2022) 13:989905. doi: 10.3389/fpsyt.2022.989905

32. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev*. (2015) 4:1. doi: 10.1186/2046-4053-4-1

33. Haem E, Doostfatemeh M, Firouzabadi N, Ghazanfari N, Karlsson MO. A longitudinal item response model for aberrant behavior checklist (ABC) data from children with autism. *J Pharmacokinet Pharmacodyn*. (2020) 47:241–53. doi: 10.1007/s10928-020-09686-0

34. Marteleto MRF, Pedromônico MRM. Validity of autism behavior checklist (ABC): preliminary study. *Braz J Psychiatry*. (2005) 27:295–301. doi: 10.1590/S1516-44462005000400008

35. Geier DA, Kern JK, Geier MR. A comparison of the autism treatment evaluation checklist (ATEC) and the childhood autism rating scale (CARS) for the quantitative evaluation of autism. *J Ment Health Res Intellect Disabil*. (2013) 6:255–67. doi: 10.1080/19315864.2012.681340

36. Moon SJ, Hwang JS, Shin AL, Kim JY, Bae SM, Sheehy-Knight J, et al. Accuracy of the childhood autism rating scale: a systematic review and meta-analysis. *Dev Med Child Neurol*. (2019) 61:1030–8. doi: 10.1111/dmcn.14246

37. Lam KSL, Aman MG. The repetitive behavior scale-revised: independent validation in individuals with autism spectrum disorders. *J Autism Dev Disord*. (2007) 37:855–66. doi: 10.1007/s10803-006-0213-z

38. Cumpston MS, McKenzie JE, Welch VA, Brennan SE. Strengthening systematic reviews in public health: guidance in the Cochrane handbook for systematic reviews of interventions, 2nd edition. *J Public Health*. (2022) 44:e588–92. doi: 10.1093/pubmed/fdac036

39. Panerai S, Tasca D, Lanuzza B, Trubia G, Ferri R, Musso S, et al. Effects of repetitive transcranial magnetic stimulation in performing eye-hand integration tasks: four preliminary studies with children showing low-functioning autism. *Autism*. (2014) 18:638–50. doi: 10.1177/1362361313495717

40. Amatachaya A, Jensen MP, Patjanasoontorn N, Auvichayapat N, Suphakunpinyo C, Janjarasjitt S, et al. The short-term effects of transcranial direct current stimulation on electroencephalography in children with autism: a randomized crossover controlled trial. *Behav Neurol*. (2015) 2015:928631. doi: 10.1155/2015/928631

41. Sun C, Zhao Z, Cheng L, Tian R, Zhao W, Du J, et al. Effect of Transcranial direct current stimulation on the mismatch negativity features of deviated stimuli in children with autism Spectrum disorder. *Front Neurosci*. (2022) 16:721987. doi: 10.3389/fnins.2022.721987

42. Han YMY, Chan MMY, Shea CKS, Lai OL-H, Krishnamurthy K, Cheung M-C, et al. Neurophysiological and behavioral effects of multisession prefrontal tDCS and concurrent cognitive remediation training in patients with autism spectrum disorder (ASD): a double-blind, randomized controlled fNIRS study. *Brain Stimul*. (2022) 15:414–25. doi: 10.1016/j.brs.2022.02.004

43. Hadoush H, Hadoush A. Modulation of resting-state brain complexity after bilateral cerebellar anodal Transcranial direct current stimulation in children with autism Spectrum disorders: a randomized controlled trial study. *Cerebellum*. (2022). Online ahead of print). doi: 10.1007/s12311-022-01481-6

44. Salehinejad MA, Paknia N, Hosseinpour AH, Yavari F, Vicario CM, Nitsche MA, et al. Contribution of the right temporoparietal junction and ventromedial prefrontal cortex to theory of mind in autism: a randomized, sham-controlled tDCS study. *Autism Res*. (2021) 14:1572–84. doi: 10.1002/aur.2538

45. Qiu J, Kong X, Li J, Yang J, Huang Y, Huang M, et al. Transcranial direct current stimulation (tDCS) over the left dorsal lateral prefrontal cortex in children with autism Spectrum disorder (ASD). *Neural Plast*. (2021) 2021:1–11. doi: 10.1155/2021/6627507

46. Amatachaya A, Auvichayapat N, Patjanasoontorn N, Suphakunpinyo C, Ngernyam N, Aree-Uea B, et al. Effect of anodal transcranial direct current stimulation on autism: a randomized double-blind crossover trial. *Behav Neurol*. (2014) 2014:173073. doi: 10.1155/2014/173073

47. Hadoush H, Nazzal M, Almasri NA, Khalil H, Alafeef M. Therapeutic effects of bilateral anodal Transcranial direct current stimulation on prefrontal and motor cortical areas in children with autism Spectrum disorders: a pilot study. *Autism Res*. (2020) 13:828–36. doi: 10.1002/aur.2290

48. Zemestani M, Hoseinpanahi O, Salehinejad MA, Nitsche MA. The impact of prefrontal transcranial direct current stimulation (tDCS) on theory of mind, emotion regulation and emotional-behavioral functions in children with autism disorder: a randomized, sham-controlled, and parallel-group study. *Autism Res*. (2022) 15:1985–2003. doi: 10.1002/aur.2803

49. Mahmoodifar E, Sotoodeh MS. Combined Transcranial direct current stimulation and selective motor training enhances balance in children with autism Spectrum disorder. *Percept Mot Skills*. (2020) 127:113–25. doi: 10.1177/0031512519888072

50. Baruth JM, Casanova MF, El-Baz A, Horrell T, Mathai G, Sears L, et al. Low-frequency repetitive Transcranial magnetic stimulation (rTMS) modulates evoked-gamma frequency oscillations in autism Spectrum disorder (ASD). *J Neurother*. (2010) 14:179–94. doi: 10.1080/10874208.2010.501500

51. Sokhadze EM, El-Baz A, Baruth J, Mathai G, Sears L, Casanova MF. Effects of low frequency repetitive transcranial magnetic stimulation (rTMS) on gamma frequency oscillations and event-related potentials during processing of illusory figures in autism. *J Autism Dev Disord*. (2009) 39:619–34. doi: 10.1007/s10803-008-0662-7

52. Casanova MF, Baruth JM, El-Baz A, Tasman A, Sears L, Sokhadze E. Repetitive Transcranial magnetic stimulation (rTMS) modulates event-related potential (ERP) indices of attention in autism. *Transl Neurosci*. (2012) 3:170–80. doi: 10.2478/s13380-012-0022-0

53. Sokhadze EM, Baruth JM, Sears L, Sokhadze GE, El-Baz AS, Casanova MF. Prefrontal neuromodulation using rTMS improves error monitoring and correction function in autism. *Appl Psychophysiol Biofeedback*. (2012) 37:91–102. doi: 10.1007/s10484-012-9182-5

54. Sokhadze EM, Lamina EV, Casanova EL, Kelly DP, Opris I, Tasman A, et al. Exploratory study of rTMS Neuromodulation effects on Electrocortical functional measures of performance in an oddball test and behavioral symptoms in autism. *Front Syst Neurosci*. (2018) 12:20. doi: 10.3389/fnsys.2018.00020

55. Ni H-C, Hung J, Wu C-T, Wu Y-Y, Chang C-J, Chen R-S, et al. The impact of single session intermittent Theta-burst stimulation over the dorsolateral prefrontal cortex and posterior superior temporal sulcus on adults with autism Spectrum disorder. *Front Neurosci*. (2017) 11:255. doi: 10.3389/fnins.2017.00255

56. Moxon-Emre I, Daskalakis ZJ, Blumberger DM, Croarkin PE, Lyon RE, Forde NJ, et al. Modulation of dorsolateral prefrontal cortex glutamate/glutamine levels following repetitive Transcranial magnetic stimulation in young adults with autism. *Front Neurosci*. (2021) 15:711542. doi: 10.3389/fnins.2021.711542

57. Ameis SH, Blumberger DM, Croarkin PE, Mabbott DJ, Lai M-C, Desarkar P, et al. Treatment of executive function deficits in autism spectrum disorder with repetitive transcranial magnetic stimulation: a double-blind, sham-controlled, pilot trial. *Brain Stimul*. (2020) 13:539–47. doi: 10.1016/j.brs.2020.01.007

58. Ni H-C, Chen Y-L, Chao Y-P, Wu C-T, Wu Y-Y, Liang SH-Y, et al. Intermittent theta burst stimulation over the posterior superior temporal sulcus for children with autism spectrum disorder: a 4-week randomized blinded controlled trial followed by another 4-week open-label intervention. *Autism*. (2021) 25:1279–94. doi: 10.1177/1362361321990534

59. Kang J, Zhang Z, Wan L, Casanova MF, Sokhadze EM, Li X. Effects of 1Hz repetitive transcranial magnetic stimulation on autism with intellectual disability: a pilot study. *Comput Biol Med*. (2022) 141:105167. doi: 10.1016/j.compbiomed.2021.105167

60. Cole EJ, Enticott PG, Oberman LM, Gwynette MF, Casanova MF, Jackson SLJ, et al. The potential of repetitive Transcranial magnetic stimulation for autism Spectrum disorder: a consensus statement. *Biol Psychiatry*. (2019) 85:e21–2. doi: 10.1016/j.biopsych.2018.06.003

61. García-González S, Lugo-Marín J, Setien-Ramos I, Gisbert-Gustemps L, Arteaga-Henríquez G, Díez-Villoria E, et al. Transcranial direct current stimulation in autism Spectrum disorder: a systematic review and meta-analysis. *Eur Neuropsychopharmacol*. (2021) 48:89–109. doi: 10.1016/j.euroneuro.2021.02.017

62. Barahona-Corrêa JB, Velosa A, Chainho A, Lopes R, Oliveira-Maia AJ. Repetitive Transcranial magnetic stimulation for treatment of autism Spectrum disorder: a systematic review and Meta-analysis. *Front Integr Neurosci*. (2018) 12:27. doi: 10.3389/fnint.2018.00027

63. Huang Y, Zhang B, Cao J, Yu S, Wilson G, Park J, et al. Potential locations for noninvasive brain stimulation in treating autism Spectrum disorders-a functional connectivity study. *Front Psych*. (2020) 11:388. doi: 10.3389/fpsyt.2020.00388

64. Javadi AH, Walsh V. Transcranial direct current stimulation (tDCS) of the left dorsolateral prefrontal cortex modulates declarative memory. *Brain Stimul*. (2012) 5:231–41. doi: 10.1016/j.brs.2011.06.007

65. Nelson JT, McKinley RA, Golob EJ, Warm JS, Parasuraman R. Enhancing vigilance in operators with prefrontal cortex transcranial direct current stimulation (tDCS). *NeuroImage*. (2014) 85:909–17. doi: 10.1016/j.neuroimage.2012.11.061

66. Bagherzadeh Y, Khorrami A, Zarrindast MR, Shariat SV, Pantazis D. Repetitive transcranial magnetic stimulation of the dorsolateral prefrontal cortex enhances working memory. *Exp Brain Res*. (2016) 234:1807–18. doi: 10.1007/s00221-016-4580-1

67. Ouerchefani R, Ouerchefani N, Allain P, Ben Rejeb MR, Le Gall D. Contribution of different regions of the prefrontal cortex and lesion laterality to deficit of decision-making on the Iowa gambling task. *Brain Cogn*. (2017) 111:73–85. doi: 10.1016/j.bandc.2016.06.010

68. Mundy P. Annotation: the neural basis of social impairments in autism: the role of the dorsal medial-frontal cortex and anterior cingulate system. *J Child Psychol Psychiatry*. (2003) 44:793–809. doi: 10.1111/1469-7610.00165

69. Thakkar KN, Polli FE, Joseph RM, Tuch DS, Hadjikhani N, Barton JJS, et al. Response monitoring, repetitive behaviour and anterior cingulate abnormalities in autism spectrum disorders (ASD). *Brain*. (2008) 131:2464–78. doi: 10.1093/brain/awn099

70. Vlamings PHJM, Jonkman LM, Hoeksma MR, van Engeland H, Kemner C. Reduced error monitoring in children with autism spectrum disorder: an ERP study. *Eur J Neurosci*. (2008) 28:399–406. doi: 10.1111/j.1460-9568.2008.06336.x

71. Ziemann U. TMS induced plasticity in human cortex. *Rev Neurosci*. (2004) 15:253–66. doi: 10.1515/REVNEURO.2004.15.4.253

72. Rushworth MFS, Johansen-Berg H, Göbel SM, Devlin JT. The left parietal and premotor cortices: motor attention and selection. *NeuroImage*. (2003) 20:S89–S100. doi: 10.1016/j.neuroimage.2003.09.011

73. Frey SH. Tool use, communicative gesture and cerebral asymmetries in the modern human brain. *Philos Trans R Soc Lond Ser B Biol Sci*. (2008) 363:1951–7. doi: 10.1098/rstb.2008.0008

74. Pezze M, McGarrity S, Mason R, Fone KC, Bast T. Too little and too much: hypoactivation and disinhibition of medial prefrontal cortex cause attentional deficits. *J Neurosci*. (2014) 34:7931–46. doi: 10.1523/JNEUROSCI.3450-13.2014

75. Wagner DD, Kelley WM, Haxby JV, Heatherton TF. The dorsal medial prefrontal cortex responds preferentially to social interactions during natural viewing. *J Neurosci*. (2016) 36:6917–25. doi: 10.1523/JNEUROSCI.4220-15.2016

frontiers | Frontiers in Psychiatry

TYPE Original Research
PUBLISHED 18 December 2023
DOI 10.3389/fpsyt.2023.1303840

OPEN ACCESS

EDITED BY
Martina Micai,
National Institute of Health (ISS), Italy

REVIEWED BY
Ann M. Neumeyer,
Massachusetts General Hospital,
United States
Xuping Gao,
Peking University Sixth Hospital, China

*CORRESPONDENCE
E. B. Warreman
✉ E.B.Warreman@lumc.nl

RECEIVED 28 September 2023
ACCEPTED 30 November 2023
PUBLISHED 18 December 2023

CITATION
Warreman EB, Nooteboom LA, Leenen PJM, Geurts HM, Terry MB, Bos JHJ, Hak E, Hoek HW, van Rossum EFC, Vermeiren RRJM and Ester WA (2023) Metabolic syndrome in adults with autistic traits: associated psychological, behavioral, and biological factors in females and males – a PharmLines initiative.
Front. Psychiatry 14:1303840.
doi: 10.3389/fpsyt.2023.1303840

Metabolic syndrome in adults with autistic traits: associated psychological, behavioral, and biological factors in females and males – a PharmLines initiative

E. B. Warreman[1]*, L. A. Nooteboom[1], P. J. M. Leenen[2], H. M. Geurts[3,4], M. B. Terry[5], J. H. J. Bos[6], E. Hak[6], H. W. Hoek[5,7,8], E. F. C. van Rossum[9,10], R. R. J. M. Vermeiren[1,8] and W. A. Ester[1,8,11]

[1]Department of Child and Adolescent Psychiatry, LUMC Curium, Leiden University Medical Center, Oegstgeest, Netherlands, [2]Department of Immunology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands, [3]Dutch Autism and ADHD Research Center, Department of Psychology, University of Amsterdam, Amsterdam, Netherlands, [4]Dr. Leo Kannerhuis, Youz, Parnassia Group, Amsterdam, Netherlands, [5]Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY, United States, [6]Groningen Research Institute of Pharmacy, PharmacoTherapy, Epidemiology and Economics, University of Groningen, Groningen, Netherlands, [7]Department of Psychiatry, University Medical Center Groningen, Groningen, Netherlands, [8]Parnassia Group, Youz, The Hague, Netherlands, [9]Department of Internal Medicine, Division of Endocrinology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands, [10]Obesity Center CGG, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands, [11]Sarr Autism Rotterdam, Youz, Parnassia Group, Rotterdam, Netherlands

Background: While cardiovascular diseases is highly prevalent and an important cause of mortality in autistic adults, knowledge on their increased cardiovascular risk is limited. Hence, this study aimed to investigate psychological, behavioral, and physical factors associated with metabolic syndrome (MetS) in adults with autistic traits.

Methods: In total, 17,705 adults from the Lifelines Cohort were included and categorized using Autism Spectrum Quotient-10 sum-scores. The quartiles with highest (HQ-traits-group females: $n = 2{,}635$; males: $n = 1803$) and lowest levels of autistic traits (LQ-traits-group, $n =$ idem) were analyzed. Using multivariable logistic regression, the associations between MetS and (self-reported and interviewed) psychological, behavioral, and physically measured factors in these stratified groups were investigated.

Results: Among females, MetS was more common in the HQ-traits-group than in the LQ-traits-group (10.0% versus 7.5%, $p<0.01$), while this was not the case among males (HQ-traits-group 13.8% versus LQ-traits-group 13.1%, $p=0.52$). In both the female and male HQ-traits-group, the presence of MetS was associated with poorer self-reported health, less daily physical activity, and altered leukocyte counts.

Conclusion: These findings underline the relevance of adequate cardiovascular prevention in adults with higher levels of autistic traits. Future research could gain more insight into the relationship between cardiovascular risk and autistic traits in females, and into tailored cardiovascular prevention.

KEYWORDS

autism, autistic traits, cardiovascular risk, metabolic syndrome, adults

Introduction

Autism spectrum disorder (ASD) is associated with an approximate two-fold increased mortality risk (1–3). In particular, cardiovascular diseases are amongst the most common causes of death in adults with ASD (1–4). Several studies have reported an elevated risk for cardiovascular diseases in adults with ASD compared to adults without ASD, with odds ratios varying approximately from 1.3 to 2.5 (5–7). Thus, the need to reduce their cardiovascular risk is evident. Furthermore, it is relevant to investigate cardiovascular risk in the general population in order to take those adults with autistic traits, specifically females, with a late or missed ASD-diagnosis into account, by analyzing them on the presence of autistic traits, rather than only on the presence of an ASD-diagnosis (8).

Metabolic syndrome (MetS) is a globally recognized set of major cardiovascular risk factors, namely hypertension, central obesity, increased fasting glucose, and dyslipidaemia (9). The prevalence of hypertension is not higher in autistic adults than in non-autistic adults, based on a recent meta-analysis (10). To our knowledge, the prevalence of central obesity, defined by increased waist circumference, has not been studied in autistic adults or in adults with autistic traits. Regarding the prevalence of diabetes in autistic people, mixed outcomes have been reported (5, 6, 11, 12). Previous studies including autistic adults investigated different or undefined outcome measures of dyslipidaemia, resulting in contradicting results (5, 7, 11, 12). Thus, the total prevalence of MetS, defined as the presence of at least three of five criteria (9), in adults with autistic traits remains unclear.

For future development of preventive cardiovascular interventions, more insight into the psychological, behavioral, and physical factors associated with cardiovascular risk (i.e., MetS) in autistic adults is needed (7, 10). Therefore, the biopsychosocial factors that will be assessed in this study include stress, anxiety, depression, alcohol consumption, smoking, physical activity, and immunological blood markers (13–18).

We hypothesize that an increased cardiovascular risk in adults with autistic traits is associated with the degree of autistic traits and related to biopsychosocial factors. Moreover, autistic males and females have different cardiovascular risk profiles (7). Therefore, the aim of this study is to investigate the prevalence of MetS and which psychological, behavioral, and physical factors are associated with MetS in female and male adults with autistic traits.

Methods

Study population

Our database consisted of data from two database: the Lifelines database and the IADB.nl pharmacy database. We first included adults from the general population in the Dutch Lifelines Cohort Study. "Lifelines is a multi-disciplinary prospective population-based cohort study examining in a unique three-generation design the health and health-related behaviors of 167,729 persons living in the North of the Netherlands. It employs a broad range of investigative procedures in assessing the biomedical, sociodemographic, behavioral, biological and psychological factors which contribute to the health and disease of the general population, with a special focus on multi-morbidity and complex genetics. The Lifelines protocol was approved by the UMCG Medical ethical committee under number 2007/152" (19). We used the second assessment of the Lifelines Study, which took place between 2014 and 2017.

Next, the Lifelines data from the 37,924 participants who submitted an autism questionnaire (AUTQ) in 2019 were combined with the medication data from the University of Groningen IADB.nl pharmacy prescription database. "This is a growing database that contains prescription data for more than 20 years from 1996 to 2016 from approximately 90 community pharmacies and covers an estimated population of 900,000 patients. Registration in the database is irrespective of health care insurance and age, gender and prescription rates among the database population have been found to be representative of the Netherlands as a whole (20), and the database has been widely used for research. Each person is individually tracked throughout the database period and prescription records contain information on the date of dispensing, the quantity dispensed, the dose regimen, the number of days the prescription is valid, the prescribing physician and the Anatomical Therapeutic Chemical code (ATC code). Each patient has a unique anonymous identifier; date of birth and gender are known. Due to the high patient-pharmacy commitment in the Netherlands, the medication records for each patient are virtually complete, except for over the counter (OTC) drugs and medication dispensed during hospitalization" (21).

For the current study (Figure 1), we included 17,705 participants, ≥18 years old at the onset of the second Lifelines assessment, who self-reported whether they had an ASD-diagnosis, and completed the short version of the Autism Spectrum Quotient (AQ-10). The 17,705 included participants were sex-stratified (10,539 females and 7,212 males) and then categorized in quartiles based on their AQ-10 sum-scores, resulting in a female quartile with highest AQ-10 sum-scores (female HQ-traits-group: $n = 2{,}635$), female quartile with lowest AQ-10 sum-scores (LQ-traits-group: $n = 2{,}635$), male quartile with highest AQ-10 sum-scores (male HQ-traits-group: $n = 1803$), and male quartile with lowest AQ-10 sum-scores (LQ-traits-group: $n = 1803$).

Of the 17,705 included participants, 198 reported having an ASD-diagnosis (1.1%). In the ASD-group ($n = 198$), 21 participants (10.6%) met the criteria for having MetS. However, G*Power analysis showed that for logistic regression using MetS as outcome and with a power of at least 0.8, in the ASD-group at least 43 participants needed to meet the criteria for MetS. Thus, the power in the diagnosed ASD-group was insufficient for performing regression.

Autistic community involvement

During several brainstorm sessions, our research team was advised about relevant research questions and variables by a project-group of the Dutch '*Academic Workplace Autism*', which consisted of both adults with ASD and clinicians with experience treating people with ASD.

Measures

Autistic traits

The AQ-10 is a valid instrument to roughly quantify the level of autistic traits in adults with average intelligence (22). It is not designed to determine the presence of an ASD-diagnosis, but it can indeed be used to investigate the degree of autistic traits in population samples (23–26). The AQ-10 consists of ten questions about the following five domains of autistic traits: attention to detail, attention

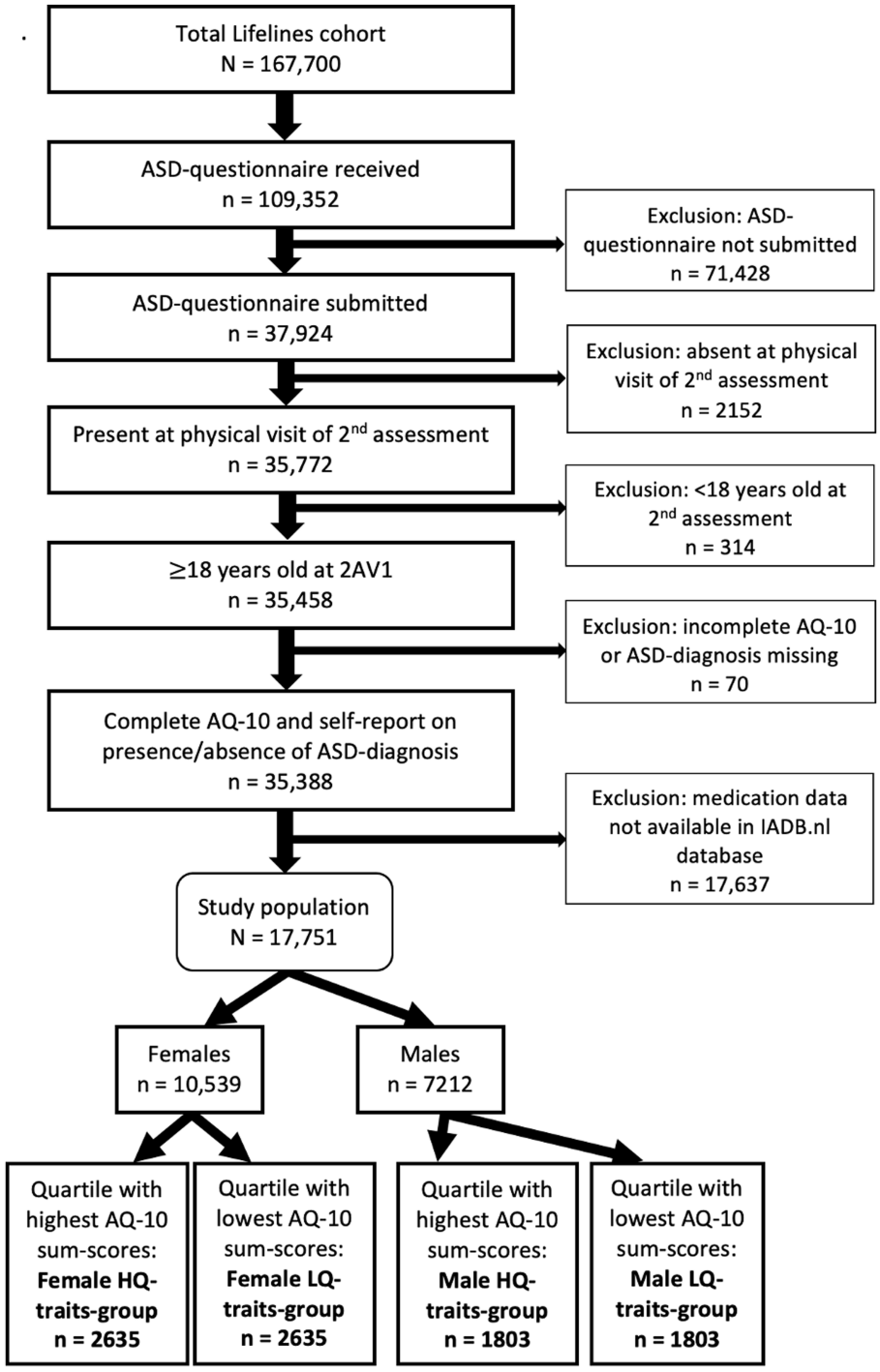


FIGURE 1
Flow diagram of study population.

switching, communication, imagination, and social skills (22). The questions are scored with a four-point Likert-scale. The minimum AQ-10 score is zero and the maximum score is 10; a higher score represents the presence of more autistic traits.

Metabolic syndrome

The definition of MetS was the presence of at least three of five criteria (9): an increased waist circumference (in males: 102 cm, in females: 88 cm; measured by trained Lifelines' staff), increased fasting glucose (serum level 5.6 mmol/L and/or use of blood glucose-lowering drugs), decreased HDL-cholesterol (in males:1.0 mmol/L, in females:1.3 mmol/L, and/or use of lipid-modifying drugs), increased triglycerides (1.7 mmol/L and/or use of lipid-modifying drugs), and/or hypertension (systolic blood pressure 130 mmHg, and/or diastolic blood pressure 85 mmHg, and/or use of antihypertensive drugs). The ATC-codes used to assess the use of blood glucose-lowering drugs,

lipid-modifying drugs, and antihypertensive drugs can be found in Supplementary Table S1. The use of these drugs was based on prescription in the IADB.nl database within a period of 180 days before the physical visit of the second Lifelines assessment.

Psychological factors

The presence of depression and anxiety were determined with a face-to-face Mini International Neuropsychiatric Interview [MINI; based on the DSM-IV-TR (27)]. Depression was defined as any current depressive disorder: major depressive disorder or dysthymia. The definition of anxiety included any current anxiety disorder: panic disorder, agoraphobia, social phobia, or generalized anxiety disorder. Long-term Difficulties Inventory (LDI) sum-scores were used to assess self-reported stress. Self-reported health was quantified with the following 5-point Likert scale RAND-question: 'How would you rate your health generally speaking?'

Behavioral factors

Physical activity was determined with the following question from the Short Questionnaire to Assess Health-enhancing physical activity: "Adding everything up, on how many days per week on average are you involved in cycling, doing odd jobs, gardening, sport, or other strenuous activities for at least 30 min?" The prevalence of an average alcohol intake of at least three glasses per day [heavy drinking (28, 29)] was measured with a question from the Flower Food Frequency questionnaire (FFQ): "During the past month, how many glasses of alcoholic drinks did you drink per day on average?" Smoking was assessed with self-report regarding smoking in the past month.

Biological factors

Leukocyte- and subtype-counts were analyzed because they are measures of (low-grade) inflammation and a biological stress response. Chronic low-grade inflammation is an essential pathogenic factor for MetS (30, 31). Blood samples were drawn by trained Lifelines' staff during a physical visit.

Covariates

Self-reported employment status and educational attainment were combined to determine socioeconomic status. Employment was defined as doing paid work for one or more hours per week. Low educational attainment included no education, primary, lower or preparatory vocational education, or lower general secondary education. Middle educational attainment was defined as: intermediate vocational education or apprenticeship, higher general secondary education, or pre-university secondary education. High educational attainment entailed higher vocational education or university. As several types psychotropic drugs can have weight gain as side effect, potentially weight-increasing antidepressants, antipsychotics, and anticonvulsants were assessed. None of the included participants used anticonvulsants. A list of the Anatomical Therapeutic Chemical (ATC) codes to identify the use of antidepressants and antipsychotics can be found in Supplementary Table S1.

Statistical analysis

We used IBM SPSS Statistics version 25 for all data analyses. Basic characteristics, including the prevalence of MetS, were compared with univariable analyses in the following groups: female HQ-traits-group versus female LQ-traits-group and male HQ-traits-group versus male LQ-traits-group (Table 1). These univariable analyses involved Chi-square tests for categorical variables and Student's *t*-tests or Mann–Whitney U tests for continuous variables. Next, multivariable analyses were performed in the female and male HQ- and LQ-traits-groups: psychological, behavioral, and biological factors were compared between these sex-stratified groups using multivariable regression, with correction for age and socioeconomic status (Table 2). Lastly, multivariable logistic regression with the presence of MetS as outcome measure was conducted (Table 3). These logistic regression models were executed for each of the included psychological, behavioral, and biological variables in the sex-stratified HQ-traits- and LQ-traits-groups. Age and socioeconomic status (employment and education) were included as covariates. Because of some missing data in the employment and educational attainment (see Supplementary Table S2), we performed step-by-step with three models (model 1 adjusted for age; model 2 adjusted for age and employment; model 3 adjusted for age, employment, and educational attainment). Model 3 was the most suitable as the point estimates remained similar. From the investigated potentially weight gain-inducing psychotropic drugs, only antidepressants were frequently used in our study population. Therefore, the latter logistic regression models were also performed with correction for the use of antidepressants. However, this did not result in outcomes leading to different conclusions, since the same significant outcomes were found. Transformation of skewed data was not indicated, because the assumptions of logistic regression were met based on the nature of the distributions and the large sample sizes.

Results

Basic characteristics

The basic characteristics of the females and males in the HQ- and LQ-traits-groups are shown in Table 1. The mean ages were not different within the female and male groups. In both the female and male HQ-traits-groups, the socioeconomic status was lower than in the female and male LQ-traits-groups.

Metabolic syndrome

MetS was more common in the female HQ-traits-group than in the female LQ-traits-group (10.0% vs. 7.5%, $p < 0.01$, see Table 1). In contrast, among males, the prevalence of MetS in the HQ-traits-group was not different from the LQ-traits-group (13.8% vs. 13.1%, $p = 0.52$). The prevalence of MetS was higher in the male HQ-traits-group than in the female HQ-traits-group (13.8% vs. 10.0%, $p < 0.01$).

Psychological, behavioral and biological factors associated with MetS

The psychological, behavioral, and biological factors in the female and male HQ- and LQ-traits-groups can be found in Table 2.

TABLE 1 Basic characteristic of HQ-traits-group, LQ-traits-group, and sex-stratified subgroups.

	Females			Males		
	HQ-traits-group *n* = 2,635	LQ-traits-group *n* = 2,635	Value of *p*[a]	HQ-traits-group *n* = 1803	LQ-traits-group *n* = 1803	Value of *p*[a]
Age (mean, SD)	49.1 (12.8)	48.6 (11.7)	N.S.	51.7 (12.9)	51.9 (11.7)	N.S.
AQ-10[b] sum score (median, IQR)	4 (4–5)	0 (0–1)	<0.01	5 (5–6)	1 (0–1)	<0.01
Ethnicity (*N*, %)						
Eastern or Western European	2,421 (91.9)	2,484 (94.3)	N.S.	1,656 (91.8)	1,677 (93.0)	N.S.
Mediterranean or Arabic	<10 (<0.4)	<10 (<0.4)		<10 (<0.6)	<10 (<0.6)	
Black	<10 (<0.4)	<10 (<0.4)		<10 (<0.6)	<10 (<0.6)	
Asian	<10 (<0.4)	<10 (<0.4)		<10 (<0.6)	<10 (<0.6)	
Other	28 (1.1)	15 (0.6)		11 (0.6)	<10 (<0.6)	
Educational attainment (*N*, %)						
Low	581 (22.0)	302 (11.5)	<0.01	389 (21.6)	186 (10.3)	<0.01
Middle	863 (32.8)	713 (27.1)	<0.01	517 (28.7)	412 (22.9)	<0.01
High	733 (27.8)	1,171 (44.4)	<0.01	546 (30.3)	860 (47.7)	<0.01
Employment (*N*, %)	1,665 (63.2)	2006 (76.1)	<0.01	1,201 (66.7)	1,348 (74.8)	<0.01
Use of antipsychotics[c]	<10 (<0.4)	<10 (<0.4)	-	<10 (<0.6)	<10 (<0.6)	-
Use of antidepressants[d]	63 (2.4)	27 (1.0)	<0.01	21 (1.2)	<10 (<0.6)	-
Metabolic syndrome[e] (*N*, %)	264 (10.0)	197 (7.5)	<0.01	248 (13.8)	236 (13.1)	N.S.
WC ≥ threshold (*N*, %)	1,135 (43.1)	1,005 (38.1)	<0.01	463 (25.7)	417 (23.1)	N.S.
Hypertension (*N*, %)	960 (36.4)	839 (31.8)	<0.01	954 (52.9)	972 (53.9)	N.S.
Triglycerides ≥ threshold (*N*, %)	273 (10.4)	209 (7.9)	<0.01	425 (23.6)	421 (23.3)	N.S.
HDL-cholesterol < threshold (*N*, %)	386 (14.6)	309 (11.7)	<0.01	196 (10.9)	163 (9.0)	N.S.
Use of lipid-modifying drugs (*N*, %)	35 (1.3)	26 (1.0)	N.S.	41 (2.3)	54 (3.0)	N.S.
Increased fasting glucose (*N*, %)	<10 (<0.4)	<10 (<0.4)	N.S.	10 (0.6)	13 (0.7)	N.S.

[a]Unadjusted value of *ps*: Chi-square tests for categorical variables and Student's *t*-tests or Mann–Whitney U tests for continuous variables. [b]AQ-10 = short version of the Autism Spectrum Quotient. [c]Only antipsychotics which are likely to have weight gain as side effect were included (corresponding ATC-codes: see Supplementary Table S1). [d]Only antidepressants which are likely to have weight gain as side effect were (corresponding ATC-codes: see Supplementary Table S1). [e]Metabolic syndrome was defined as the presence of three or more of the following criteria: (1) waist circumference (WC) above threshold: ≥88 cm in females and ≥102 cm in males, (2) hypertension: systolic blood pressure ≥130 mmHg, diastolic blood pressure ≥85 mmHg, and/or use of antihypertensive drugs, (3) triglycerides ≥1.7 mmol/L and/or use of lipid-modifying drugs, (4) HDL-cholesterol <1.3 mmol/L in females and < 1.0 in males, and/or use of lipid-modifying. Drugs, (5) fasting serum glucose ≥5.6 mmol/L and/or use of blood glucose-lowering drugs.

Table 3 shows the associations between these psychological, behavioral, and biological factors and the presence of MetS. In the female HQ-traits-group, the presence of MetS was associated with higher stress levels, poorer self-reported health, and the presence of a depressive disorder (OR 1.07, 95% CI 1.01–1.13; OR 0.53, 95% CI 0.43–0.66; OR 1.65, 95% CI 1.03–2.63; see Table 3). To explain, for example, a one-point higher score on the LDI stress questionnaire increases the odds of having MetS 1.07 times. Regarding behavioral factors, the presence of MetS was associated with less physical activity and smoking in the female HQ-traits-group (OR 0.88, 95% CI 0.91–0.95; OR 1.53, 95% CI 1.01–2.30). In other words, one more day of at least 30 min of physical activity per week decreases the odds of having MetS 0.88 times. In addition, higher total leukocyte-, neutrophil-, lymphocyte-, and monocyte-counts were associated with MetS in the female HQ-traits-group. However, in the female HQ-traits-group, the presence of anxiety disorders, alcohol use of more than two glasses per day, eosinophil-counts, and the neutrophil-to-lymphocyte ratio were not associated with the presence of MetS.

In the male HQ-traits-group (see Table 3), the presence of MetS was associated with poorer self-reported health, less physical activity, and higher total leukocyte-, neutrophil-, lymphocyte-, and monocyte-counts (OR 0.59, 95% CI 0.48–0.72; OR 0.84, 95% CI 0.78–0.92; OR 1.31, 95% CI 1.21–1.43; OR 1.39, 95% CI 1.24–1.57; OR 2.00, 95% CI 1.54–2.59; OR 13.83, 95% CI 5.39–35.49). In this male HQ-group, MetS was not associated with stress levels, the presence of anxiety or depressive disorders, alcohol use, smoking, eosinophil-counts, and the neutrophil-to-lymphocyte ratio.

Discussion

Our study showed that in the general population, MetS is more common in females with higher levels of autistic traits than in females with lower levels of autistic traits. When comparing males with higher and lower levels of autistic traits, their prevalence of MetS was not

TABLE 2 Psychological, behavioral and biological factors: HQ-traits-group versus LQ-traits-group.

	Females				Males			
	HQ-traits-group, *n* = 2,635	LQ-traits-group, *n* = 2,635	Value of *p*[a]	Adjusted OR (95% CI)[b]	HQ-traits-group, *n* = 1803	LQ-traits-group, *n* = 1803	Value of *p*[a]	Adjusted OR (95% CI)[b]
Psychological								
Stress (median, IQR)	2 (1–4)	2 (1–3)	<0.01	1.17 (1.14–1.21)	2 (0–3)	1 (0–3)	<0.01	1.17 (1.13–1.22)
Self-reported health (median, IQR)	3.0 (3.0–4.0)	3.0 (3.0–4.0)	<0.01	0.64 (0.59–0.70)	3.0 (3.0–4.0)	4.0 (3.0–4.0)	<0.01	0.65 (0.59–0.71)
Anxiety disorder (*N*, %)	331 (12.6)	127 (4.8)	<0.01	2.80 (2.23–3.52)	146 (8.1)	41 (2.3)	<0.01	3.48 (2.39–5.05)
Depressive disorder (*N*, %)	190 (7.2)	55 (2.1)	<0.01	3.39 (2.42–4.74)	80 (4.4)	27 (1.5)	<0.01	2.85 (1.77–4.59)
Behavioral								
Alcohol use, >2 glasses/day (*N*, %)	259 (9.8)	275 (10.4)	N.S.	0.98 (0.80–1.21)	412 (22.9)	502 (27.8)	0.02	0.70 (0.58–0.84)
Physical activity, days/week (median, IQR)	4.5 (3.0–6.0)	5.0 (3.0–6.0)	<0.01	0.94 (0.91–0.98)	4.0 (2.5–6.0)	4.5 (3.0–6.0)	<0.01	0.95 (0.91–0.99)
Smoking (*N*, %)	308 (11.7)	255 (9.7)	0.02	1.14 (0.93–1.39)	212 (11.8)	250 (13.9)	N.S.	0.73 (0.58–0.91)
Biological								
Total leukocytes (10^E9/L) (median, IQR)	5.80 (4.90–6.90)	5.70 (4.90–6.80)	<0.01	1.04 (1.00–1.08)	5.80 (4.90–6.83)	5.80 (5.00–6.90)	N.S.	0.99 (0.95–1.03)
Neutrophils (10^E9/L) (median, IQR)	3.11 (2.48–3.92)	3.03 (2.43–3.78)	0.01	1.05 (0.99–1.10)	3.03 (2.49–3.76)	3.05 (2.50–3.77)	N.S.	1.00 (0.93–1.06)
Lymphocytes (10^E9/L) (median, IQR)	1.92 (1.58–2.33)	1.91 (1.55–2.32)	N.S.	1.00 (0.91–1.11)	1.89 (1.55–2.27)	1.90 (1.54–2.28)	N.S.	0.89 (0.78–1.02)
Monocytes (10^E9/L) (median, IQR)	0.46 (0.38–0.55)	0.45 (0.37–0.54)	<0.01	1.94 (1.24–3.04)	0.52 (0.43–0.62)	0.51 (0.42–0.62)	N.S.	0.93 (0.57–1.51)
Eosinophils (10^E9/L) (median, IQR)	0.15 (0.10–0.23)	0.15 (0.10–0.23)	N.S.	1.01 (0.62–1.65)	0.17 (0.11–0.26)	0.18 (0.12–0.27)	N.S.	1.13 (0.65–1.96)
Neutrophil-to-lymphocyte ratio (median, IQR)	1.62 (1.26–2.12)	1.60 (1.21–2.05)	N.S.	1.06 (0.98–1.14)	1.64 (1.25–2.14)	1.63 (1.27–2.08)	N.S.	1.04 (0.95–1.14)

[a]Unadjusted *p*-values: Chi-square tests for categorical variables and Student's *t*-tests or Mann–Whitney U tests for continuous variables. [b]Adjusted for age and socioeconomic status (employment and educational attainment).

10.3389/fpsyt.2023.1303840

10.3389/fpsyt.2023.1303840

TABLE 3 Multivariable logistic regression with the presence of metabolic syndrome as outcome.

	Females		Males	
	HQ-traits-group, *n* = 2,635	LQ-traits-group, *n* = 2,635	HQ-traits-group, *n* = 1803	LQ-traits-group, *n* = 1803
	Metabolic syndrome (OR, 95% CI)[a]	Metabolic syndrome (OR, 95% CI)[a]	Metabolic syndrome (OR, 95% CI)[a]	Metabolic syndrome (OR, 95% CI)[a]
Psychological				
Stress	1.07 (1.01–1.13)	1.05 (0.97–1.13)	1.01 (0.94–1.08)	1.12 (1.03–1.22)
Self-reported health	0.53 (0.43–0.66)	0.54 (0.44–0.68)	0.59 (0.48–0.72)	0.47 (0.38–0.58)
Anxiety disorder	1.13 (0.74–1.72)	1.68 (0.91–3.10)	1.44 (0.88–2.38)	1.42 (0.58–3.50)
Depressive disorder	1.65 (1.03–2.63)	1.93 (0.79–4.69)	1.58 (0.85–2.91)	1.06 (0.31–3.65)
Behavioral				
Alcohol use of >2 glasses/day	1.00 (0.57–1.78)	1.87 (1.12–3.12)	1.28 (0.85–1.95)	1.84 (1.25–2.70)
Physical activity (days/week)	0.88 (0.91–0.95)	0.90 (0.83–0.98)	0.84 (0.78–0.92)	0.85 (0.78–0.92)
Smoking	1.53 (1.01–2.30)	1.51 (0.93–2.45)	1.05 (0.66–1.66)	1.69 (1.12–2.53)
Biological				
Total leukocytes (10^E9/L)	1.41 (1.30–1.52)	1.42 (1.29–1.55)	1.31 (1.21–1.43)	1.20 (1.09–1.31)
Neutrophils (10^E9/L)	1.49 (1.34–1.65)	1.56 (1.38–1.77)	1.39 (1.24–1.57)	1.45 (1.28–1.65)
Lymphocytes (10^E9/L)	2.32 (1.87–2.87)	1.64 (1.29–2.09)	2.00 (1.54–2.59)	1.47 (1.15–1.87)
Monocytes (10^E9/L)	6.76 (2.81–16.28)	7.11 (2.35–21.50)	13.83 (5.39–35.49)	9.50 (3.71–24.35)
Eosinophils (10^E9/L)	2.40 (0.84–6.87)	2.94 (0.98–8.89)	1.84 (0.69–4.86)	2.28 (0.71–7.33)
Neutrophil-to-lymphocyte ratio	1.07 (0.90–1.26)	1.25 (1.04–1.49)	1.12 (0.94–1.34)	1.17 (0.99–1.39)

[a]Adjusted for age and socioeconomic status (employment and educational attainment).

different. These findings are concordant with a previous sex-stratified study including adults with an ASD-diagnosis (7).

With respect to the investigated psychological factors, in both females and males with higher levels of autistic traits, the presence of MetS was strongly associated with poorer self-reported health. Also, stress levels and the presence of anxiety disorders were moderately associated with MetS in females with higher levels of autistic traits. To our knowledge, these findings cannot directly be compared to other studies, since the relation between these psychological variables and MetS in adults with autistic traits has not been examined previously. It does seem that autistic traits, self-reported health, stress and anxiety disorders are interrelated, based on previous research (32–34).

Regarding the assessed behavioral factors, the presence MetS was strongly associated with less physical activity in both females and males with higher levels of autistic traits. Moreover, females and males with higher levels of autistic traits were less physically active than females and males with lower levels of autistic traits. In previous studies, adults either with an ASD-diagnosis or autistic traits also reported less physical activity (35, 36). Smoking was moderately associated with MetS in the females with higher levels of autistic traits from our study. However, in our study, females with higher levels of autistic traits did not smoke more than females with lower levels of autistic traits, which is in line with previous research in autistic adults (37). Together, especially enhancement of physical activity should be taken into account in the prevention of cardiovascular risk for adults with autistic traits.

From the investigated biological factors, MetS was strongly associated with leukocyte and several -subtype counts in both males and females with higher levels of autistic traits. This association could be explained by increased chronic stress levels in adults with higher levels of autistic traits, as psychological stress can alter these immunological variables through the hypothalamic–pituitary–adrenal axis (18). Altered immune responses due to chronic stress are interrelated with metabolic activity and increased risk for cardiovascular diseases (31, 38, 39). However, MetS itself is also related to low-grade systemic inflammation, since the total leukocyte and -subtype counts were also associated with MetS in males and females with lower levels of autistic traits.

Strengths and limitations

The large sample size is the main strength of this study, reporting on a wide range of biopsychosocial variables in adults from a general population cohort. Furthermore, our analyses based on the participants' level of autistic traits is a first step to better understand the increased risk for cardiovascular diseases in autistic adults and to identify cardiovascular risk profiles associated with higher level of autistic traits. Another strength of this study is the use of physically measured variables (e.g., blood pressure, fasting glucose, waist circumference, cholesterol levels) and linked medication data from the IADB.nl database to define the presence of MetS in participants.

Temporality was not examined in our study, because of the cross-sectional design. Also, the AQ-10 scores were assessed on a later moment in time (on average 4 years later) than the measures of MetS and psychological, behavioral, and biological factors. However, it has

previously been investigated that the AQ-10 test–retest reliability was adequate with a time interval of 6 to 12 months (40). It could be debated whether differences in AQ-10 scores between males and females had an effect on the found associations. However, the statistical AQ-10 variance was smaller in males than in females from the HQ-traits-groups. Also, the adult AQ-10 was validated for both men and women (22). Moreover, categorization of our study population in reversed order (first into HQ-/LQ-traits-groups and then sex-categorization) did not lead to other main study results. Next, it should be noted that in the Lifelines Cohort, only people with the ability to fill in self-report questionnaires were eligible for inclusion. Thus, our study results cannot be generalized to adults with (cognitive) disabilities impacting self-report. Lastly, since 25 (12.6%) of the participants with ASD from the 198 participants with ASD in the total study population were not included in the final analysis of female and male HQ- and LQ-traits-groups, our study was not able to cover all people diagnosed with ASD in our Lifelines Cohort sample.

Implications

Healthcare providers, such as general practitioners and psychiatrists, should be alert to assess cardiovascular risk factors when providing care for females with autistic traits, because of their increased prevalence of MetS. This implies that a wider range of females with higher levels of autistic traits, other than only those with an ASD-diagnosis based on previous research (7), should be included in timely cardiovascular preventive interventions. Next, adults with autistic traits and their healthcare providers should be educated about the factors associated with MetS in this population. Future studies could gain more insight into the pathway through which autistic traits, biopsychosocial factors, and cardiovascular risk factors interact, especially in females.

Conclusion

In females with higher levels of autistic traits, the prevalence of MetS is higher than in females with lower levels of autistic traits. In both males and females with higher levels of autistic traits, the presence of MetS is strongly associated with poorer self-reported health, less physical activity, and altered leukocyte and -subtype counts. Earlier and adequate cardiovascular preventive measures are indicated for adults with relatively more autistic traits. To decrease morbidity and mortality of adults with high levels of autistic traits, future research should focus on implementation of cardiovascular prevention for adults with autistic traits.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: All data collected for the study, including individual (pseudonymized) participant data and a data dictionary defining each field in the set, are available via the Lifelines Research Office and Statistics Netherlands (CBS). Access to this dataset and other available data from the Lifelines cohort and CBS can be requested by scientists. Access will be granted after evaluation of an application form describing the research proposal (including a data selection) and a signed Data and Material Transfer Agreement. Data will be released in a secure environment. Requests to access these datasets should be directed to Director General of Statistics Netherlands (CBS), AanvraagMicrodata@cbs.nl; https://www.lifelines.nl/researcher/how-to-apply.

Ethics statement

The studies involving humans were approved by University Medical Center Groningen, Medical Ethical Committee. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

EW: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. LN: Conceptualization, Supervision, Writing – review & editing. PL: Conceptualization, Writing – review & editing. HG: Conceptualization, Writing – review & editing. MT: Conceptualization, Methodology, Supervision, Writing – review & editing. JB: Data curation, Writing – review & editing. EH: Writing – review & editing. HH: Funding acquisition, Writing – review & editing. ER: Conceptualization, Writing – review & editing. RV: Conceptualization, Supervision, Writing – review & editing. WE: Conceptualization, Methodology, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. "This work, the Lifelines initiative, was supported by subsidy from the Dutch Ministry of Health, Welfare and Sport, the Dutch Ministry of Economic Affairs, the University Medical Centre Groningen and the Provinces in the North of the Netherlands (Drenthe, Friesland, Groningen)" (19). Our study was supported by a grant from the Netherlands Organisation for Health Research and Development (NWO-ZonMw) (grant number 639003101). "The IADB.nl and the PharmLines Initiative are funded by the University of Groningen, Groningen Research Institute of Pharmacy" (20).

Acknowledgments

We wish to acknowledge the services of the Lifelines Cohort Study, all study participants, the contributing research centres delivering data to Lifelines, and the participating IADB.nl pharmacies for providing their data for research. We also thank the members of Dutch 'Academic Workplace for Autism' for contributing to this study by sharing useful insights regarding the importance of selected study outcomes for the autistic population.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2023.1303840/full#supplementary-material

References

1. Hirvikoski T, Mittendorfer-Rutz E, Boman M, Larsson H, Lichtenstein P, Bölte S. Premature mortality in autism spectrum disorder. *Br J Psychiatry*. (2016) 208:232–8. doi: 10.1192/bjp.bp.114.160192

2. Hwang YIJ, Srasuebkul P, Foley KR, Arnold S, Trollor JN. Mortality and cause of death of Australians on the autism spectrum. *Autism Res*. (2019) 12:806–15. doi: 10.1002/aur.2086

3. Schendel DE, Overgaard M, Christensen J, Hjort L, Jørgensen M, Vestergaard M, et al. Association of Psychiatric and Neurologic Comorbidity with Mortality among Persons with Autism Spectrum Disorder in a Danish population. *JAMA Pediatr*. (2016) 170:243–50. doi: 10.1001/jamapediatrics.2015.3935

4. Shavelle RM, Strauss DJ, Pickett J. Causes of death in autism. *J Autism Dev Disord*. (2001) 31:569–76. doi: 10.1023/a:1013247011483

5. Croen LA, Zerbo O, Qian Y, Massolo ML, Rich S, Sidney S, et al. The health status of adults on the autism spectrum. *Autism*. (2015) 19:814–23. doi: 10.1177/1362361315577517

6. Hand BN, Angell AM, Harris L, Carpenter LA. Prevalence of physical and mental health conditions in Medicare-enrolled, autistic older adults. *Autism*. (2020) 24:755–64. doi: 10.1177/1362361319890793

7. Weir E, Allison C, Warrier V, Baron-Cohen S. Increased prevalence of non-communicable physical health conditions among autistic adults. *Autism*. (2021) 25:681–94. doi: 10.1177/1362361320953652

8. Lai MC, Baron-Cohen S. Identifying the lost generation of adults with autism spectrum conditions. *Lancet Psychiatry*. (2015) 2:1013–27. doi: 10.1016/S2215-0366(15)00277-1

9. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the international diabetes federation task force on epidemiology and prevention; National Heart, Lung, and Blood Institute; American Heart Association; world heart federation; international atherosclerosis society; and International Association for the Study of obesity. *Circulation*. (2009) 120:1640–5. doi: 10.1161/CIRCULATIONAHA.109.192644

10. Dhanasekara CS, Ancona D, Cortes L, Hu A, Rimu AH, Robohm-Leavitt C, et al. Association between autism Spectrum disorders and cardiometabolic diseases: a systematic review and meta-analysis. *JAMA Pediatr*. (2023) 177:248–57. doi: 10.1001/jamapediatrics.2022.5629

11. Fortuna RJ, Robinson L, Smith TH, Meccarello J, Bullen B, Nobis K, et al. Health conditions and functional status in adults with autism: a cross-sectional evaluation. *J Gen Intern Med*. (2016) 31:77–84. doi: 10.1007/s11606-015-3509-x

12. Vohra R, Madhavan S, Sambamoorthi U. Comorbidity prevalence, healthcare utilization, and expenditures of Medicaid enrolled adults with autism spectrum disorders. *Autism*. (2017) 21:995–1009. doi: 10.1177/1362361316665222

13. Denollet J, Maas K, Knottnerus A, Keyzer JJ, Pop VJ. Anxiety predicted premature all-cause and cardiovascular death in a 10-year follow-up of middle-aged women. *J Clin Epidemiol*. (2009) 62:452–6. doi: 10.1016/j.jclinepi.2008.08.006

14. Harshfield EL, Pennells L, Schwartz JE, Willeit P, Kaptoge S, Bell S, et al. Association between depressive symptoms and incident cardiovascular diseases. *JAMA*. (2020) 324:2396–405. doi: 10.1001/jama.2020.23068

15. Rosengren A, Hawken S, Ôunpuu S, Sliwa K, Zubaid M, Almahmeed WA, et al. Association of psychosocial risk factors with risk of acute myocardial infarction in 11119 cases and 13648 controls from 52 countries (the INTERHEART study): case-control study. *Lancet*. (2004) 364:953–62. doi: 10.1016/S0140-6736(04)17019-0

16. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet*. (2004) 364:937–52. doi: 10.1016/S0140-6736(04)17018-9

17. Cole SW. Social regulation of leukocyte homeostasis: the role of glucocorticoid sensitivity. *Brain Behav Immun*. (2008) 22:1049–55. doi: 10.1016/j.bbi.2008.02.006

18. Dhabhar FS, Malarkey WB, Neri E, McEwen BS. Stress-induced redistribution of immune cells--from barracks to boulevards to battlefields: a tale of three hormones--Curt Richter award winner. *Psychoneuroendocrinology*. (2012) 37:1345–68. doi: 10.1016/j.psyneuen.2012.05.008

19. Scholtens S, Smidt N, Swertz MA, Bakker SJL, Dotinga A, Vonk JM, et al. Cohort profile: life lines, a three-generation cohort study and biobank. *Int J Epidemiol*. (2015) 44:1172–80. doi: 10.1093/ije/dyu229

20. Visser ST, Schuiling-Veninga CC, Bos JH, de Jong-van den Berg LT, Postma MJ. The population-based prescription database IADB.Nl: its development, usefulness in outcomes research and challenges. *Expert Rev Pharmacoecon Outcomes Res*. (2013) 13:285–92. doi: 10.1586/erp.13.20

21. Sediq R, van der Schans J, Dotinga A, Alingh R, Wilffert B, Bos JHJ, et al. Concordance assessment of self-reported medication use in the Netherlands three-generation lifelines cohort study with the pharmacy database ia DB.Nl: the pharm lines initiative. *Clin Epidemiol*. (2018) 10:981–9. Published 2018 Aug 16. doi: 10.2147/CLEP.S163037

22. Allison C, Auyeung B, Baron-Cohen S. Toward brief "red flags" for autism screening: the short autism Spectrum quotient and the short quantitative checklist for autism in toddlers in 1,000 cases and 3,000 controls. *J Am Acad Child Adolesc Psychiatry*. (2012) 51:202–212.e7. doi: 10.1016/j.jaac.2011.11.003

23. Ashwood KL, Gillan N, Horder J, Hayward H, Woodhouse E, McEwen FS, et al. Predicting the diagnosis of autism in adults using the autism-Spectrum quotient (AQ) questionnaire. *Psychol Med*. (2016) 46:2595–604. doi: 10.1017/S0033291716001082

24. Lundin A, Kosidou K, Dalman C. Measuring autism traits in the adult general population with the brief autism-Spectrum quotient, AQ-10: findings from the Stockholm public health cohort. *J Autism Dev Disord*. (2019) 49:773–80. doi: 10.1007/s10803-018-3749-9

25. Sizoo BB, Horwitz EH, Teunisse JP, Kan CC, Vissers CTWM, Forceville EJM, et al. Predictive validity of self-report questionnaires in the assessment of autism spectrum disorders in adults. *Autism*. (2015) 19:842–9. doi: 10.1177/1362361315589869

26. Warrier V, Greenberg DM, Weir E, Buckingham C, Smith P, Lai MC, et al. Elevated rates of autism, other neurodevelopmental and psychiatric diagnoses, and autistic traits in transgender and gender-diverse individuals. *Nat Commun*. (2020) 11:3959. Published 2020 Aug 7. doi: 10.1038/s41467-020-17794-1

27. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. 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. *J Clin Psychiatry*. (1998) 59:22–57.

28. Wouters HJCM, van Zeventer IA, van der Klauw MM, Wolffenbuttel BHR, Huls G. Association between peripheral blood cell count abnormalities and health-related quality of life in the general population. *Hema*. (2020) 5:e503. Published 2020 Dec 21. doi: 10.1097/HS9.0000000000000503

29. Rausch C, van Zon SKR, Liang Y, Laflamme L, Möller J, de Rooij SE, et al. Geriatric syndromes and incident chronic health conditions among 9094 older community-dwellers: findings from the lifelines cohort study. *J Am Med Dir Assoc*. (2022) 23:54–59.e2. doi: 10.1016/j.jamda.2021.02.030

30. Del Giudice M, Gangestad SW. Rethinking IL-6 and CRP: why they are more than inflammatory biomarkers, and why it matters. *Brain Behav Immun*. (2018) 70:61–75. doi: 10.1016/j.bbi.2018.02.013

31. Dijkstra-de Neijs L, Leenen PJM, Hays JP, van der Valk ES, Kraaij R, van Rossum EFC, et al. Biological consequences of psychological distress in caregivers of children with autism Spectrum disorder and its potential relevance to other chronic diseases including cancer. *Curr Epidemiol Rep*. (2020) 7:139–48. doi: 10.1007/s40471-020-00237-2

32. Moseley RL, Turner-Cobb JM, Spahr CM, Shields GS, Slavich GM. Lifetime and perceived stress, social support, loneliness, and health in autistic adults. *Health Psychol*. (2021) 40:556–68. doi: 10.1037/hea0001108

33. Amos GA, Byrne G, Chouinard PA, Godber T. Autism traits, sensory over-responsivity, anxiety, and stress: a test of explanatory models. *J Autism Dev Disord*. (2019) 49:98–112. doi: 10.1007/s10803-018-3695-6

34. Warreman EB, Nooteboom LA, Terry MB, Hoek HW, Leenen P, van Rossum E, et al. Psychological, behavioural and biological factors associated with gastrointestinal symptoms in autistic adults and adults with autistic traits [published online ahead of print, 2023 Feb 16]. *Autism*. (2023) 27:2173–86. doi: 10.1177/13623613231155324

35. McCoy SM, Jakicic JM, Gibbs BB. Comparison of obesity, physical activity, and sedentary Behaviors between adolescents with autism Spectrum disorders and without. *J Autism Dev Disord*. (2016) 46:2317–26. doi: 10.1007/s10803-016-2762-0

36. Hillier A, Buckingham A, Schena D 2nd. Physical activity among adults with autism: participation, attitudes, and barriers. *Percept Mot Skills*. (2020) 127:874–90. doi: 10.1177/0031512520927560

37. Weir E, Allison C, Baron-Cohen S. Understanding the substance use of autistic adolescents and adults: a mixed-methods approach. *Lancet Psychiatry*. (2021) 8:673–85. doi: 10.1016/S2215-0366(21)00160-7

38. Babio N, Ibarrola-Jurado N, Bulló M, Martínez-González MÁ, Wärnberg J, Salaverría I, et al. White blood cell counts as risk markers of developing metabolic syndrome and its components in the PREDIMED study. *PLoS One*. (2013) 8:e58354. doi: 10.1371/journal.pone.0058354

39. Dominguez-Andres J, Netea MG. Long-term reprogramming of the innate immune system. *J Leukoc Biol*. (2019) 105:329–38. doi: 10.1002/JLB.MR0318-104R

40. Broadbent J, Galic I, Stokes MA. Validation of autism spectrum quotient adult version in an Australian sample. *Autism Res Treat*. (2013) 2013:984205. doi: 10.1155/2013/984205

frontiers | Frontiers in Psychiatry

TYPE Brief Research Report
PUBLISHED 10 June 2024
DOI 10.3389/fpsyt.2024.1385947

OPEN ACCESS

EDITED BY
Mila Vulchanova,
NTNU, Norway

REVIEWED BY
Cecilia Brynskov,
University of Copenhagen, Denmark
Rein Ove Sikveland,
NTNU, Norway

*CORRESPONDENCE
Shu H. Yau
shu.yau@murdoch.edu.au

RECEIVED 14 February 2024
ACCEPTED 16 May 2024
PUBLISHED 10 June 2024

CITATION
Yau SH, Choo K, Tan J, Monson O and Bovell S (2024) Comparing and contrasting barriers in augmentative alternative communication use in nonspeaking autism and complex communication needs: multi-stakeholder perspectives. *Front. Psychiatry* 15:1385947. doi: 10.3389/fpsyt.2024.1385947

Comparing and contrasting barriers in augmentative alternative communication use in nonspeaking autism and complex communication needs: multi-stakeholder perspectives

Shu H. Yau[1,2]*, Kaylynn Choo[1], Jane Tan[1], Olivia Monson[1] and Stephanie Bovell[1]

[1]School of Psychology, Murdoch University, Perth, WA, Australia, [2]School of Education, University of Bristol, Bristol, United Kingdom

Augmentative alternative communication (AAC) devices or systems are often prescribed to minimally verbal or nonspeaking autistic individuals and other individuals with complex communication needs to facilitate communication or as an alternative to spoken language. AAC use can result in communication gains and improved quality of life for minimally verbal or nonspeaking individuals. Despite this, AAC abandonment is high, limiting societal participation of the individual on the autism spectrum with complex communication needs. Our study is a novel exploration of the barriers of AAC use from a multi-stakeholder perspective, and a qualitative analysis of similarities and differences between stakeholders. We conducted semi-structured interviews and focus groups with 30 parent-carers, educators and clinicians currently supporting AAC users in Western Australia and analysed the data using reflexive thematic analysis. Barriers from each stakeholder group were coded, resulting in 17 subthemes forming five main themes common to all stakeholders: Stakeholder Knowledge, Stakeholder Attitudes and Stigma, Resources, AAC User Engagement, and Device Fit. Contrasting perspectives included actual and perceived stigma associated with AAC use (parent-carers vs clinicians); different struggles with resources and knowledge (parent-carers vs clinicians and educators); and a lack of clinician communication in the processes that determined AAC-fit for school environments (educators only). Findings are discussed in the context of improving inter-stakeholder collaboration and capacity building in Australian health service and practice to better support minimally verbal or nonspeaking autistic individuals and individuals with complex communication needs. Suggestions are also offered for communication partner training.

KEYWORDS

augmentative alternative communication, autism, communication partners, complex communication needs, minimally verbal, nonspeaking, stakeholder perspectives

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental condition characterised by challenges with social communication and heterogeneous language ability (1). About a third of autistic children and youth are minimally verbal or nonspeaking and are often neglected in autism research (2). Minimally verbal or nonspeaking autistic individuals and individuals with significant language needs are encompassed by the broader term complex communication needs (CCN), which affects an estimated 1 in 500 people in Australia (3).

Augmentative and alternative communication (AAC) is typically prescribed as communication interventions for minimally verbal autistic individuals and individuals with CCN (4, 5). AAC encompasses systems and devices that supplement/augment language development or act as a replacement/alternative to verbal speech, or both (6). AAC interventions include unaided (e.g., hand signs) or aided (e.g., speech generating devices) systems, and can range from light-tech (or no-tech) to high-tech systems. The current study focuses on the latter (e.g., iPad, eye-gaze systems), and is aligned with the belief that the goal of AAC is to allow users to communicate independently without a facilitator being present (7, 8).

Research on AAC use shows improved communication skills in autistic children (9) – including those with intellectual disabilities and CCN (10, 11) – decreased challenging behaviours (12, 13), increased requesting skills (14), increased social participation (15), and increased language and communication development (16–19). When interviewed, AAC users and stakeholders reported qualitative benefits like improved communication (20), better parent-child relationships (21), and increased independence (22).

Despite the potential positive outcomes from AAC use, 30%-50% of users abandon or under-use their AACs (23). In a systematic review on barriers and facilitators of light-tech AAC use, Moorcroft et al. (24) identified environmental factors (e.g., attitudes and supports by professionals, family and society) and personal factors (e.g., AAC user's attitude, socioeconomic status and culture) as the main barriers to provision and use of AAC by people with CCN. Research on barriers of AAC use have found similar themes but often focuses only on AAC users (25), parents only (26–29), or parents and clinicians only (30–32). Our study takes a novel multi-stakeholder approach (parent-carers, educators and clinicians) to capture nuances between stakeholder views on AAC barriers across a wider range of real-life settings (i.e., home, school, clinical therapy).

Methods

Participants

This study was approved by the Murdoch University Human Research Ethics Committee. Participants were recruited through purposive sampling through autism-specific services and disability service providers in Western Australia (WA), word-of-mouth, and social media. Participants included nine parent-carers, ten educators and eleven clinicians from metropolitan Perth and regional Western Australia.

Participant demographics can be found in Table 1. The parent-carer group comprised primary carers of high-tech AAC users who are predominantly autistic (minimally verbal/nonspeaking) pre-school and school-aged children. The educator group comprised school principals, mainstream and special schoolteachers and assistants. The clinician group comprised speech and language pathologists (SLPs) and psychologists. Educators and clinicians varied in work-experience with AAC users, and types of AAC supports they had engaged with. All clinicians and educators had worked with minimally verbal autistic AAC users of all ages and with varied co-occurring diagnoses. Few had experience with unaided systems (Makaton, AUSLAN), many had experience with light-tech (e.g., PODD) and all had experience with high-tech AAC (e.g., eye gaze, iPad speech generating devices).

Data collection

We used semi-structured interviews and focus groups separated by stakeholder group (i.e., parent-carers, educators, and clinicians) to encourage open conversation in the absence of the other stakeholder groups and therefore a deeper understanding of the challenges unique to each group (e.g., 33). Interviews were targeted to each stakeholder group as per focus group/interview recommendations (34). Participants were asked ten questions in three sections. Section 1 and 2 focused on a stakeholder's experiences when supporting AAC users. In section 2, participants were also shown nine barriers identified by previous research (21, 24, 35, 36), and asked to rank the three biggest barriers in AAC they had faced (barrier cards are provided in the Supplementary File). These rankings had two purposes: first as a conversation tool, inviting participants to agree or disagree on previously found barriers and to elaborate on their points; second as a tool in our analysis to compare and contrast the barriers across the stakeholder groups. Section 3 focused on overcoming barriers which forms a separate study. An assistant moderator was present to record field notes and provide a summary at the end of each focus group or interview. Participants could confirm or correct the accuracy of the summary.

Procedure

Due to COVID-19 restrictions in WA during 2022, four sessions were in-person and 16 sessions were online. Participants were given the option to attend focus groups (n = 18) or individual interviews (n = 12) as flexibility is needed when collecting data from these populations. This format variability is not uncommon (30, 37) and a breakdown of attendance format by participant group is provided in the Supplementary File. To maintain consistency, all researchers piloted sessions with Murdoch University Child Cognition and Autism Research Laboratory members (including those with lived-experience of ASD and/or CCN) who were not involved in the study. Consent was obtained from all participants

TABLE 1 Demographics of 30 participants in focus groups and interviews.

Stakeholder Group	Participant	Role	Child Age (years)	Child Diagnosis	Highest Education Level	AAC experience (years)
Parent-Carer	1	Mother	14	Down syndrome	bachelor	>10
Parent-Carer	2	Mother	14	cerebral palsy	bachelor	NA
Parent-Carer	3	Mother	7	ASD	postgraduate	3-5
Parent-Carer	4	Mother	13	ASD, Bainbridge-Ropers syndrome	bachelor	>5
Parent-Carer	5	Mother	26	ASD	bachelor	>10
Parent-Carer	6	Grandmother	27	ASD	Year 10	>10
Parent-Carer	7	Mother	8	ASD	bachelor	3-5
Parent-Carer	8	Mother	4	ASD	N/A	N/A
Parent-Carer	9	Mother	3.5	Angelman syndromic form of ASD	N/A	N/A
Educator	1	educator	Varies	Varies	postgraduate diploma	>5
Educator	2	educator	Varies	Varies	masters	>10
Educator	3	educator	Varies	Varies	advanced diploma	>5
Educator	4	educator	Varies	Varies	TAFE	3-5
Educator	5	educator	Varies	Varies	graduate diploma	>5
Educator	6	educator	Varies	Varies	bachelor; graduate certificate	NA
Educator	7	educator	Varies	Varies	bachelor; graduate certificate	10-15
Educator	8	educator	Varies	Varies	diploma	10-15
Educator	9	educator	Varies	Varies	PhD	>5
Educator	10	educator	Varies	Varies	diploma	>10
Clinician	1	SLP	Varies	Varies	graduate certificate	5
Clinician	2	SLP	Varies	Varies	postgraduate	>10
Clinician	3	SLP	Varies	Varies	bachelor	<2
Clinician	4	SLP	Varies	Varies	masters	>10
Clinician	5	SLP	Varies	Varies	masters	3-5
Clinician	6	SLP	Varies	Varies	masters	<2
Clinician	7	SLP	Varies	Varies	bachelor	<2
Clinician	8	SLP	Varies	Varies	bachelor	>5
Clinician	9	SLP	Varies	Varies	bachelor	<2
Clinician	10	school psychologist	Varies	Varies	masters	>10
Clinician	11	school psychologist	Varies	Varies	masters	>10

SLP, speech language pathologist; ASD, autism spectrum disorder; ID, intellectual delay.

prior to the study and participants were given a $20 grocery voucher each as a token of appreciation. No participants chose to withdraw post-interview, therefore the final analysis consisted of the full dataset of responses. Participant sessions were recorded and transcribed verbatim.

Data analysis

Themes were identified as per Braun and Clarke's (38, 39) reflexive thematic analysis procedures which included researchers' engagement with semantic content of the data. Using a codebook

approach, we first combed the transcripts for potential codes, on MAXQDA 2022 (40). Then, through iterative discussions and inductive data engagement the research team generated and refined themes from the initial codes (41). Finally, we looked for similarities and differences between the stakeholder groups for each of the 18 codes, while also referring to assistant moderator field notes and participants' top three barrier rankings. Through reflexive discussions, all researchers could debate and challenge different researcher standpoints of the themes (39, 42).

Results

Table 2 shows the subthemes generated from all stakeholders that formed the five main themes: Stakeholder Knowledge, Stakeholder Attitudes and Stigma, Resources, AAC User Engagement, and Device Fit. There were differences between stakeholder groups in how each of the five themes were experienced. Subthemes were highlighted if identified as being unique to a particular stakeholder group (italicised in Table 2).

Theme 1: Stakeholder Knowledge

All stakeholders mentioned a lack of AAC knowledge as a barrier. 44% of parent-carers, 90% educators and 55% clinicians ranked this in their top three barriers of AAC use. When stakeholders lacked technical and practical knowledge in AAC, there would be fewer and briefer AAC conversations with users. For example, Parent-Carer 1 said that because she did not know how to operate the AAC device efficiently, it shortens the conversations she has with her child: *"…I try to find the words [on AAC], it takes a long time and [child] loses his patience."* Lack of knowledge among professionals also leads to poorer support and learning opportunities for AAC users. For example, Educator 5 felt limited in his ability to teach his students who use AAC if he is not fluent in using the device *"How are you supposed to model it and make it useful and valuable to the students?"*.

All stakeholder groups unanimously raised that their lack of knowledge was attributable to difficulty accessing training. Some parent-carers faced hurdles at the beginning of their AAC journey, as they could not readily access AAC or speech and language services and relevant training, *"We were very new to NDIS [National Disability Insurance Scheme] and we didn't know where we were meant to get any of the services from."* (Parent-Carer 6). Many clinicians cited a lack of depth in AAC training during tertiary education made them feel poorly prepared to serve AAC users and their families. *"[There are] theoretical things in university, but there's not much opportunity to apply in practice the actual selection of device, selection of vocabulary, display."* (Clinician 3). This sentiment is echoed by Clinician 1, *"(at university) we never touched a device … never done a disability practical…"*.

A lack of AAC knowledge in stakeholders in our study also extended to other communication partners such as peers, siblings and the broader society. Parent-Carer 5's son communicates with his parents, support worker and SLP using AAC, however, his AAC interactions are limited when he is within community settings (e.g., work and peer-support groups) *"…takes time for him to get [AAC] out and start it up. If they don't know he's using it, it can end the conversation a bit quickly."* The same occurs in therapy,*"…a lot of talking by the occupational therapist and [name]'s got less opportunity to talk because he's not using the device … unless the support worker specifically intervenes and suggests the use of the iPad, it's not used at all."* (Parent-Carer 5).

Additionally, psychologists in our clinician sample questioned their role in the AAC space, *"Is it to advocate for its use? Is it to incorporate its use in what we do? This is a speech space and we're a bit turf aware…(knowing the boundary) can help us be more respectful of our clients and more consistent with APS (Australian Psychological Society) ethics … the ethical code has things on disrespectful communication and respect, and I think AAC is part of respectful communication*." (Clinician 10).

Stakeholder Knowledge: parent-carer specific barrier

Four parent-carers (44%) described facing additional challenges, such as understanding AAC training, autism research and in navigating high-tech AAC devices: *"I'm not terribly well-educated. But (AAC trainers) presume that everyone's got a university degree … a lot of us … left school in year ten, and we really aren't up with all this modern stuff. They assume you know a lot about the current research on autism…"* (Parent-Carer 6).

Theme 2: Stakeholder Attitudes and Stigma

88% of parent-carers, 80% of educators and all clinicians mentioned experiencing negative attitudes towards AAC use and uptake. Stakeholders raised concerns that AAC would hamper a child's development and potential, and more pragmatic concerns around being responsible for damaging expensive equipment. For example, parent-carers cited their own initial hesitation when AAC was suggested as an intervention: *"I worried my son will lose his will to speak, so better to make him speak clearly, use more speech therapy."* (Parent-Carer 1). However, parents in our sample eventually jumped onboard with AAC intervention when they experience success communicating with their child, *"I wasn't ready for it (AAC), seems confronting (to think) oh she'll never speak, but she took to it well and it really let us see that she is thinking about things and has things to tell us. She just can't say that verbally … it's given her a voice."* (Parent-Carer 2).

Parents cited further hesitation to AAC intervention when clinicians they rely on for advice held beliefs that a young child was not 'capable' or ready for AAC: *"The paediatrician (said) you can't give her [child] a communication system, she won't understand it. She needs to first show that she can understand pictures."* (Parent-Carer 8). Clinicians noticed that this then causes delays to communication intervention, *"Often paediatricians won't refer for*

TABLE 2 Barriers of AAC use identified by carers, educators and clinicians.

Themes	Codes – Barriers
Stakeholder Knowledge	1. Insufficient access to specialised training (AAC, profound Autism) 2. Lack of awareness or access to disability services. 3. Societal AAC knowledge or awareness 4. *Difficulties in accessing knowledge and technology [parent-carers only].*
Stakeholder Attitudes and Stigma	1. Reluctance in AAC uptake as early intervention 2. Device is withheld for safekeeping *3. Experienced stigma versus perceived stigma [parent-carers and clinicians].*
Resources	1. Limited time 2. Limited financial resources. *3. Time and funds for upskilling and to buy spare devices [educators and clinicians]*
AAC User Engagement	1. Acute factors 2. Ingrained factors 3. Late AAC introductory age. *4. User characteristics and willingness not a barrier [educators and clinicians].*
Device Fit	1. Poor fit to users due to features 2. Poor fit to context and communication partners. 3. *Poor fit in school due to lack of inter-stakeholder communication [educators only].*

Differences between stakeholder groups are denoted in italics. Differences that arose from or pertained to a specific stakeholder group is noted in square brackets.

an assessment for AAC and will try manage what I would call complex and challenging behaviours through medications." (Clinician 11).

Lastly, all stakeholder groups experienced communication partners withholding the device, due to a fear the device would 'break on their watch', "*…mum that doesn't let her daughter use her device outdoors because she's afraid it will get broken … fear of that 'gap' when it's broken, and you have to get it replaced or fixed."* (Clinician 5). Parent-carer 3 states, *"…a few times our therapist mentions that A's tablet is not easily accessible for him at school, like it's up in the cupboard … the school was concerned the tablet got damaged, not necessarily by A, but you know, because there's other kids*".

Stakeholder Attitudes and Stigma: experienced stigma versus perceived stigma

Another concern that was evident across the dataset was the stigma associated with being seen to use an AAC device. Parent-carers were worried about negative societal perceptions or societal stigma against their children using AACs. *"…(people) don't expect him to have a device, or don't understand why he doesn't talk straight away because he looks like everyone else…"* (Parent-Carer 6). Some parent-carers experienced barriers in communication from extended family members and peers because of the AAC, *"… cousins don't try at all to communicate with (child), and kids in general don't, they just sort of go, 'oh she can't talk'"* (Parent-Carer 4).

In contrast, clinicians believed this to be an issue of 'perceived stigma',*"I don't think it's society not accepting it. I think it's parents thinking society won't accept it."* (Clinician 1). Clinicians mentioned stories of AACs going unused due to stigma that carrying an AAC signals that one is 'incapable', *"For years … nobody would ever set up his device for him because they [believed] … makes him look more disabled or makes him look more different [in school]."* (Clinician 1). Clinicians also reported having seen acceptance and patience: *"A lot of adults generally are accepting, they would wait … and would give [AAC users] the time to say what he needs"* (Clinician 5). This was also reported in children, *"Their peers absolutely love it (speech generating AAC) … other kids are like, what have you got and can I press it, can I play too?"* (Clinician 4).

As Clinician 10 surmises, stigma may be due to a lack of visibility of high profile AAC users in Australia, *"We have Dylan Alcott, but we don't really have a champion for AAC".*

Theme 3: Resources

All stakeholders cited competing financial and time demands that limit the quantity and quality of AAC opportunities they could create. Parent-carers experienced competing financial and time demands, and need to prioritise care-related needs over AAC communication: "*…her needs are very large so we don't have enough money to have a speechie and also have a physio, ABA … I haven't got the time or the resources to create little booklets and read with her with her device … everything takes a very long time, plus working and my other child … you're so busy trying to feed her, toilet her, get her to sleep…"* (Parent-Carer 4).

Educators and clinicians also cite a lack of resources as a barrier, specifically in family members and support workers. "*Support workers are paid to prioritize housework over working with people on their communication."* (Clinician 8). Clinician 3 adds, "*(families) have so much going on in their lives, so many stressors than learning a new language system … they're sleep poor, time poor. It's hard to be adding more.*"

While educators and clinicians do not think financial resources are a barrier for them per se, most raised that organisational decisions ultimately affect whether they can attend AAC training or have resources to work with: *"It's really hard to get to know [AAC system names] when you're in class with 30 kids … already under the pump to get your curriculum boxes ticked, let alone stop and try to learn a device … when that child goes, the device goes. When do you ever practice? Do you take it off a child during recess and practice? No, I need spare devices."* (Educator 4).

Theme 4: AAC User Engagement

In our coding, most stakeholders (89% of parent-carers, 60% of educators and 55% of clinicians) identified a user's willingness to use the AAC as a barrier. *"(Name) has the ability and knows where most of the words are. But his willingness to engage … Or wanting to use it as a communication. That's the biggest barrier."* (Parent-Carer 7).

When an AAC user does not initiate or reciprocate communication using AAC, it can be due to either acute or ingrained reasons. An acute example is when a child is emotionally

dysregulated: *"If he is sad or angry … he doesn't have the concentration to look at the AAC … the time that you really want to communicate to find out what is wrong is the time that we have the most difficulty in in trying to reach him*" (Parent-Carer 3). Autistic AAC users may rely on ingrained or internalised routines, and not spontaneously initiate AAC use: "*… their routine of using their device is, someone tells me to press the button and then I press it … they internalize that as how they use their device … prompt dependent …"* (Clinician 4). Other autistic children may use AAC only in specific or predictable contexts: *"… when I use the AAC with him (to chat), he just doesn't want to use it. If I said let's do your homework … then he will use his AAC."* (Parent-Carer 7). User engagement in our sample was compounded by the age at which the AAC device was introduced: "*When devices are introduced late, they [users] have already established pretty effective means of communicating their needs and wants."* (Educator 5).

AAC User Engagement: more of a barrier to parent-carers than educators and clinicians

When asked to rate the biggest three barriers that affected their AAC use, nine parent-carers (100%) picked 'user engagement/willingness' compared to educators (30%) and clinicians (27%). When asked to elaborate, educators and clinicians believed that user (dis)engagement is 'perceived' and the real barrier is poor device fit and support.

"…individual's abilities or willingness should not be up there [of top barriers]. It might look like that, but it's because they've been given the wrong system or the people around them haven't got adequate training to support its use." (Educator 6).

Theme 5: Device Fit and features

'Device Fit' was rated as a top three barrier to AAC use by all parent-carers, 80% of eight educators and 73% clinicians, due to poorly customised fit or sensory overwhelm to the user.

"Ideally we'll have the AAC on him all the time, like having the tablet sling on his shoulder … but it does hinder his movement." (Parent-Carer 3 on the bulkiness of the AAC).

"There were just too many (distracting) icons" (Educator 8 on why they abandoned a high-tech AAC for an autistic child).

*"… the voice … is robotic or American (in an Australian context) … not representative voice" (*Educator 10*)*

Other times, the device or AAC system prescribed for a child is not the 'supported AAC type' in their school, which hinders their daily use *"… it just happened that LAMP [prescribed AAC] is not the preferred system, [school] prefers Proloquo … but it's not like we can swap our system, the time and energy costs of switching systems is not worth it"* (Parent-Carer 3)

Device Fit educator specific barrier of poor inter-stakeholder communication

While all groups discussed poor device fit to the child's daily use, only the educator group brought up that this was due to a lack of inter-stakeholder communication. Educators mentioned that schools are generally not involved or consulted when a child's device is being chosen by clinicians, resulting in a device presenting barriers at school, where a child spends a significant amount of time, *"The devices would just turn up … (we all agree) that is not an appropriate choice for that person (in school)."* (Educator 5).

Discussion

In this study, we set out to identify and contrast barriers to AAC use in a multi-stakeholder group (parent-carers, educators and clinicians) who support an autistic child who is nonspeaking or minimally verbal, as well as those with CCN.

The first theme on poor *Stakeholder Knowledge* was consistent with findings in parents (29), educators (36) and clinicians (43). In a reversal of findings to ours, 'lack of knowledge' was brought up in 80% of caregivers but only 40% of clinicians in Romano and Chun's (31) study. This difference is likely due to work experience in our sample, with many of our educators and clinicians having two or less years of AAC experience whereas Romano and Chun sampled 'experienced' speech pathologists. This reflects the role of work experience in increasing confidence and efficacy in clinicians and suggests the importance of pre-service (44) and in-service training. Higher knowledge and self-efficacy in clinicians are often related to more experience working with autistic clients and specific training in autism and complex co-occurring conditions (45). While all our stakeholders cite difficulties accessing specialized training and disability services, our parent-carers mentioned additional challenges in understanding the content within AAC training and navigating high-tech AAC devices. Ganz et al. (46) suggested that service provision and AAC selection need to consider the individual with CCN, as well as the preferences of the key stakeholders that support them. In this case, it is imperative for clinicians to take extra time to identify parents' level of knowledge and consider the technology they are comfortable with, when prescribing AAC and designing training for them.

Our second theme was on *Stakeholder Attitudes and Stigma.* As with past studies (4, 9, 13, 16, 20, 22, 24, 27, 34, 35, 46–52), our parent-carers initially worried that their children will lose their potential for speech if they rely on AAC devices to 'speak for them', or that AAC devices will single their child out to peers as being different. The former is linked to stakeholder attitudes (i.e., belief in myths; 53), and the latter to stigma. Within our second theme, there was a sharp contrast in the experience of societal stigma: parent-carers acknowledged a real impact, whereas clinicians attributed it to perception.

It is possible that the discrepancy between 'real' and 'perceived' societal stigma is largely context-dependent. In the broader community (parent-carer context), there is limited knowledge of and exposure to AACs and people with disabilities, in comparison to structured school programs or speech-language clinics (educator and clinician context). The communities within the latter context are inherently more inclusive due to relevant professional training and/or exposure to disability, making it less likely for educators and clinicians to encounter the stigma and isolation that parent-carers experience: an area that warrants future research. Future research should also investigate the potential influence of the AAC user's age

or AAC device type on the different experiences of stigma. For instance, clinicians who work with younger children or children with mainstream devices (e.g., ipads) may have more positive experiences. Societal stigma may be reduced by improving AAC interventions through peer-mediated interventions with explicit teaching of AAC use and turn-taking in children (49, 54). When neurotypical peers were taught AAC interaction strategies, students using AAC enjoyed their interactions, saw their peers as friends, and were more involved in class activities (55).

Also concerning is that our parent-carers and clinicians were discouraged from requesting AAC intervention for children by physicians who cited inaccurate attitudes and beliefs that children needed 'pre-requisite' cognitive and sensorimotor skills to use AAC. This is likely linked to physicians' self-reported lack of autism-specific knowledge and confidence in managing care of autistic individuals with co-occurring intellectual delays or other severe impairments (51, 56). This calls for targeted development of autism and complex communication training programs focusing on improving physician awareness, efficacy and behaviours. Lastly, there were pragmatic concerns around being responsible for expensive AAC equipment, and being fearful of being penalized or going without, in the event the AAC is damaged or lost. This suggests a need for clarity in NDIS provisions [e.g., device replacements; (57)] and is linked to the next theme on resources.

Our third theme on *Resources* (a lack of or competing) was consistent throughout parent-carer, educator and clinician groups. Our parent-carer group mentioned competing demands on their time and finances, making it hard for them to commit to AAC partner training. Indeed, it is not uncommon to find parents in AAC families performing the roles of caregivers, communication partners, teachers, advocates, therapy coordinators and AAC programmers (58). Our educators and clinicians were also affected by a resource constraints, such that the time available for training families and communication partners is dependent on a family's funding (NDIS or otherwise). Moreover, their own time and ability to access resources to upskill and practice on devices are tied to organizational decisions.

The fourth theme on *User Engagement* describes barriers to effective AAC use when a child is dysregulated or prompt-dependent – which was also found by Donato et al. (47). Parent-carers in our study who find that their autistic children have a prescribed use of AAC is consistent with research in families with minimally verbal children with autism, where AAC use is primarily transactional (e.g., food/drink requests; 33). Unexpectedly, our educators and clinicians did not consider user engagement as a barrier to AAC use, which contrasts with previous findings (e.g., 43, 59, 60), as well as our parent-carer experiences. This could be explained by our earlier finding on autistic children's lower engagement in social communication versus their preference or better performance in task-oriented communication and context-bound routines. Educators and clinicians often interact with autistic children within a structured program with goal-oriented tasks, which autistic children typically perform better at/engage in more (61), hence professionals in our study may not experience the user (dis)engagement that parent-carers do.

The fifth and final theme is on *Device Fit* for the user, specifically physical or sensory mismatch, also found in other studies (28, 59). A few parents and clinicians mentioned AAC users eschewing their speech generating devices due to the identity or pitch of the voice. Promisingly, AAC technology is developing, where 'voices' can be customized using vocalizations from the user combined with recordings of a matched-speech donor (62). To prevent a family giving up on AAC altogether due to poor fit, it is important that clinicians and service providers communicate clearly with parents and educators that 'finding the right fit' is often an ongoing process (50), and encourage them to be flexible and open-minded when trialling AAC systems. Legislation on AAC access and service providers should ideally support changing AAC needs as circumstances and skills inevitably change. This may mean an AAC user needs two different types of AAC concurrently, so they have the freedom to swap to the communication method that works for them in the moment (63).

To be used effectively, AACs also need to fit the main contexts where they will be used daily, such as school. However, our parent-carers reported their child getting less support in school if they had a 'less supported' AAC system. Related to this, educators lamented the lack of collaboration and communication when clinicians decide on AAC fit. Such barriers can be eased by implementing an interprofessional collaboration (IPC) framework (64), which is both patient-centred and population-oriented. In IPC, problem solving is shared at the community level to ensure appropriate access and fit to services for autistic individuals. This may demand more time from educators and clinicians – and could be constrained by limited knowledge and professional boundaries – but is deserving of additional time from employers and funding through government bodies. Implementing ICP can ease the burden of care coordination for caregivers by eliminating care silos. Clinicians who engage in shared decision-making with schools are more knowledgeable about feasible interventions within the constraints of a school setting (52), which better supports the child with the AAC.

The comparative approach of our study highlighted common experiences across the three stakeholder groups as well as contrasting perspectives. These views can be used in future training with the different stakeholders to help break down boundaries and foster the connections needed to improve inter-stakeholder collaboration. It is important to include AAC users themselves in future studies, to further understand their views on barriers in relation to their communication partners. It could also be useful to extend on our findings through micro-ethnography of AAC users with neurotypical peers. Future researchers should also aim to recruit the voices of fathers, support workers and other therapists who are also frequent communication partners.

Conclusion

While AAC use is beneficial in fostering communication in minimally verbal and nonspeaking individuals, up to 50% of users and families abandon or underuse their AAC. Our study explored barriers to AAC use in different stakeholder groups and found that

barriers fell into five themes: *Stakeholder Knowledge*, *Stakeholder Attitudes and Stigma, Resources, User Engagement, and Device Fit.* By employing a multi-stakeholder approach, we uncovered nuanced differences between stakeholders in supporting autistic AAC users and those with complex communication needs. Such insights are useful in tailoring training to meet each stakeholder group's needs to better support an AAC user. Our findings are important for ongoing Australian NDIS legislative amendments, specifically to improve access to resources and training, and inter-agency collaboration.

Data availability statement

The raw data is not available due to the video format of the recordings, and potentially identifiable information shared by stakeholders, particularly information from parent-carers on children. However, de-identified transcriptions or data codes supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Murdoch University Human Research Ethics Committee. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of de-identifiable data included in this article.

Author contributions

SY: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. KC: Data curation, Formal analysis, Project administration, Writing – original draft, Writing – review & editing. JT: Data curation, Formal analysis, Investigation, Project administration, Resources, Writing – review & editing. OM: Conceptualization, Methodology, Validation, Writing – review & editing. SB: Data curation, Formal analysis, Investigation, Project administration, Resources, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was funded by a Murdoch University gender equity leadership grant (2022) and a Lotterywest Building Communities Grant (420174241).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2024.1385947/full#supplementary-material

References

1. American Psychiatric Association. *Diagnostic and statistical manual of mental disorders, 5th ed.* (Washington DC: American Psychiatric Association) (2013). doi: 10.1176/appi.books.9780890425596.

2. Tager-Flusberg H, Kasari C. Minimally verbal school-aged children with autism spectrum disorder: The neglected end of the spectrum. *Autism Res.* (2013) 6:468–76. doi: 10.1002/aur.1329

3. Perry A, Reilly S, Bloomberg K, Johnson H. *An Analysis of Needs for people with a disability who have Complex Communication Needs.* Melbourne: La Trobe university; School of Human Communication Sciences, Bundoora (2002). p. 3086.

4. Iacono T, Trembath D, Erickson S. The role of augmentative and alternative communication for children with autism: current status and future trends. *Neuropsychiatr Dis Treat.* (2016) 12:2349–61. doi: 10.2147/NDT.S95967

5. Speech Pathology Australia. *Augmentative and Alternative Communication Clinical Guideline.* Melbourne: Speech Pathology Australia (2020).

6. Schlosser RW, Wendt O. Effects of augmentative and alternative communication intervention on speech production in children with autism: A systematic review. *Am J Speech-Language Pathol.* (2008) 17:212–30. doi: 10.1044/1058-0360(2008/021

7. Travers JC, Tincani MJ, Lang R. Facilitated communication denies people with disabilities their voice. *Res Pract Persons Severe Disabil.* (2014) 39:195–202. doi: 10.1177/1540796914556778

8. Schlosser RW, Hemsley B, Shane H, Todd J, Lang R, Lilienfeld SO, et al. Rapid prompting method and autism spectrum disorder: Systematic review exposes lack of evidence. *Rev J Autism Dev Disord.* (2019) 6:403–12. doi: 10.1007/s40489-019-00175-w

9. Logan K, Iacono T, Trembath D. A systematic review of research into aided AAC to increase social-communication functions in children with autism spectrum disorder. *Augmentative Altern Communication.* (2017) 33:51–64. doi: 10.1080/07434618.2016.1267795

10. Sigafoos J, O'Reilly MF, Lancioni GE, Sutherland D. Augmentative and alternative communication for individuals with autism spectrum disorder and intellectual disability. *Curr Dev Disord Rep.* (2014) 1:51–7. doi: 10.1007/s40474-013-0007-x

11. Morin KL, Ganz JB, Gregori EV, Foster MJ, Gerow SL, Genç-Tosun D, et al. A systematic quality review of high-tech AAC interventions as an evidence-based practice. *Augment Altern Comm.* (2018) 34(2):104–17. doi: 10.1080/07434618.2018.1458900

12. Walker VL, Snell ME. Effects of augmentative and alternative communication on challenging behavior: A meta-analysis. *Augmentative Altern Communication*. (2013) 29:117–31. doi: 10.3109/07434618.2013.785020

13. Kasari C, Kaiser A, Goods K, Nietfeld J, Mathy P, Landa R, et al. Communication interventions for minimally verbal children with autism: A sequential multiple assignment randomized trial. *J Am Acad Child Adolesc Psychiatry*. (2014) 53:635–46. doi: 10.1016/j.jaac.2014.01.019

14. Srinivasan S, Patel S, Khade A, Bedi G, Mohite J, Sen A, et al. Efficacy of a novel augmentative and alternative communication system in promoting requesting skills in young children with Autism Spectrum Disorder in India: A pilot study. *Autism Dev Lang Impairments*. (2022) 7:1–22. doi: 10.1177/23969415221120749

15. Collette D, Brix A, Brennan P, DeRoma N, Muir BC. Proloquo2go enhances classroom performance in children with autism spectrum disorder. *OTJR: Occupation, Participation and Health*. (2019) 39(3):143–50. doi: 10.1177/1539449218799451

16. Millar DC, Light JC, Schlosser RW. The impact of augmentative and alternative communication intervention on the speech production of individuals with developmental disabilities: A research review. *J Speech Language Hearing Res*. (2006) 49:248–64. doi: 10.1044/1092-4388(2006/021

17. DiStefano C, Shih W, Kaiser A, Landa R, Kasari C. Communication growth in minimally verbal children with ASD: The importance of interaction. *Autism Res*. (2016) 9:1093–102. doi: 10.1002/aur.1594

18. Almirall D, DiStefano C, Chang YC, Shire S, Kaiser A, Lu X, et al. Longitudinal effects of adaptive interventions with a speech-generating device in minimally verbal children with ASD. *J Clin Child Adolesc Psychol*. (2016) 45(4):442–56. doi: 10.1080/15374416.2016.1138407

19. Brady NC, Thiemann-Bourque K, Fleming K, Matthews K. Predicting language outcomes for children learning augmentative and alternative communication: Child and environmental factors. *J Speech Lang Hear Res*. (2013) 56(5):1595–612. doi: 10.1044/1092-4388(2013/12-0102

20. Joginder Singh S, Hussein NH, Mustaffa Kamal R, Hassan FH. Reflections of Malaysian parents of children with developmental disabilities on their experiences with AAC. *Augmentative Altern Communication*. (2017) 33:110–20. doi: 10.1080/07434618.2017.1309457

21. Park H. Parents' experiences and acceptance factors of AAC intervention for children with complex communication needs. *Communication Sci Disord*. (2020) 25:318–33. doi: 10.12963/csd.20729

22. McNaughton D, Richardson L. Supporting positive employment outcomes for individuals with autism who use AAC. *Perspect Augmentative Altern Communication*. (2013) 22:164–72. doi: 10.1044/aac22.3.164

23. Webb EJ, Lynch Y, Meads D, Judge S, Randall N, Goldbart J, et al. Finding the best fit: examining the decision-making of augmentative and alternative communication professionals in the UK using a discrete choice experiment. *BMJ Open*. (2019) 9(11):e030274. doi: 10.1136/bmjopen-2019-030274

24. Moorcroft A, Scarinci N, Meyer C. A systematic review of the barriers and facilitators to the provision and use of low-tech and unaided AAC systems for people with complex communication needs and their families. *Disability Rehabilitation: Assistive Technol*. (2019) 14:710–31. doi: 10.1080/17483107.2018.1499135

25. Cooper L, Balandin S, Trembath D. The loneliness experiences of young adults with cerebral palsy who use alternative and augmentative communication. *Augment Altern Comm*. (2009) 25(3):154–64. doi: 10.1080/07434610903036785

26. Anderson K, Balandin S, Stancliffe RJ. Australian parents' experiences of speech generating device (SGD) service delivery. *Dev Neurorehabilitation*. (2014) 17:75–83. doi: 10.3109/17518423.2013.857735

27. Johnson H, Van Nierop M, Iacono T. Parents' perspectives of an Australian augmentative and alternative communication service:"I clapped for my child". *Res Pract Intellectual Dev Disabil*. (2021) 8:46–59. doi: 10.1080/23297018.2020.1861552

28. Moorcroft A, Scarinci N, Meyer C. "I've had a love-hate, I mean mostly hate relationship with these PODD books": parent perceptions of how they and their child contributed to AAC rejection and abandonment. *Disability Rehabilitation: Assistive Technol*. (2021) 16:72–82. doi: 10.1080/17483107.2019.1632944

29. Berenguer C, Martínez ER, De Stasio S, Baixauli I. Parents' perceptions and experiences with their children's use of augmentative/alternative aommunication: A systematic review and qualitative meta-synthesis. *Int J Environ Res Public Health*. (2022) 19:8091–101. doi: 10.3390/ijerph19138091

30. Anderson KL, Balandin S, Stancliffe RJ. "It's got to be more than that". Parents and speech-language pathologists discuss training content for families with a new speech generating device. *Disability Rehabilitation: Assistive Technol*. (2016) 11:375–84. doi: 10.3109/17483107.2014.967314

31. Romano N, Shon Chun RY. Augmentative and alternative communication use: Family and professionals' perceptions of facilitators and barriers. *CoDAS (São Paulo)*. (2018) 30:e20170138–e20170138. doi: 10.1590/2317-1782/20162017138

32. Moorcroft A, Scarinci N, Meyer C. 'We were just kind of handed it and then it was smoke bombed by everyone': How do external stakeholders contribute to parent rejection and the abandonment of AAC systems? *Int J Lang Communication Disord*. (2020) 55:59–69. doi: 10.1111/1460-6984.12502

33. Doak L. Rethinking family (dis) engagement with augmentative & alternative communication. *J Res Special Educ Needs*. (2021) 21:198–210. doi: 10.1111/1471-3802.12510

34. Krueger RA, Casey MA. Designing and conducting focus group interviews (2002). Available at: http://lucascountyhealth.com/wp-content/uploads/2019/10/Designing-and-Conducting-Focus-Group-Interviews.pdf.

35. Johnson JM, Inglebret E, Jones C, Ray J. Perspectives of speech language pathologists regarding success versus abandonment of AAC. *Augmentative Altern Communication*. (2006) 22:85–99. doi: 10.1080/07434610500483588

36. Chung YC, Stoner JB. A meta-synthesis of team members' voices: What we need and what we do to support students who use AAC. *Augmentative Altern Communication*. (2016) 32:175–86. doi: 10.1080/07434618.2016.1213766

37. Saggers B, Tones M, Dunne J, Trembath D, Bruck S, Webster A, et al. Promoting a collective voice from parents, educators and allied health professionals on the educational needs of students on the autism spectrum. *J Autism Dev Disord*. (2019) 49:3845–65. doi: 10.1007/s10803-019-04097-8

38. Braun V, Clarke V. Using thematic analysis in psychology. *Qual Res Psychol*. (2006) 3:77–101. doi: 10.1191/1478088706qp063oa

39. Braun V, Clarke V. Reflecting on reflexive thematic analysis. *Qual Res Sport Exercise Health*. (2019) 11:589–97. doi: 10.1080/2159676X.2019.1628806

40. VERBI Software. *MAXQDA 2022*. Berlin, Germany: VERBI Software (2021).

41. Braun V, Clarke V. To saturate or not to saturate? Questioning data saturation as a useful concept for thematic analysis and sample-size rationales. *Qual Res Sport Exerc Health*. (2021) 13(2):201–16. doi: 10.1080/2159676X.2019.1704846

42. Wild CE, Rawiri NT, Willing EJ, Hofman PL, Anderson YC. Determining barriers and facilitators to engagement for families in a family-based, multicomponent healthy lifestyles intervention for children and adolescents: a qualitative study. *BMJ Open*. (2020) 10:e037152. doi: 10.1136/bmjopen-2020-037152

43. Moorcroft A, Scarinci N, Meyer C. Speech pathologist perspectives on the acceptance versus rejection or abandonment of AAC systems for children with complex communication needs. *Augmentative Altern Communication*. (2019) 35:193–204. doi: 10.1080/07434618.2019.1609577

44. Pitt KM, Brennan S, Sauerwein AM, Weissling K. Preservice training in augmentative and alternative communication for speech-language pathologists and special education teachers: prevalence and preferences. *Perspect ASHA Special Interest Groups*. (2023) 8:1456–68. doi: 10.1044/2023_PERSP-23-00023

45. Corden K, Brewer R, Cage E. A systematic review of healthcare professionals' knowledge, self-efficacy and attitudes towards working with autistic people. *Rev J Autism Dev Disord*. (2022) 9:386–99. doi: 10.1007/s40489-021-00263-w

46. Ganz JB, Pustejovsky JE, Reichle J, Vannest KJ, Foster M, Pierson LM, et al. Participant characteristics predicting communication outcomes in AAC implementation for individuals with ASD and IDD: A systematic review and meta-analysis. *Augmentative Altern Communication*. (2023) 39:7–22. doi: 10.1080/07434618.2022.2116355

47. Donato C, Spencer E, Arthur-Kelly M. A critical synthesis of barriers and facilitators to the use of AAC by children with autism spectrum disorder and their communication partners. *Augmentative Altern Communication*. (2018) 34:242–53. doi: 10.1080/07434618.2018.1493141

48. Ganz JB. AAC interventions for individuals with autism spectrum disorders: State of the science and future research directions. *Augmentative Altern Communication*. (2015) 31:203–14. doi: 10.3109/07434618.2015.1047532

49. Jensen E, Douglas SN, Gerde HK. Dispelling myths surrounding AAC use for children: recommendations for professionals. *Inclusive Practices*. (2023) 2:30–6. doi: 10.1177/27324745221144308

50. Laubscher E, Pope L, Light J. "You just want to be able to communicate with your child": parents' Perspectives on communication and AAC use for beginning communicators on the autism spectrum. *Am J Speech-Language Pathol*. (2023) 33 (2):716–35. doi: 10.1044/2023_AJSLP-23-00254

51. Malik-Soni N, Shaker A, Luck H, Mullin AE, Wiley RE, Lewis ME, et al. Tackling healthcare access barriers for individuals with autism from diagnosis to adulthood. *Pediatr Res*. (2022) 91:1028–35. doi: 10.1038/s41390-021-01465-y

52. McClain MB, Haverkamp CR, Holt J, Peacock GG, Winter S. Interprofessional education and training. In: *Interprofessional care coordination for pediatric autism spectrum disorder*. Cham: Springer International Publishing AG (2020). p. 369–83.

53. Romski M, Sevcik RA. Augmentative communication and early intervention: Myths and realities. *Infants Young Children*. (2005) 18:174–85. doi: 10.1097/00001163-200507000-00002

54. Trembath D, Balandin S, Togher L, Stancliffe RJ. Peer-mediated teaching and augmentative and alternative communication for preschool-aged children with autism. *J Intellectual Dev Disability*. (2009) 34:173–86. doi: 10.1080/13668250902845210

55. Biggs EE, Carter EW, Gustafson J. Efficacy of peer support arrangements to increase peer interaction and AAC use. *Am J Intellectual Dev Disabil*. (2017) 122:25–48. doi: 10.1352/1944-7558-122.1.25

56. Clarke L, Fung LK. The impact of autism-related training programs on physician knowledge, self-efficacy, and practice behavior: A systematic review. *Autism*. (2022) 26:1626–40. doi: 10.1177/13623613221102016

57. National Disability Insurance Scheme. Summary report – outcomes of the request for information for designing an early childhood assistive technology approach (2022). Available at: https://www.ndis.gov.au/media/4470/download?attachment.

58. Caron JG. "We Bought an iPad": Considering family priorities, needs, and preferences as an AAC support provider. *Perspect Augmentative Altern Communication*. (2015) 24:5–11. doi: 10.1044/aac24.1.5

59. Baxter S, Enderby P, Evans P, Judge S. Barriers and facilitators to the use of high-technology augmentative and alternative communication devices: a systematic review and qualitative synthesis. *Int J Lang Communication Disord*. (2012) 47:115–29. doi: 10.1111/j.1460-6984.2011.00090.x

60. Sievers SB, Trembath D, Westerveld MF. Speech-language pathologists' knowledge and consideration of factors that may predict, moderate, and mediate AAC outcomes. *J Autism Dev Disord*. (2020) 50(1):238–49. doi: 10.1007/s10803-019-04217-4

61. Logan K, Iacono T, Trembath D. A systematic search and appraisal of intervention characteristics used to develop varied communication functions in children with autism who use aided AAC. *Res Autism Spectr Disord*. (2022) 90 (1):101896–915. doi: 10.1016/j.rasd.2021.101896

62. Pullin G, Treviranus J, Patel R, Higginbotham J. Designing interaction, voice, and inclusion in AAC research. *Augmentative Altern Communication*. (2017) 33:139–48. doi: 10.1080/07434618.2017.1342690

63. Donaldson AL, Corbin E, McCoy J. "Everyone deserves AAC": preliminary study of the experiences of speaking autistic adults who use augmentative and alternative communication. *Perspect ASHA Special Interest Groups*. (2021) 6(2):315–26. doi: 10.1044/2021_PERSP-20-00220

64. Gardner L, Campbell JM, Gilchrest C, McClain MB, Shahidullah JD. Identification of autism spectrum disorder and interprofessional collaboration between school and clinical settings. *Psychol Sch*. (2022) 59(7):1308–18. doi: 10.1002/pits.22673

frontiers | Frontiers in Psychiatry

TYPE Review
PUBLISHED 24 June 2024
DOI 10.3389/fpsyt.2024.1333717

OPEN ACCESS

EDITED BY
Mila Vulchanova,
NTNU, Norway

REVIEWED BY
Margarita Stankova,
New Bulgarian University, Bulgaria
Rekha Jagadapillai,
University of Louisville, United States

*CORRESPONDENCE
Harumi Jyonouchi
hjyonouchi@saintpetersuh.com

RECEIVED 05 November 2023
ACCEPTED 03 June 2024
PUBLISHED 24 June 2024

CITATION
Jyonouchi H (2024) Autism spectrum disorder and a possible role of anti-inflammatory treatments: experience in the pediatric allergy/immunology clinic. *Front. Psychiatry* 15:1333717. doi: 10.3389/fpsyt.2024.1333717

Autism spectrum disorder and a possible role of anti-inflammatory treatments: experience in the pediatric allergy/immunology clinic

Harumi Jyonouchi[1,2]*

[1]Department of Pediatrics, Saint Peter's University Hospital, New Brunswick, NJ, United States, [2]Department of Pediatrics, Rutgers University-Robert Wood Johnson School of Medicine, New Brunswick, NJ, United States

Autism spectrum disorder (ASD[1]) is a behaviorally defined syndrome encompassing a markedly heterogeneous patient population. Many ASD subjects fail to respond to the 1st line behavioral and pharmacological interventions, leaving parents to seek out other treatment options. Evidence supports that neuroinflammation plays a role in ASD pathogenesis. However, the underlying mechanisms likely vary for each ASD patient, influenced by genetic, epigenetic, and environmental factors. Although anti-inflammatory treatment measures, mainly based on metabolic changes and oxidative stress, have provided promising results in some ASD subjects, the use of such measures requires the careful selection of ASD subjects based on clinical and laboratory findings. Recent progress in neuroscience and molecular immunology has made it possible to allow re-purposing of currently available anti-inflammatory medications, used for autoimmune and other chronic inflammatory conditions, as treatment options for ASD subjects. On the other hand, emerging anti-inflammatory medications, including biologic and gate-keeper blockers, exert powerful anti-inflammatory effects on specific mediators or signaling pathways. It will require both a keen understanding of the mechanisms of action of such agents and the careful selection of ASD patients suitable for each treatment. This review will attempt to summarize the use of anti-inflammatory agents already used in targeting ASD patients, and then emerging anti-inflammatory measures applicable for ASD subjects based on scientific rationale and clinical trial data, if available. In our experience, some ASD patients were treated under diagnoses of autoimmune/autoinflammatory conditions and/or post-infectious neuroinflammation. However, there are little clinical trial data specifically for ASD subjects. Therefore, these emerging immunomodulating agents for potential use for ASD subjects will be discussed based on preclinical data, case reports, or data generated in patients with other medical conditions. This review will hopefully highlight the expanding scope of immunomodulating agents for treating neuroinflammation in ASD subjects.

KEYWORDS

ASD (autism spectrum disorder), biologics, immunomodulating agents, neuroinflammation, COVID-19 (coronavirus disease 2019)

1 Introduction

ASD is a complex developmental disorder, mostly defined by behavioral symptoms and its onset and progress is likely to be affected by multiple genetic and environmental factors (1). Such genetic and environmental factors likely vary in ASD subjects, resulting in markedly heterogeneous patients that all fall under the current diagnostic criteria of ASD. This makes it difficult to treat ASD subjects with 'one size fits all' measures. It would be ideal if tailor-made approaches based on each ASD subject's genetic/epigenetic/environmental conditions could be created. Instead, the 1st line treatment measures for ASD are behavioral and pharmacological interventions. However, these measures are not universally effective. Primary care providers may be consulted by frustrated parents regarding other treatment options which are often promoted by practitioners of complementary and alternative medicine (CAM). However, such CAM measures are often not based on sound scientific rationale and rigorous clinical trials. In contrast, treatment measures targeting specific molecules or pathways of neuroinflammation may provide alternative treatment options for some ASD subjects who are found to have evidence of neuroinflammation associated with specific mechanisms. This review will discuss anti-inflammatory measures that have been tried or can be applied to ASD subjects based on scientific rationale.

Inflammation has long been indicated in the pathogenesis of ASD through multiple lines of evidence. Epidemiological studies have indicated that maternal inflammation caused by infectious and non-infectious triggers during pregnancy are associated with an increased risk of ASD (2, 3). As direct evidence of neuroinflammation, neuroglial activation in the presence of inflammatory mediators has been shown in the brain of ASD subjects (4). Further analysis revealed that maternal inflammation occurring in the 1st and 2nd trimesters has a role in developmental impairment of offspring, irrespective of triggering events (5, 6). Such findings led to the creation of one of the most rigorously studied animal models of autism, maternal immune activation (MIA). In this rodent model, maternal sterile inflammation is induced by injection of endotoxin during the 2nd trimester, and this leads to ASD like developmental symptoms in offspring later in life (1, 7). Such prolonged effects of maternal inflammation not associated with specific pathogens is partly explained by the reprogramming of innate immune responses. That is, epigenetic changes following potent immune stimuli result in persistent changes in innate immunity, referred as to innate immune memory (IIM) (8–10) MIA may cause inflammation skewed IIM, referred as to trained immunity (TI) (8, 10). In fact, mal-adapted TI is implicated in the pathogenesis of numbers of chronic neuropsychiatric conditions (8, 9). It has also been shown that maternal derived interleukin-6 (IL-6) plays a key intermediary in the MIA model (11). Further study revealed the importance of placental IL-6 for the development of the fetal brain and subsequent behavioral changes (12). These results indicate that maternal sterile inflammation can cause profound and lasting effects on offspring.

Apart from MIA, cognitive development is known to be affected by genetically altered immune responses prone to neuroinflammation. Gene variants associated with increased risk of ASD often cause aberrant immune responses and subsequent inflammatory condition (1). For example, variants of tuberous sclerosis complex 1 and 2 (TSC1/TSC2) are associated with inflammatory conditions caused by aberrant activation of the mTOR (mammalian target of rapamycin) pathway (13). ASD subjects are also characterized by a high frequency of comorbid inflammatory conditions such as chronic GI inflammation, which may also be indicative of inflammation prone immune conditions (14–17).

If neuroinflammation does play a role in the pathogenesis of ASD, questions may arise as to whether there is direct evidence of neuroinflammation in the brain of these individuals, and if so, what type of cells are contributing to neuroinflammation. Microglial cells are one of the key innate immune cells in the brain, and they are resident macrophages in the CNS. They are grossly classified as either proinflammatory (M1) or anti-inflammatory alternatively activated (M2) microglial cells, although they exhibit diverse phenotypes (18). Embryogenic microglial cells, along with astrocytes, are thought to play a crucial role in brain development, regulating neurogenesis, neuronal migration, and synaptic plasticity (19–21). Microglial cells also act as major innate immune cells in the CNS throughout life, serving as a sensor of the CNS microenvironment, and they are easily activated by various stimuli (20). Such activated microglial cells play a pivotal role in controlling infection, inflammation, and injury in the CNS (19, 20). However, dysregulated activation of microglial cells can cause chronic neuroinflammation. Bone marrow (BM) derived macrophages can also infiltrate to the CNS under conditions when the blood brain barrier (BBB) is impaired, and can transform into BM derived microglial cells, exerting proinflammatory actions (22). For example, a pathogenic role of microglial cells is illustrated in Rett syndrome. *MECP2* pathogenic variants cause Rett syndrome which is characterized by progressive developmental disorder and ASD behavioral symptoms. It has been shown that loss of function (LOF) *MECP2* pathogenic variants cause dysregulated inflammatory responses of microglial cells, partly due to both an increase in mitochondrial production of reactive oxygen species (ROS) and a decrease in ATP production (19, 23–25).

It is also of note that conditions affecting other organs can exert significant effects on CNS inflammation in ASD patients. This may be partly explained by the effects of BM derived microglial cells (22), circulating mediators released by the affected organs, and stimuli through the sensory nervous system. In fact, microglial cells in the CNS are known to be affected by stimuli derived from other organs (19–21). MIA induced developmental impairment are in part attributed to the effects of maternally derived inflammatory mediators that affect fetal brain cells, especially microglial cells (1). The gut microbiome is also likely to affect brain development through various mechanisms and its role in the pathogenesis of neurodegenerative disease has been a focus of intense research (26).

The evidence that supports a role for neuroinflammation mediated by innate immune cells in the brain, at least in some ASD patients, are summarized as described above. However, in each ASD individual, the mechanisms of neuroinflammation may vary

depending on various genetic, epigenetic, and environmental factors that affect development of the brain and the immune system. Understanding the dynamic mechanisms of neuroinflammation will help us apply anti-inflammatory measures as treatment options for ASD patients. In the following section, anti-inflammatory measures used for targeting ASD subjects, based on scientific rationale and trial data, will be discussed first. Then, possible application of other immunomodulating agents for treating ASD subjects will be discussed. This will include emerging agents developed recently for treating autoimmune/autoinflammatory and other chronic inflammatory conditions. For these agents, secondary to scant data of clinical trials in ASD subjects, discussion will be based on pre-clinical data, case reports, results generated from use in other medical conditions.

2 Metabolic factors associated with neuroinflammation in ASD and treatment measures targeting metabolic changes or factors causing such changes

2.1 Lipid metabolites and their signaling pathways

1) *COX1 and COX2*: Lipids are major components of the brain and lipid metabolites act as regulatory molecules for both brain development and homeostasis. Major lipid metabolites that serve as lipid mediators are prostaglandins (PGs) and leukotrienes (LTs) metabolized by arachidonic acid (AA) and other unsaturated fatty acids by cyclooxygenases (COXs) and lipoxygenases (LOXs), respectively (27). PGE2 signaling has been known to play a role in brain morphogenesis (28, 29) and impaired COX2/PEG2 signaling has been associated with ASD pathogenesis in the MIA model (29, 30). The COX pathway involves two rate limiting enzymes, COX-1 and COX-2. COX-1 is expressed constitutively in all the cells, while COX-2 is induced by inflammatory mediators and expressed mainly in the CNS, kidney, thymus, and gastrointestinal (GI) tract (31). Both endotoxin which is used for inducing MIA, and inflammatory mediators generated in the MIA model (interleukin-1ß (IL-1ß), IL-6, tumor necrosis factor-α (TNF-α), type 1interferons (IFNs), and AA), induce COX-2 (31, 32). In addition, evidence suggests that COX-2 mediates N-methyl-D aspartate (NMDA) neurotoxicity (33).
2) *COX2 and ASD*: In the previous studies that used peripheral blood monocytes from ASD subjects, as surrogates of microglial cells, increased production of the above-described cytokines in response to innate immune stimuli were observed (34). Such increases occur in ASD subjects that have a history of fluctuating behavioral symptoms and cognitive functioning following microbial infection (34). Interestingly, COX-2 and PGE2 were reported to be elevated in plasma of ASD subjects along with lower levels of α-synuclein (35). Therefore, blocking COX-2 may have the potential to attenuate neuroinflammation and subsequent neuronal damage in ASD subjects. On the other hand, up-regulation of COX-2 in the brain may be neuroprotective, partly regulating blood flow in the brain (31). It is of note that major adverse reactions associated with COX-2 inhibitors are cardiovascular events (36).

Clinical trial data of COX-2 inhibitors in ASD subjects has been scant. Only one randomized, double-blind, placebo-controlled trial addressed the efficacy of celecoxib, a COX-2 inhibitor, on behavioral symptoms in ASD subjects when celecoxib was given as an adjuvant treatment for risperidone administered for 10 weeks; behavioral symptoms were evaluated with the use of the aberrant behavior checklist (ABC) (37). The authors report statistically significant improvement in ABC subscales of irritability, lethargy, and stereotypy (37). In the author's clinic, attenuation of behavioral symptoms by celecoxib, a COX2 inhibitor, were also observed frequently when celecoxib was administered for controlling exacerbation of ASD behavioral symptoms, following viral syndromes, like influenza. Pioglitazone exerts various anti-inflammatory effects including suppression of COX-2 expression on microglial cells (38). Beneficial effects of pioglitazone have been reported in traumatic brain injury (38). In one study, pioglitazone is also reported to have attenuated ASD behavioral symptoms (39).

In summary, COX-2 inhibitors may be beneficial for ASD subjects with evidence of COX2 activation, especially in the acute and/or subacute stages. However, a caution is necessary for its long-term use.

2.2 Tryptophan metabolism

1) *Kynurenine pathway*: Tryptophan is an essential amino acid and a precursor of the metabolites that affect the functioning of multiple organs, including the brain. In the immune system, most tryptophan is mainly metabolized in the kynurenine pathway (40, 41). Multiple cytokines generated by innate immune responses including type 1 IFNs, IL-1ß, and TNF-α, activate rate-limiting enzymes of indoleamine 2,3-dioxygenases (IDO1/IDO2) in the kynurenine pathway (40). Most cells including immune cells express IDO1 and this enzyme often remains up-regulated in chronic inflammation. Tryptophan depletion caused by IDO activation and accumulation of kynurenine pathway metabolites result in immunosuppression, partly through facilitating induction of regulatory T (Treg) cells (40–43). However, metabolites of the kynurenine pathway can also cause toxic effects on the brain and such toxic effects are implicated in the pathogenesis of neuropsychiatric conditions such as schizophrenia (42). Therefore, in the acute and subacute stages of

neuroinflammation, modulating IDO activity may provide protection against neuroinflammation.

2) *Serotonin pathway*: Another pathway of tryptophan metabolism is the serotonin pathway, in which tryptophan is metabolized to serotonin (5-hydroxytryptophan, 5-HT). 5-HT is mainly produced by enterochromaffin cells in the gut, but it is also produced in the brain (40, 42). 5-HT is then metabolized to melatonin, a circadian hormone regulating sleep (42). Serotonin produced in the gut conveys signals to intestinal neurons, affecting various functions of the GI tract (42). 5-HT production in the gut is greatly affected by the gut microbiome (44). Both kynurenine and 5-HT pathways compete each other, and subtle changes in tryptophan metabolism are expected to affect the functions of multiple organs.

3) *Potential effects of maladapted IIM and Tryptophan metabolism on neuroinflammation*: Tryptophan metabolized by IDO1/2 will be metabolized to kynurenic acid (KYNA), which is thought to be neuroprotective, by exerting antagonistic effects on NMDA. On the other hand, quinolinic acid (QUIN), a down-stream metabolite of KYNA, activates NMDA receptors (41, 45). QUIN produced by microglial cells, is implicated in the pathogenesis of neuropsychiatric diseases such as depression (45, 46). In contrast, in the disorders of dopaminergic transmission like schizophrenia, excessive KYNA may be harmful, causing down-regulation of NMDAR signaling (45). In addition, general immunosuppressive actions exerted by activation of the kynurenine pathway may cause chronic immunosuppression through facilitating differentiation of Treg cells (42). Excessive actions of Treg cells make subjects vulnerable to recurrent infection and more susceptible to malignancy.

Changes in kynurenine pathway metabolites have also been reported in those diagnosed with ASD. For example, increased urinary concentrations of neurotoxic tryptophan metabolites were reported in ASD subjects (47). Polymorphism of NMDAR subunits, target molecules of kynurenine metabolites, have also been reported in subjects with ASD (48), along with altered levels of other tryptophan metabolites (49, 50).

High circulating levels of 5-HT, which essentially reflects 5-HT produced in the gut and stored in platelets, have been reported in about one third of ASD subjects (51). Changes in 5-HT levels may be associated with changes in gut serotonin metabolism and/or changes in clearance of 5-HT from both the liver and the lung (52). However, associations between hyperserotonemia and characteristic ASD behavioral symptoms have not been consistently shown (52). Likewise, selective serotonin reuptake inhibitors (SSRIs) that inhibit the actions of the serotonin reuptake transporter (SERT) do not exert universally beneficial effects on those diagnosed with ASD. These findings indicate that there are complex underlying mechanisms at play. Interestingly, an analysis of principal pathogenetic components and biological endophenotypes including serotonin blood levels, identified associations with immune dysfunction in ASD subjects (53). In the same study population, the immune component provided the largest contributions to phenotypic variance (54); these results support the effects of the immune activation on serotonin metabolism in ASD subjects. Notable findings from studies addressing changes in serotonin metabolites in ASD subjects at molecular levels are summarized below:

2.2.1 SERT polymorphism

Since tryptophan is a highly charged molecule, it requires SERT, an active transporter, that brings 5-HT through cell membranes and the transported 5-HT is then inactivated by monoamine oxidase (18). The modulation of actions of SERT impacts the development of the centric and enteric nervous systems (52, 55). The gene Slc6a4, which encodes SERT, has been the focus of intensive research, examining its association with the pathogenesis of common neuropsychiatric conditions. A finding of a gain of function (GOF) pathogenic variant of the SERT gene led to the development of an animal model of ASD, 'SERT Ala56 mouse' (56). In this murine ASD model, the GOF SERT gene mutation causes excessive activation of p38MAPK (57), and resultant hyper-clearance of 5-HT and hyper-sensitivity to 5-HT receptors in the nervous system (56). The GOF SERT mutation causes hyperserotonemia as was observed in SERT knockout mice (55, 56). Altered levels of 5-HT in both the CNS and gut, have also been reported in the MIA, a rodent model of autism (58). In ASD subjects with frequent activation of innate immunity through immune insults, changes in tryptophan metabolism are likely to occur, affecting or exacerbating pre-existing neuropsychiatric symptoms. This is partly because IL-1β and other inflammatory cytokines generated by innate immune responses activate both IDO1 and SERT (41, 42, 56, 57).

2.2.2 Effects of minocycline on tryptophan metabolism

One of the immunomodulating agents that can affect the kynurenine pathway is minocycline. This second-generation tetracycline antibiotic exerts anti-inflammatory and immunoregulatory actions partly through suppressing cytokine production and microglial cell activation. Minocycline's unique neuroprotective effects have been partly attributed to its direct suppression of IDO1 in the presence of retinoic acid (vitamin A): IDO inhibition by minocycline results in reduced productions of neurotoxic tryptophan metabolites (59). The neuroprotective effects of minocycline are also thought to be associated with its inhibitory effects on GSK3ß (60). Neuroprotective effects of minocycline are best demonstrated in neuroinflammation caused by reperfusion injury (61). In the animal model of white matter disease induced by intracerebral hemorrhage, protective effects of minocycline are attributed to its suppressive action on MAPK signaling mediated by TGF-ß (62). Neuroprotective effects of minocycline has also been shown to improve stress-induced behavioral changes (60).

Although the effects of minocycline appear promising in animal models and in other medical conditions, the therapeutic effects of minocycline on ASD have not been consistently shown. Recent meta-analysis of minocycline as treatment for neuropsychiatric conditions revealed beneficial effects of minocycline on schizophrenia, favoring its use as an adjunctive treatment (63). As for ASD, only one randomized, double-blind, placebo-controlled trial of minocycline evaluated its effects as an adjunctive treatment to risperidone (64). In this study, 46 medication-naïve ASD children were treated for 10 weeks with risperidone plus placebo or risperidone plus minocycline (N=23 in each group). Authors reported significant improvement in scores of ABC hyperactivity and irritability subscales, but no effect on inappropriate speech, lethargy, and stereotypy ABC subscales (64).

The above-described findings on the use of minocycline may be partly attributed to the complex effects that multiple genetic and environmental factors have on tryptophan metabolism. It is also possible that prolonged use of minocycline may block the immunosuppressive effects of tryptophan metabolites of KYNA and Treg cells, subsequently increasing the risk of autoimmune conditions. Minocycline may be effective for specific conditions when intricate homeostasis of tryptophan metabolites is impaired, resulting in worsening toxic effects of kynurenine metabolites. Careful selection of ASD subjects will be required for applying minocycline as a treatment option for ASD subjects.

2.3 PI3K/Akt/mTOR signaling pathway

Pathogenic variants of multiple genes are known to impose a significant risk for developing ASD and are often associated with regulation of the signaling pathways. Hyperactivation of PI3K (phosphatidylinositol 3 kinase)/Akt (protein kinase B)/mTOR pathway is one of such pathways affected by ASD pathogenic variants (65). This pathway has been shown to exert a crucial role in brain development including corticogenesis and synaptogenesis (65).

1) *PI3K/Akt/mTOR pathway*: mTOR is expressed ubiquitously in all cells as two types of mTOR complexes (mTORC1 and mTORC2) and it plays a major role in cell proliferation and activation. Activation of mTORC1 leads to inhibition of autophagy and Treg cell differentiation (65, 66). mTORC1 responds to signals from nutrients, metabolites and growth factors and it controls cellular functions including energy metabolism and autophagy (66). mTORC2 is positively regulated by the TSC1/2 (tuberous sclerosis complex 1 and 2) and up-stream PI3K signaling. mTORC1 is positively regulated by Akt, and the Akt phosphorylation by mTORC2 result in activation of mTORC1. Such complex interactions are essential for brain morphogenesis, synaptic plasticity, and neuronal regeneration. Therefore, pathogenic variants of genes coding for proteins associated with the PI3K/Akt/mTOR pathway cause various neuropsychiatric and neurodegenerative conditions (66). In addition, activation of this pathway is implicated in the pathogenesis of epilepsy, as typically seen in patients diagnosed with tuberous sclerosis (TS) (67).
2) *ASD phenotype and mTOR signaling pathway*: Previous genetic studies for detecting candidate genes associated with ASD risk have identified several genes that are associated with PI-3K/Akt/mTOR signaling pathway; these include FMR1, PTEN, TSC1, and TSC2 (68). This is not surprising, since patients with pathogenic variants of these genes exhibit ASD phenotypes at high frequency (65). Additional evidence of the role of the PI-3K/Akt/mTOR pathway in ASD pathogenesis has emerged in the murine model of ASD generated by fetal exposure to valproic acid. In this model, multiple mechanisms appear to be involved for the development of the ASD phenotype (69). Involvement of the PI-3K/Akt/mTOR pathway is supported by the finding of improved behavioral symptoms with post-natal administration of rapamycin, an mTOR blocker that predominantly blocks mTORC1 (70). This protective action of rapamycin is partly attributed to prevention of impaired autophagy caused by the over-activation of the PI-3K/Akt/mTOR signaling (70). Others also reported a possible link between the increase in plasma levels of CCL5 (chemokine C-C motif ligand 5) in ASD subjects and activation of the mTOR signaling pathway (71).

The above-described findings may indicate that it is feasible to apply mTOR inhibitors in controlling ASD symptoms. However, mTOR inhibitors exert significant immunosuppressive actions and may not be readily applicable to general ASD population. Current available reports focus on its use in controlling seizure activity and other neurological manifestation in patients who are identified to carry pathogenic variants of TSC and PTEN (67, 72–74). The author reported one ASD case with treatment-resistant seizures for whom sirolimus (rapamycin) was successfully used as an adjunctive treatment to IVIg and anakinra for seizure control (75). In summary, mTOR inhibitors likely have a role in a subset of ASD subjects with identified genetic variants associated with PI-3K/Akt/mTOR signaling pathway and those with specific conditions such as treatment-resistant seizures.

It is also of note, that activation of the PI-3K/Akt/mTOR signaling pathway will impair differentiation of Treg cells as well as autophagy. In such conditions, agents that help recovering autophagic deficiency will provide therapeutic effects. One of such agents is N-acetylcysteine (NAC). NAC has been used as an inexpensive dietary supplements for treating glutathione deficiency associated with multiple medical conditions, by improving glutamatergic neurotransmission through glutathione synthesis (76). In addition, NAC is reported to improve autism like behavior by recovering autophagic deficiency and decreasing Notch-1/Hes-1 pathway activity in rodent models (77). Potential therapeutic effects on NAC on ASD behavioral symptoms (irritability and hyperactivity) have been shown by the recent meta-analysis of randomized controlled trials (8–12 weeks trials) (78) Therefore, NAC may be safely tried in ASD patients with evidence of activation of PI-3K/Akt/mTOR signaling pathway as an adjunct treatment.

2.4 Microbiome

In the previous sections, we have discussed the possible roles played by lipid metabolites, tryptophan metabolites, and mTOR signaling activation, in association with neuroinflammation observed in ASD. One factor that may affect all the above-described metabolisms and signaling pathways is the microbiome. Bidirectional communications between the CNS and gut microbiota, often referred as to gut-brain axis, have been studied extensively. Evidence supports a role for the microbiota in the pathogenesis of common neurodevelopmental disorders, including ASD (26). A role of microbiome in neurodevelopmental conditions has been reviewed by many others (79–82). Known key roles that microbiome plays in neuronal development in association with ASD pathogenesis are summarized below:

1) *The role of microbiota in brain development*: The role of microbiota in brain development has been extensively studied in the germ-free mice. Evidence supports its role in multiple neurodevelopmental processes that include maturation and functioning of microglial cells (83, 84). Microbiota implicated in the pathogenesis of impaired growth in preterm newborns were shown to cause up-regulation of markers of neuroinflammation in germ-free mice (85).
2) *The role of microbiome metabolites on the Gut-Brain Axis:* The gut microbiome produces various metabolites that not only affect the development of the gut immune system, but also affect the Gut-Brain Axis. For example, indigestible oligosaccharides are fermented in the colon, resulting in production of short chain fatty acids (SCFAs) which affect homeostasis of the Gut-Brain Axis (26, 86). This action is partly exerted through modulating tryptophan metabolism (44). Namely, the microbiome in the gut metabolizes tryptophan to tryptamine, indole, and indol-3-proprionic acid (IPA), and all these metabolites exert either activating or inhibitory actions on aryl hydrocarbon receptors (AHRs) (44), subsequently affecting the gut immune system, and the gut-brain axis.
3) *Abnormalities in the gut microbiome and their effects on the brain in ASD*: It has been shown that GI symptoms are one of the most common comorbid conditions, which is partly attributed to dysbiosis, as reviewed elsewhere (87). Changes in the gut microbiome in ASD subjects has been repeatedly shown by multiple authors (82). One study showed that transplanting gut microbiota from ASD patients to the germ-free mice induced ASD like behavioral symptoms (88). Butyrate (BT) is one of the major components of SCFAs. BT is thought to be neuroprotective, partly through suppressing inflammatory responses in the macrophage lineage cells in the gut (26). One study showed that bacterial taxa producing BT are lower in ASD subjects than controls (89). Others also reported that 4-ethylphenylsulfate (4-EPS), a toxic metabolite of the gut microbiota, which has been implicated in the pathogenesis of ASD behavioral symptoms, was high in the MIA model, a rodent model of autism (90). This was normalized by colonization of *Bacteroides Fragilis* (90). SCFAs are also known to affect the metabolism of tryptophan and will further affect the brain when an imbalance of SCFAs develop due to dysbiosis (87). In ASD subjects, reduced biodiversity of the microbiota has been reported which may be associated with SCFAs such as propionic acid in the gut (91, 92). Propionic acid has been implicated in the ASD pathogenesis with creation of the rat model of autism as a propionic acid-induced autism (93). In fact, both propionate and butyrate are known to modify the expression of numerous genes in neuronal cells and will impact their functions (94, 95).
4) *Intestinal Barrier Dysfunction and the Microbiota-Gut-Brain-Axis:* ASD subjects may be more easily affected by the changes in the gut microbiome secondary to their increased gut permeability, allowing increased flux of chemical mediators and even inflammatory cells from the gut (80). Based on findings in the propionic acid-induced animal model of autism, increased gut permeability is attributed to worsening ASD symptoms in certain conditions (96). Previous study also reported increase in the gut permeability at a higher frequency in ASD subjects than neurotypical controls (97).

As summarized above, abnormalities in the gut microbiome have been widely recognized in ASD. However, the treatment options to correct such abnormalities of microbiome have not been extensively tested. The study results may also be affected by marked heterogeneity of ASD subjects. The recent meta-analysis of the use of probiotics revealed overall favorable responses to probiotics in ASD subjects, but these data are generated in the studies with small numbers of subjects (82). It may be promising, but more information is needed to better understand which commensal floras are more beneficial and can aid in controlling ASD behavioral symptoms. The studies addressing effects of prebiotics, synbiotics, and a combination of prebiotics, probiotics, and synbiotics, yielded mixed and inconclusive results by recent meta-analysis (82). Likewise, butyrate, one of SCFAs, is reported to have anti-inflammatory and neuroprotective effects in animal models in BTBR mice (98) and the rat MIA model induced by maternal injection of LPS (99). However, there are no clinical trials of butyrate that have reproduced the favorable effects of butyrate seen in the preclinical data regarding ASD subjects.

The use of fecal microbiota transplant (FMT) for correcting dysbiosis in ASD patients has recently begun. Recent studies of FMT have yielded promising results: Kang, et al. reported improvement of GI symptoms and ASD behavioral symptoms following treatment of FMT for 8 weeks in 80% of participants (100). The same group also reported that in the follow-up of the 18 participants who were responsive to the FMT, improvement of GI and behavioral symptoms was maintained in most of the subjects

for 2 years after the completion of the FMT (101). Similar beneficial effects of FMT have also been reported by another group (102). The recent review of meta-analysis reported significant improvement in ABC as well as Child Autism Rating Scale scores following FMT (103), FMT may provide another treatment option in ASD subjects who exhibit evidence of chronic GI inflammation. However, it is also of note that in the published studies, ASD subjects with other notable co-morbid conditions such as seizure disorders have been excluded in these studies. However, in epilepsy, a potential effect of gut microbiome has been suspected (104). Epilepsy has become a major co-morbid condition in older ASD children and young adults suffering from ASD. FMT may eventually be expanded for treating ASD subjects with other serious co-morbid conditions associated with CNS inflammation such as seizures.

3 Immunomodulating agents utilized for autoimmune/autoinflammatory and post-infectious encephalopathy – potential applications to ASD subjects

Recently, more and more immunomodulating agents have been used for controlling neuroinflammation associated with autoimmune/autoinflammatory and/or chronic inflammatory conditions. Some of these agents have been used in ASD subjects under diagnosis of autoimmune and autoinflammatory conditions. However, except for corticosteroids and intravenous immunoglobulin (IVIg), most of these agents are not specifically used for targeting ASD subjects. In this section, immunomodulating agents that could be potentially applied for controlling neuroinflammation in ASD subjects will be discussed. The effects of corticosteroids and IVIg will be discussed first, since published studies for treating ASD subjects are available. Then other immunomodulating agents that have been used for controlling neuroinflammation in various conditions, but not specifically used for ASD subjects will be discussed. ASD subjects referred to the author's clinic have often been treated with these agents by other providers under diagnoses of various conditions. These include autoimmune encephalitis (AE), pediatric acute-onset neuropsychiatric syndrome (PANS), pediatric acute neuropsychiatric disorders associated with Streptococcal infection (PANDAS), and post-infectious inflammation associated with COVID-19, which is commonly referred as to long COVID. Since newly emerging immunomodulating agents exert potent actions with the possibility of even more hazardous side effects than described in the previous section, it will be necessary for clinicians to understand the underlying mechanisms of action and scientific rationale for their use. Nevertheless, it should be noted that these additional treatment options will be welcome to those ASD subjects who have difficulty in responding to the treatment measures described in section 2.

3.1 Corticosteroids

Corticosteroids (CS) have been used in numerous autoimmune and inflammatory conditions including conditions described in the previous paragraph. They exerts a wide variety of anti-inflammatory and immunosuppressive actions (105). Although CS provide quick symptomatic relief in autoimmune and inflammatory conditions, they are typically used in the initial stage aiming for inducing remission, secondary to significant side effects (105). CS have also been used for treating various psychiatric disorders associated with neuroinflammation (106). CS play pivotal roles in the stress responses mediated by the hypothalamic pituitary adrenal (HPA) axis. In some ASD subjects, altered or impaired function of the HPA axis has been reported (106, 107). Therefore, in certain conditions, CS may provide some therapeutic effects in ASD subjects.

There are two reports of randomized, placebo-controlled trials of CS on small numbers of ASD subjects. One study (108) tried 1 mg/kg/day prednisolone for 12 weeks as an add-on treatment to risperidone in 37 ASD subjects (single blinded): the authors report improvement of ABC subscale scores (irritability, hyperactivity, lethargy, and stereotypy) (108). Another study (109) used 1 mg/kg/dose prednisolone for 24 weeks and tapering off over 9 weeks (N=20 in the placebo group and N=18 in the trial group); authors report significant improvement of language scores in ASD subjects treated with prednisolone (109). However, in these studies, there was no careful evaluation for components of neuroinflammation in the ASD study subjects. Various case reports or case series reported some benefits of CS. However, CS is also known to cause significant short-term and long-term side effects. One of the concerning side effects of CS in ASD subjects is psychomotor agitation, often manifested as mood swings, especially with a high dose of CS (105). Therefore, CS may be a suitable measure for acute care, controlling the initial stage and/or acute exacerbation of neuroinflammation.

3.2 Intravenous immunoglobulin

In addition to providing functional antibodies, IVIg exerts a wide range of immunoregulatory actions, and has been used for treating autoimmune and post-infectious inflammatory conditions involving the CNS and/or peripheral nervous system. Such neurological conditions treated by IVIg include Guillain-Barré syndrome (GBS), chronic inflammatory demyelinating polyneuropathy (CIDP), AE, and PANS/PANDAS.

1) *Effects of IVIg on innate immunity*: A part of the anti-inflammatory effects of IVIg has been attributed to antibodies interacting with the activated form of complement 3 (C3b), blocking downstream cascade of complement activation, thereby inhibiting complement mediated inflammatory processes. This action of IVIg was shown to have prevented neuronal cell death in an animal model of stroke (110). IVIg is also known to inhibit the activation of innate immune cells [macrophage/monocyte lineage cells and dendritic cells (DCs)] through induction of Fas-mediated apoptosis and triggering the production of counter-regulatory cytokines. In addition, IVIg contains neutralizing antibodies that bind to cytokines produced by

innate immune cells (111). It also modifies both activating and inhibitory signaling through Fcγ receptors (111). Such effects of IVIg on Fcγ receptors are attributed to α2,6-sialic acid portion of immunoglobulins that exert actions via the DC-SIGN signaling in murine models (112, 113). However, it is unclear whether the same actions take place in human cells. IVIg has also been shown to suppress B cell activation/proliferation by agonistic binding to inhibitory cell surface receptors on B cells (111). Antibodies contained in IVIg have also been shown to directly neutralize pathogenic autoantibodies (111).

2) *Effects of IVIg on adaptive immunity*: IVIg has been shown to exert a regulatory effect on the balance on Treg and inflammatory-prone, type 17 T-helper (Th17) cell differentiation and function. In Kawasaki disease (KD) for which IVIg serves as the 1st line treatment measure, IVIg promotes Treg cell function/differentiation and suppresses the production of inflammatory cytokines by Th17 cells (114, 115). Effects of IVIg on the balance of Th1/Th17 and Treg cells has been reported in GBS and CIDP patients, as well (116, 117).

IVIg is reported to exert actions on many other immune cells, including neutrophils and NK cells. However, the actions of IVIg may vary depending on donor pool and how the product was prepared. This makes it difficult to assess the effects of IVIg on ASD subjects, whose degrees of neuroinflammation may also vary. Previous studies assessing the effects of IVIg on ASD children reveled mixed results (118, 119). In one double-blind, placebo-controlled crossover study, 12 ASD subjects without known immunodeficiency were given a single dose of IVIg. Authors report no improvement of clinical ratings as compared to controls treated with placebo (120). On the other hand, in 31 ASD children treated under diagnosis of AE, authors reported modest, but statistically significant improvement in subscale scores of ABC and SRS (social responsiveness scores) (121). In this study, AE diagnosis appears not based on the standard measures for seropositive AE. Instead, ASD subjects were recommended to have IVIg treatment, based on positive non-specific inflammatory markers. These results indicates that IVIg may be a feasible option for ASD subjects with clear evidence of antibody deficiency and/or evidence of immune-dysregulatory conditions that have been shown to benefit from the immunoregulatory actions of IVIg.

3.3 B cell targeted therapy

1) *Rituximab (B cell ablation therapy):* AE is a rare autoimmune condition affecting the CNS through the production on autoantibodies that attack neuronal cells (seropositive AE) and/or through cell mediated immunity (seronegative AE). AE is rare in all age groups and clinical presentation may vary markedly. In addition, presenting symptoms may overlap ASD behavioral symptoms. AE was initially reported in patients with antibodies against the NMDA receptor, which remains the leading cause of AE (122). Onset of AE symptoms may be rapid. However, in some cases, AE symptoms can be subtle and may have symptoms such as developmental regression, resembling developmental issues often seen in ASD (123). AE diagnosis requires extensive neuroimaging, analysis of cerebrospinal fluid (CSF) including autoantibodies associated with AE, and electroencephalogram (EEG) to support AE diagnosis. The possibility of misdiagnosing someone with AE with ASD exists, since there may be common underlying mechanisms associated with both AE and ASD (123). In addition to the agents commonly used to control the acute stage (steroid, plasmapheresis, and IVIg), other immunomodulating agents have been utilized to achieve long-term remission in AE. Rituximab has been frequently used as the 2nd line treatment measure for this purpose (124).

Rituximab is a chimeric monoclonal antibody targeting CD20 which is expressed predominantly on mature B cells. The therapeutic effects of rituximab are attributed to the deletion of antibody producing memory B cells, and also prevention on generation of new plasma cells (125). Long lived mature plasma cells which does not express CD20 will keep producing autoantibodies, so that autoantibody production, albeit low in degree, is expected to continue in AE patients (125, 126). Repopulation of deleted B lineage cells is reported to start to occur 26 weeks after the initial treatment (127). Thus, rituximab is typically given every 6 months in AE patients (127). Rituximab has also been used for treating refractory patients with PANDAS/PANS in select cases (128, 129). However, no double blinded, placebo-controlled trials have been reported in AE or PANS/PANDAS subjects. In the author's experience, there are ASD subjects treated with rituximab under diagnosis of AE, PANS, or PANDAS by other providers, but their responses appear to be mixed. Rituximab use may be limited to ASD patients for whom there is clear-cut evidence that autoantibodies or autoreactive B cells play a role in their comorbid medical conditions.

2) *Tocilizumab*: In AE patients without detectable autoantibodies (seronegative AE) or refractory to B cell targeted treatments, other immunomodulating agents that target other pathways have been tried. Blockers targeting IL-6 signaling have been used for treating AE, especially seronegative AE (130). Tocilizumab is a humanized monoclonal antibody (mAb) that targets soluble, and membrane attached IL-6 receptors (IL-6R) (130, 131). It blocks the function of IL-6, a pleotropic cytokine, that affects not only B cell differentiation, but also affects the function of T cells and innate immune cells (131). In a cohort study conducted at a single institution, tocilizumab was reported to have favorable effects in patients diagnosed with AE and refractory to rituximab (132). The same group reported better outcomes (better modified ranking scales) in the escalation treatment using tocilizumab, compared to controls: 60 out of 80 AE patients that were included in this study were categorized as

seronegative AE (133). It is unclear whether any reported studies included ASD subjects. Currently, no published data exists regarding trials of tocilizumab in ASD subjects diagnosed with AE or other autoimmune conditions. Our clinic has experienced some ASD subjects treated with tocilizumab elsewhere, but their responses appear to be mixed. It is unclear at this time which ASD subjects, if any, would benefit from tocilizumab, in the absence of good biomarkers.

3.4 Biologics and blockers of inflammasome signaling pathway

As blockers of inflammasome, colchicine and anakinra, IL-1ß blocker, have been extensively used for controlling autoinflammatory syndromes caused by pathogenic gene variants that render dysregulation inflammasome activation (134, 135). Colchicine and anakinra have also been used for treating multiple autoimmune and autoinflammatory conditions. Activation of innate immunity is often associated with activation of inflammasome signaling pathways and blockers of inflammasome may thus provide additional therapeutic options for ASD subjects who have evidence of innate immune abnormalities. Therapeutic utility of inflammasome blockers (colchicine and anakinra) have also been illustrated in patients with long COVID (136–139), since activation of type 1 IFN signaling pathway by sars-cov-2 leads to inflammasome activation (140). These agents are readily applicable for treating ASD subjects suffering from long COVID. Indeed, the author also observed favorable effects of colchicine and anakinra in ASD subjects suffering from long COVID in the author's clinic. The therapeutic actions of colchicine and anakinra are summarized below:

1) *Colchicine – an old medication for autoinflammatory conditions*: Colchicine is known to affect the actions of tubulins, which play key roles in chemotaxis and phagocytosis of innate immune cells (136). It also inhibits NLRP3, and subsequently blocks IL-1ß induced inflammasome activation and production of TNF-α and IL-6 (141, 142). Colchicine's action on neutrophils also inhibits neutrophil-platelet interactions, preventing thrombosis triggered by neutrophilic inflammation (142, 143). Given these actions, colchicine, an established medication commonly used for autoimmune/autoinflammatory conditions, is expected to be useful for controlling neutrophilic inflammation triggered by Th17 cells and/or innate immune cells. Since colchicine is a strong inhibitor of P450 3A4, it is necessary to evaluate all drugs that patients are already on for possible drug-interactions. Nevertheless, the above-described actions of colchicine appear promising for controlling COVID-19 induced neuroinflammation. Indeed, several studies reported decreased mortality in severe COVID-19 cases with the use of colchicine (136, 144). However, the current published reports of colchicine focus on its effects on cardiovascular conditions associated with long COVID, and little information is available regarding its actions on neuropsychiatric symptoms. The author has experienced favorable effects with the use of colchicine in ASD subjects suffering from long COVID, with improvement in behavioral symptoms shown by the ABC (145). However, at this time, there is no published data of a trial of colchicine in ASD subjects, with or without long COVID.
2) *Anakinra*: Anakinra, a soluble IL-1 receptor antagonist (IL-1Ra), is a recombinant product of human IL-1ra. It has been used for treating various autoimmune and autoinflammatory conditions (146). Following the COVID-19 pandemic, IL-1ß has been shown to be a key cytokine, causing cytokine storm and subsequent hyper-immune activation in severe COVID-19 cases (137, 139). These findings indicate that there is utility of anakinra for treating long COVID. One study reported marked increase in spontaneous production of IL-1ß from NLRP3 inflammasome in severe COVID-19 cases and subsequent favorable responses to anakinra (138). IL-1ß is also implicated in the pathogenesis of epilepsy associated with neuroinflammation and this prompted the use of anakinra for treatment of refractory seizure disorders, since anakinra is a small molecule that can pass through the intact blood brain barrier (BBB) (147, 148). The author has experienced favorable effects with anakinra in patients with refractory seizure disorder as an adjunctive treatment (75). IL-1ß has also been implicated in the pathogenesis of MIA, as described in the Introduction section. In animal models of chorioamnionitis induced by Group B streptococcal infection, neurobehavioral impairment was attenuated by an IL-1ß blockade by anakinra (149). Anakinra may be a reasonable therapeutic option for ASD subjects suffering from refractory seizures, long COVID, and autoinflammatory conditions refractory to the 1st intervention measures.

3.5 Blockers of mTOR pathways

As described earlier, candidate genes implicated in the pathogenesis of ASD include those associated with PI-3K/Akt/mTOR signaling pathway (65, 66). Importance of this signaling pathway was illustrated in patients with TS, and mTOR inhibitors have been used for controlling refractory seizures in TS patients (67). Given the proposed roles of the PI-3K/Akt/mTOR signaling pathway in ASD pathogenesis, mTOR inhibitors may also have favorable effects on neurodevelopment or cognitive functioning. In the rodent model of haplo-insufficient TS complex (TSC), everolimus, a mTOR inhibitor that was developed for TS seizure control, was reported to have attenuated impairment of social deficits (150). Such favorable effects of mTOR inhibitors were also shown in the rodent model of ASD created by silencing the *Cntnap2* gene, thereby causing hyperactivation of the Akt-mTOR signaling (151).

Hyperactivation of PI-3K/Akt/mTOR signaling pathway has also been reported in patients with COVID-19. Blockers of this signaling pathway has also been proposed as possible therapeutic

options for severe COVID-19 (152). It was proposed that mTOR inhibitor can exert favorable therapeutic effects on severe COVID-19 patients by augmenting autophagy along with inhibiting viral replication (153, 154). However, at this time, there is no clinical trials of mTOR inhibitors in ASD subjects without identified mutations of PI-3K/Akt/mTOR pathway. In ASD subjects with clear evidence of hyperactivation of this signaling pathway and/or in those with long COVID refractory to other measures, inhibitors targeting this pathway may provide an additional treatment option (155, 156). Likewise, there are no data of a trial of mTOR inhibitors in ASD subjects without pathogenic mutations of PI-3K/Akt/mTOR pathways. ASD with treatment resistant seizures may benefit from the use of mTOR inhibitors.

3.6 Blockers of type 1 IFN signaling and downstream signaling

As summarized in the introduction section, epigenetic changes in innate immunity can cause prolonged effects, which are now referred as to IIM (8, 157). Previous studies reported by the author and her colleagues revealed evidence of on-going innate immune abnormalities associated with altered IIM in some ASD subjects (158–160). One of the innate immune pathways triggered by various viruses including sars-cov-2 is the type 1 IFN signaling pathways. Induction of dysregulated IIM by SARS-CoV-2 may partly explain the long term sequelae of long COVID (161).

Patients with primary immunodeficiency caused by excessive production of type 1 IFNs called as interferonopathies, present with autoinflammatory and subsequent autoimmune conditions (162). These patients frequently present with neuropsychiatric symptoms. Long COVID patients often reveal neuropsychiatric symptoms and neurological deficits (163, 164). In animal models of long COVID, persistent neuroinflammation has been shown to affect multiple neuronal cells, including microglial cells which appear to play a crucial role in COVID induced persistent neuroinflammation (164, 165). AE like symptoms associated with COVID-19 have also been reported (166, 167). In these patients described above, neuroinflammation is expected to be better managed by immunomodulating agents that target signaling pathways activated by type 1 IFNs. It is also of note that type 1 signaling pathways and resultant Th17 cell activation have been implicated in the pathogenesis of various autoimmune conditions as seen in patients diagnosed with interferonopathies (162). Key down-stream signaling molecules in this pathway are Janus kinases (JAK). In fact, JAK inhibitors have emerged for controlling the above described autoimmune/autoinflammatory conditions triggered by type 1 IFN signaling (168, 169). In this section, JAK inhibitors and Th17 cell targeted treatment measures will be discussed.

1) *JAK inhibitors*: Janus kinases (JAKs) act as signal transducers and 4 mammalian members are identified: JAK1, JAK2, JAK3, and TYK2 (170, 171). All JAKs, except for JAK3, which is expressed only in hematopoietic and lymphoid cells, are expressed ubiquitously, and play a crucial role in the JAK-STAT (signal transducers and activators of the transcription) pathways (171). The JAK-STAT pathway transduces signaling from multiple cytokine receptors. JAK3 mediates signals from type 1 cytokines and thus deficiency of JAK3 lead to severe combined immunodeficiency (SCID) (172). On the other hand, dysregulated activation of the JAK-STAT pathway will lead to chronic inflammatory conditions, implicated in autoimmune, allergic, and autoinflammatory conditions (173). In patients with interferonopathies, type 1 IFN signaling involving JAK1/JAK2 may be therapeutic targets for these patients (162). Likewise, cytokines noted to be up-regulated in patients with severe COVID-19 utilize the JAK/STAT pathway and levels of these cytokines are reported to be positively associated with the disease outcome/mortality of COVID-19 (174). Therefore, JAK inhibitors have been used for treating COVID-19 as well (170).

   Baricitinib, a JAK1/JAK2 inhibitor, has been used for treating severe COVID-19 cases requiring hospitalization. In a randomized, double-blind, placebo-controlled trial for severe COVID-19 patients, baricitinib was reported to have caused less adverse reactions when used as an adjunctive therapy to remdesivir and dexamethasone (175). Multiple open-label studies have also reported beneficial effects of baricitinib for treating severe COVID-19 (176). Similar beneficial effects of tofacitinib, a JAK3/1 inhibitor, was reported in severe COVID-19 cases (177, 178). Beneficial effects of ruxolitinib, a JAK1/2 inhibitor, have also been reported in severe COVID-19 cases (179). With the increase in reports of the favorable effects of baricitinib, FDA issued the EUA (emergency use authorization) for its use as an adjunct treatment with remdesivir for the treatment of hospitalized COVID-19 patients older than 2 years of age. Newly available JAK1/2 inhibitors (upadacitinib and abrocitinib) may even be more effective for controlling type 1 IFN induced immune activation (180, 181).

   There are no reports of clinical trials of JAK inhibitors in ASD patients. In ASD subjects with pre-existing immunodysregulatory conditions involving the JAK/STAT pathways, JAK inhibitor may provide an additional treatment option for controlling neuropsychiatric symptoms, especially in those suffering from long COVID. Oral intake and the acceptable safety profiles of JAK inhibitors may make it an easier option for ASD subjects (168, 169).

2) *Th17 cell targeted therapy*: Type 17 T-helper (Th17) cells differentiate from pluripotent T cells through induction of Th17 specific transcription factor RORγt (RAR related orphan receptor-γt) which signals through the STAT3/JAK3 pathway, rendering Th17 cell differentiation in the presence of IL-1ß, IL-6 and TGF-ß (182). RORγt augments expression of IL-17 and IL-23 receptors and

IL-23 produced by innate immune cells binds to IL-23R, further augmenting induction of RORγt and IL-17 (183). Although IL-23 does not induce Th17 differentiation, it stabilizes Th17 cells. Th17 cells are known to exert a major defense against fungal pathogens through activation of neutrophils, but dysregulation of IL-23/IL-17 axis has been implicated in the pathogenesis of various autoimmune and inflammatory conditions (184, 185). Immunomodulating agents targeting the IL-17/IL-23 axis have been successfully used for treating multiple autoimmune conditions (185).

After the onset of the COVID-19 pandemic, marked activation of the IL-17/IL-23 axis following COVID-19 has become also apparent (186–189). This may also be the results of an imbalance in regulatory T (Treg) and Th17 cells during the acute stage of COVID-19 as well as in long COVID (190). Restoring the Treg/Th17 balance with the use of a Treg cell inducing cytokine (IL-2) or IL-17 inhibitors may be a possible treatment option for long COVID patients (190). There are report of increase in serum levels of IL-17 along with IL-2 in long COVID patients (191). JAK inhibitors inhibit STAT mediated signaling of IL-17 inducing cytokines, as well as Th17 produced cytokines. Therefore, JAK inhibitors may also exert beneficial effects by suppressing Th17 differentiation/functions in ASD subjects suffering from autoimmune conditions or chronic inflammatory conditions like long COVID. Immunomodulating agents targeting Th17/IL-23 axis have been developed for controlling IBD and skin inflammatory conditions like plaque psoriasis (192, 193). ASD subjects are known to suffer from chronic GI symptoms (16). In addition, skin pruritus/discomfort are expected to cause worsening behavioral symptoms (194). ASD subjects suffering from the above-described medical conditions are likely to benefit from medications targeting IL-17/IL-23 axis. There are no reports of Th17 targeted therapies tried specifically in ASD subjects. However, the author experienced that several ASD subjects treated with Th17/IL-23 targeted therapies (ustekinumab, secukinumab, and risankizumab) for co-morbid conditions, as described above, revealed beneficial effects on their ASD behavioral symptoms. ASD subjects suffering from long COVID may also benefit from these medications. However, it will be necessary to carefully select ASD subjects for a trial using inhibitors of the IL-17/IL-23 axis.

4 Conclusions

In this review, the author discussed various immunomodulating agents as possible treatments for neuroinflammation in ASD subjects based on solid scientific rationale and clinical trial data that were available at the time of preparation of this manuscript. In addition, this review also discussed the potential use of emerging immunomodulating agents including biologics. These agents were developed in recent years and many new agents are now in the pipeline. These agents may provide additional treatment options for ASD subjects. However, published data of clinical trials in ASD subjects are scant for emerging biologics and other immunomodulating agents. Therefore, discussion regarding scientific rationale for biologics for this review was based on pre-clinical studies (animal models), and results of studies from other medical conditions. It cannot be emphasized enough that ASD subjects are markedly heterogenous, and therefore, careful evaluation must take place for assessing treatment options of immunomodulating agents based on clinical and laboratory findings. In addition, it is imperative for medical providers to have a good understanding of the mechanisms of actions for these agents in order to provide optimal treatment measures safely.

Author contributions

HJ: Conceptualization, Funding acquisition, Validation, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work is partly funded by the Jonty Foundation, St. Paul, MN, the Brain Foundation, Pleasanton, CA, and O'Sullivan Foundation, Princeton, NJ.

Acknowledgments

The author was thankful for Dr. L. Huguienin for critically reviewing the manuscript.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Zawadzka A, Cieslik 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. doi: 10.3390/ijms222111516

2. Ornoy A, Weinstein-Fudim L, Ergaz Z. Prenatal factors associated with autism spectrum disorder (ASD). *Reprod Toxicol*. (2015) 56:155–69. doi: 10.1016/j.reprotox.2015.05.007

3. Massrali A, Adhya D, Srivastava DP, Baron-Cohen S, Kotter MR. Virus-induced maternal immune activation as an environmental factor in the etiology of autism and schizophrenia. *Front Neurosci*. (2022) 16:834058. doi: 10.3389/fnins.2022.834058

4. 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. doi: 10.1002/ana.20315

5. Jiang HY, Xu LL, Shao L, Xia RM, Yu ZH, Ling ZX, et al. Maternal infection during pregnancy and risk of autism spectrum disorders: A systematic review and meta-analysis. *Brain Behav Immun*. (2016) 58:165–72. doi: 10.1016/j.bbi.2016.06.005

6. Hornig M, Bresnahan MA, Che X, Schultz AF, Ukaigwe JE, Eddy ML, et al. Prenatal fever and autism risk. *Mol Psychiatry*. (2018) 23:759–66. doi: 10.1038/mp.2017.119

7. Kwon HK, Choi GB, Huh JR. Maternal inflammation and its ramifications on fetal neurodevelopment. *Trends Immunol*. (2022) 43:230–44. doi: 10.1016/j.it.2022.01.007

8. Bekkering S, Dominguez-Andres J, Joosten LAB, Riksen NP, Netea MG. Trained immunity: reprogramming innate immunity in health and disease. *Annu Rev Immunol*. (2021) 39:667–93. doi: 10.1146/annurev-immunol-102119–073855

9. Montalvo-Martinez L, Cruz-Carrillo G, Maldonado-Ruiz R, Trujillo-Villarreal LA, Garza-Villarreal EA, Camacho-Morales A. Prenatal programing of motivated behaviors: can innate immunity prime behavior? *Neural Regener Res*. (2023) 18:280–3. doi: 10.4103/1673–5374.346475

10. Al B, Suen TK, Placek K, Netea MG. Innate (learned) memory. *J Allergy Clin Immunol*. (2023) 152:551–66. doi: 10.1016/j.jaci.2023.06.014

11. Smith SE, Li J, Garbett K, Mirnics K, Patterson PH. Maternal immune activation alters fetal brain development through interleukin-6. *J Neurosci*. (2007) 27:10695–702. doi: 10.1523/JNEUROSCI.2178–07.2007

12. Wu WL, Hsiao EY, Yan Z, Mazmanian SK, Patterson PH. The placental interleukin-6 signaling controls fetal brain development and behavior. *Brain Behav Immun*. (2017) 62:11–23. doi: 10.1016/j.bbi.2016.11.007

13. Mizuguchi M, Ohsawa M, Kashii H, Sato A. Brain symptoms of tuberous sclerosis complex: pathogenesis and treatment. *Int J Mol Sci*. (2021) 22. doi: 10.3390/ijms22136677

14. Hughes HK, Moreno RJ, Ashwood P. Innate immune dysfunction and neuroinflammation in autism spectrum disorder (ASD). *Brain Behav Immun*. (2023) 108:245–54. doi: 10.1016/j.bbi.2022.12.001

15. Usui N, Kobayashi H, Shimada S. Neuroinflammation and oxidative stress in the pathogenesis of autism spectrum disorder. *Int J Mol Sci*. (2023) 24. doi: 10.3390/ijms24065487

16. KhaChadourian V, Mahjani B, Sandin S, Kolevzon A, Buxbaum JD, Reichenberg A, et al. Comorbidities in autism spectrum disorder and their etiologies. *Transl Psychiatry*. (2023) 13:71. doi: 10.1038/s41398-023-02374-w

17. Madra M, Ringel R, Margolis KG. Gastrointestinal issues and autism spectrum disorder. *Child Adolesc Psychiatr Clin N Am*. (2020) 29:501–13. doi: 10.1016/j.chc.2020.02.005

18. Thion MS, Garel S. Microglial ontogeny, diversity and neurodevelopmental functions. *Curr Opin Genet Dev*. (2020) 65:186–94. doi: 10.1016/j.gde.2020.06.013

19. Komada M, Nishimura Y. Epigenetics and neuroinflammation associated with neurodevelopmental disorders: A microglial perspective. *Front Cell Dev Biol*. (2022) 10:852752. doi: 10.3389/fcell.2022.852752

20. Hu C, Li H, Li J, Luo X, Hao Y. Microglia: Synaptic modulator in autism spectrum disorder. *Front Psychiatry*. (2022) 13:958661. doi: 10.3389/fpsyt.2022.958661

21. Xiong Y, Chen J, Li Y. Microglia and astrocytes underlie neuroinflammation and synaptic susceptibility in autism spectrum disorder. *Front Neurosci*. (2023) 17:1125428. doi: 10.3389/fnins.2023.1125428

22. Zhou K, Han J, Wang Y, Xu Y, Zhang Y, Zhu C. The therapeutic potential of bone marrow-derived macrophages in neurological diseases. *CNS Neurosci Ther*. (2022) 28:1942–52. doi: 10.1111/cns.13964

23. Zhao D, Mokhtari R, Pedrosa E, Birnbaum R, Zheng D, Lachman HM. Transcriptome analysis of microglia in a mouse model of Rett syndrome: differential expression of genes associated with microglia/macrophage activation and cellular stress. *Mol Autism*. (2017) 8:17. doi: 10.1186/s13229-017-0134-z

24. Pecorelli A, Cervellati C, Cordone V, Hayek J, Valacchi G. Compromised immune/inflammatory responses in Rett syndrome. *Free Radic Biol Med*. (2020) 152:100–6. doi: 10.1016/j.freeradbiomed.2020.02.023

25. Wittrahm R, Takalo M, Marttinen M, Kuulasmaa T, Makinen P, Kemppainen S, et al. MECP2 Increases the Pro-Inflammatory Response of Microglial Cells and Phosphorylation at Serine 423 Regulates Neuronal Gene Expression upon Neuroinflammation. *Cells*. (2021) 10. doi: 10.3390/cells10040860

26. Nandwana V, Nandwana NK, Das Y, Saito M, Panda T, Das S, et al. The role of microbiome in brain development and neurodegenerative diseases. *Molecules*. (2022) 27. doi: 10.3390/molecules27113402

27. Yui K, Imataka G, Yoshihara S. Lipid-based molecules on signaling pathways in autism spectrum disorder. *Int J Mol Sci*. (2022) 23. doi: 10.3390/ijms23179803

28. Wong CT, Ahmad E, Li H, Crawford DA. Prostaglandin E2 alters Wnt-dependent migration and proliferation in neuroectodermal stem cells: implications for autism spectrum disorders. *Cell Commun Signal*. (2014) 12:19. doi: 10.1186/1478–811X-12–19

29. Wong CT, Bestard-Lorigados I, Crawford DA. Autism-related behaviors in the cyclooxygenase-2-deficient mouse model. *Genes Brain Behav*. (2019) 18:e12506. doi: 10.1111/gbb.12506

30. Rai-Bhogal R, Wong C, Kissoondoyal A, Davidson J, Li H, Crawford DA. Maternal exposure to prostaglandin E(2) modifies expression of Wnt genes in mouse brain - An autism connection. *Biochem Biophys Rep*. (2018) 14:43–53. doi: 10.1016/j.bbrep.2018.03.012

31. Sethi R, Gomez-Coronado N, Walker AJ, Robertson OD, Agustini B, Berk M, et al. Neurobiology and therapeutic potential of cyclooxygenase-2 (COX-2) inhibitors for inflammation in neuropsychiatric disorders. *Front Psychiatry*. (2019) 10:605. doi: 10.3389/fpsyt.2019.00605

32. Lampiasi N, Bonaventura R, Deidda I, Zito F, Russo R. Inflammation and the potential implication of macrophage-microglia polarization in human ASD: an overview. *Int J Mol Sci*. (2023) 24. doi: 10.3390/ijms24032703

33. Tian J, Kim SF, Hester L, Snyder SH. S-nitrosylation/activation of COX-2 mediates NMDA neurotoxicity. *Proc Natl Acad Sci U S A*. (2008) 105:10537–40. doi: 10.1073/pnas.0804852105

34. Jyonouchi H, Geng L. Associations between monocyte cytokine profiles and co-morbid conditions in autism spectrum disorders. *In Autism Spectr Disord -Profile Heterogeneity Etiological Core Outcome (Edited by M. Fitzeald)*. (2020), 1–16. doi: 10.5772/intechopen95548

35. El-Ansary A, Alhakbany M, Aldbass A, Qasem H, Al-Mazidi S, Bhat RS, et al. Alpha-Synuclein, cyclooxygenase-2 and prostaglandins-EP2 receptors as neuroinflammatory biomarkers of autism spectrum disorders: Use of combined ROC curves to increase their diagnostic values. *Lipids Health Dis*. (2021) 20:155. doi: 10.1186/s12944–021-01578–7

36. Chen W, Zhong Y, Feng N, Guo Z, Wang S, Xing D. New horizons in the roles and associations of COX-2 and novel natural inhibitors in cardiovascular diseases. *Mol Med*. (2021) 27:123. doi: 10.1186/s10020–021-00358–4

37. Asadabadi M, Mohammadi MR, Ghanizadeh A, Modabbernia A, Ashrafi M, Hassanzadeh E, et al. Celecoxib as adjunctive treatment to risperidone in children with autistic disorder: a randomized, double-blind, placebo-controlled trial. *Psychopharmacol (Berl)*. (2013) 225:51–9. doi: 10.1007/s00213–012-2796–8

38. Zamanian MY, Taheri N, Opulencia MJC, Bokov DO, Abdullaev SY, Gholamrezapour M, et al. Neuroprotective and anti-inflammatory effects of pioglitazone on traumatic brain injury. *Mediators Inflammation*. (2022) 2022:9860855. doi: 10.1155/2022/9860855

39. Boris M, Kaiser CC, Goldblatt A, Elice MW, Edelson SM, Adams JB, et al. Effect of pioglitazone treatment on behavioral symptoms in autistic children. *J Neuroinflamm*. (2007) 4:3. doi: 10.1186/1742–2094-4–3

40. Barik S. The uniqueness of tryptophan in biology: properties, metabolism, interactions and localization in proteins. *Int J Mol Sci*. (2020) 21. doi: 10.3390/ijms21228776

41. Savino R, Carotenuto M, Polito AN, Di Noia S, Albenzio M, Scarinci A, et al. Analyzing the potential biological determinants of autism spectrum disorder: from neuroinflammation to the kynurenine pathway. *Brain Sci*. (2020) 10. doi: 10.3390/brainsci10090631

42. Modoux M, Rolhion N, Mani S, Sokol H. Tryptophan metabolism as a pharmacological target. *Trends Pharmacol Sci*. (2021) 42:60–73. doi: 10.1016/j.tips.2020.11.006

43. Lemos H, Mohamed E, Ou R, McCardle C, Zheng X, McGuire K, et al. Co-treatments to boost IDO activity and inhibit production of downstream catabolites induce durable suppression of experimental autoimmune encephalomyelitis. *Front Immunol*. (2020) 11:1256. doi: 10.3389/fimmu.2020.01256

44. Roth W, Zadeh K, Vekariya R, Ge Y, Mohamadzadeh M. Tryptophan metabolism and gut-brain homeostasis. *Int J Mol Sci*. (2021) 22. doi: 10.3390/ijms22062973

45. Savitz J. The kynurenine pathway: a finger in every pie. *Mol Psychiatry*. (2020) 25:131–47. doi: 10.1038/s41380–019-0414–4

46. Bartoli F, Misiak B, Callovini T, Cavaleri D, Cioni RM, Crocamo C, et al. The kynurenine pathway in bipolar disorder: a meta-analysis on the peripheral blood levels of tryptophan and related metabolites. *Mol Psychiatry*. (2021) 26:3419–29. doi: 10.1038/s41380–020-00913–1

47. 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. doi: 10.1186/s13229–016-0109–5

48. Lee EJ, Choi SY, Kim E. NMDA receptor dysfunction in autism spectrum disorders. *Curr Opin Pharmacol*. (2015) 20:8–13. doi: 10.1016/j.coph.2014.10.007

49. Bilgic A, Abusoglu S, Sadic Celikkol C, Oflaz MB, Akca OF, Sivrikaya A, et al. Altered kynurenine pathway metabolite levels in toddlers and preschool children with autism spectrum disorder. *Int J Neurosci*. (2022) 132:826–34. doi: 10.1080/00207454.2020.1841187

50. Carpita B, Nardi B, Palego L, Cremone IM, Massimetti G, Carmassi C, et al. Kynurenine pathway and autism spectrum phenotypes: an investigation among adults with autism spectrum disorder and their first-degree relatives. *CNS Spectr*. (2022) 27:1–12. doi: 10.1017/S1092852922000840

51. Gabriele S, Sacco R, Persico AM. Blood serotonin levels in autism spectrum disorder: a systematic review and meta-analysis. *Eur Neuropsychopharmacol*. (2014) 24:919–29. doi: 10.1016/j.euroneuro.2014.02.004

52. Israelyan N, Margolis KG. Serotonin as a link between the gut-brain-microbiome axis in autism spectrum disorders. *Pharmacol Res*. (2018) 132:1–6. doi: 10.1016/j.phrs.2018.03.020

53. Sacco R, Curatolo P, Manzi B, Militerni R, Bravaccio C, Frolli A, et al. Principal pathogenetic components and biological endophenotypes in autism spectrum disorders. *Autism Res*. (2010) 3:237–52. doi: 10.1002/aur.151

54. Sacco R, Lenti C, Saccani M, Curatolo P, Manzi B, Bravaccio C, et al. Cluster analysis of autistic patients based on principal pathogenetic components. *Autism Res*. (2012) 5:137–47. doi: 10.1002/aur.1226

55. Tanaka M, Sato A, Kasai S, Hagino Y, Kotajima-Murakami H, Kashii H, et al. Brain hyperserotonemia causes autism-relevant social deficits in mice. *Mol Autism*. (2018) 9:60. doi: 10.1186/s13229–018-0243–3

56. Stilley SE, Blakely RD. Rare opportunities for insights into serotonergic contributions to brain and bowel disorders: studies of the SERT ala56 mouse. *Front Cell Neurosci*. (2021) 15:677563. doi: 10.3389/fncel.2021.677563

57. Robson MJ, Quinlan MA, Margolis KG, Gajewski-Kurdziel PA, Veenstra-VanderWeele J, Gershon MD, et al. p38alpha MAPK signaling drives pharmacologically reversible brain and gastrointestinal phenotypes in the SERT Ala56 mouse. *Proc Natl Acad Sci U S A*. (2018) 115:E10245–54. doi: 10.1073/pnas.1809137115

58. Knuesel I, Chicha L, Britschgi M, Schobel SA, Bodmer M, Hellings JA, et al. Maternal immune activation and abnormal brain development across CNS disorders. *Nat Rev Neurol*. (2014) 10:643–60. doi: 10.1038/nrneurol.2014.187

59. Clemens V, Regen F, Le Bret N, Heuser I, Hellmann-Regen J. Anti-inflammatory effects of minocycline are mediated by retinoid signaling. *BMC Neurosci*. (2018) 19:58. doi: 10.1186/s12868-018-0460-x

60. Wang W, Wang R, Xu J, Qin X, Jiang H, Khalid A, et al. Minocycline attenuates stress-induced behavioral changes via its anti-inflammatory effects in an animal model of post-traumatic stress disorder. *Front Psychiatry*. (2018) 9:558. doi: 10.3389/fpsyt.2018.00558

61. Naderi Y, Panahi Y, Barreto GE, Sahebkar A. Neuroprotective effects of minocycline on focal cerebral ischemia injury: a systematic review. *Neural Regener Res*. (2020) 15:773–82. doi: 10.4103/1673–5374.268898

62. Yang H, Gao XJ, Li YJ, Su JB, E TZ, Zhang X, et al. Minocycline reduces intracerebral hemorrhage-induced white matter injury in piglets. *CNS Neurosci Ther*. (2019) 25:1195–206. doi: 10.1111/cns.13220

63. Panizzutti B, Skvarc D, Lin S, Croce S, Meehan A, Bortolasci CC, et al. Minocycline as treatment for psychiatric and neurological conditions: A systematic review and meta-analysis. *Int J Mol Sci*. (2023) 24. doi: 10.3390/ijms24065250

64. Ghaleiha A, Alikhani R, Kazemi MR, Mohammadi MR, Mohammadinejad P, Zeinoddini A, et al. Minocycline as adjunctive treatment to risperidone in children with autistic disorder: A randomized, double-blind placebo-controlled trial. *J Child Adolesc Psychopharmacol*. (2016) 26:784–91. doi: 10.1089/cap.2015.0175

65. Thomas SD, Jha NK, Ojha S, Sadek B. mTOR signaling disruption and its association with the development of autism spectrum disorder. *Molecules*. (2023) 28. doi: 10.3390/molecules28041889

66. Deneubourg C, Ramm M, Smith LJ, Baron O, Singh K, Byrne SC, et al. The spectrum of neurodevelopmental, neuromuscular and neurodegenerative disorders due to defective autophagy. *Autophagy*. (2022) 18:496–517. doi: 10.1080/15548627.2021.1943177

67. Schubert-Bast S, Strzelczyk A. Review of the treatment options for epilepsy in tuberous sclerosis complex: towards precision medicine. *Ther Adv Neurol Disord*. (2021) 14:17562864211031100. doi: 10.1177/17562864211031100

68. Onore C, Yang H, Van de Water J, Ashwood P. Dynamic Akt/mTOR signaling in children with autism spectrum disorder. *Front Pediatr*. (2017) 5:43. doi: 10.3389/fped.2017.00043

69. Kuo HY, Liu FC. Pathophysiological studies of monoaminergic neurotransmission systems in valproic acid-induced model of autism spectrum disorder. *Biomedicines*. (2022) 10. doi: 10.3390/biomedicines10030560

70. Lieberman OJ, Cartocci V, Pigulevskiy I, Molinari M, Carbonell J, Broseta MB, et al. mTOR suppresses macroautophagy during striatal postnatal development and is hyperactive in mouse models of autism spectrum disorders. *Front Cell Neurosci*. (2020) 14:70. doi: 10.3389/fncel.2020.00070

71. Wang B, Qin Y, Wu Q, Li X, Xie D, Zhao Z, et al. mTOR signaling pathway regulates the release of proinflammatory molecule CCL5 implicated in the pathogenesis of autism spectrum disorder. *Front Immunol*. (2022) 13:818518. doi: 10.3389/fimmu.2022.818518

72. Saffari A, Brosse I, Wiemer-Kruel A, Wilken B, Kreuzaler P, Hahn A, et al. Safety and efficacy of mTOR inhibitor treatment in patients with tuberous sclerosis complex under 2 years of age - a multicenter retrospective study. *Orphanet J Rare Dis*. (2019) 14:96. doi: 10.1186/s13023–019-1077–6

73. Srivastava S, Jo B, Zhang B, Frazier T, Gallagher AS, Peck F, et al. A randomized controlled trial of everolimus for neurocognitive symptoms in PTEN hamartoma tumor syndrome. *Hum Mol Genet*. (2022) 31:3393–404. doi: 10.1093/hmg/ddac111

74. Mizuguchi M, Ikeda H, Kagitani-Shimono K, Yoshinaga H, Suzuki Y, Aoki M, et al. Everolimus for epilepsy and autism spectrum disorder in tuberous sclerosis complex: EXIST-3 substudy in Japan. *Brain Dev*. (2019) 41:1–10. doi: 10.1016/j.braindev.2018.07.003

75. Jyonouchi H, Geng L. Resolution of EEG findings and clinical improvement in a patient with encephalopathy and ESES with a combination of immunomodulating agents other than corticosteroids: A case report. *Epilepsy Behav Rep*. (2020) 14:100379. doi: 10.1016/j.ebr.2020.100379

76. Schwalfenberg GK. N-acetylcysteine: A review of clinical usefulness (an old drug with new tricks). *J Nutr Metab*. (2021) 2021:9949453. doi: 10.1155/2021/9949453

77. Zhang YH, Wang T, Li YF, Deng YN, He XL, Wang LJ. N-acetylcysteine improves autism-like behavior by recovering autophagic deficiency and decreasing Notch-1/Hes-1 pathway activity. *Exp Biol Med (Maywood)*. (2023) 248:966–78. doi: 10.1177/15353702231179924

78. 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) 55:196–206. doi: 10.1177/0004867420952540

79. Sterling KG, Dodd GK, Alhamdi S, Asimenios PG, Dagda RK, De Meirleir KL, et al. Mucosal immunity and the gut-microbiota-brain-axis in neuroimmune disease. *Int J Mol Sci*. (2022) 23. doi: 10.3390/ijms232113328

80. Dargenio VN, Dargenio C, Castellaneta S, De Giacomo A, Laguardia M, Schettini F, et al. Intestinal barrier dysfunction and microbiota-gut-brain axis: possible implications in the pathogenesis and treatment of autism spectrum disorder. *Nutrients*. (2023) 15. doi: 10.3390/nu15071620

81. Montagnani M, Bottalico L, Potenza MA, Charitos IA, Topi S, Colella M, et al. The crosstalk between gut microbiota and nervous system: A bidirectional interaction between microorganisms and metabolome. *Int J Mol Sci*. (2023) 24. doi: 10.3390/ijms241210322

82. Inchingolo AM, Patano A, Piras F, Mancini A, Inchingolo AD, Paduanelli G, et al. Interconnection between microbiota-gut-brain axis and autism spectrum disorder comparing therapeutic options: A scoping review. *Microorganisms*. (2023) 11. doi: 10.3390/microorganisms11061477

83. Dinan TG, Cryan JF. Gut instincts: microbiota as a key regulator of brain development, ageing and neurodegeneration. *J Physiol*. (2017) 595:489–503. doi: 10.1113/JP273106

84. Diaz Heijtz R, Wang S, Anuar F, Qian Y, Bjorkholm B, Samuelsson A, et al. Normal gut microbiota modulates brain development and behavior. *Proc Natl Acad Sci U.S.A*. (2011) 108:3047–52. doi: 10.1073/pnas.1010529108

85. Lu J, Lu L, Yu Y, Cluette-Brown J, Martin CR, Claud EC. Effects of intestinal microbiota on brain development in humanized gnotobiotic mice. *Sci Rep*. (2018) 8:5443. doi: 10.1038/s41598-018-23692-w

86. Sivaprakasam S, Prasad PD, Singh N. Benefits of short-chain fatty acids and their receptors in inflammation and carcinogenesis. *Pharmacol Ther*. (2016) 164:144–51. doi: 10.1016/j.pharmthera.2016.04.007

87. Murakami Y, Imamura Y, Kasahara Y, Yoshida C, Momono Y, Fang K, 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. doi: 10.3390/cells12071087

88. Sharon G, Cruz NJ, Kang DW, Gandal MJ, Wang B, Kim YM, et al. Human gut microbiota from autism spectrum disorder promote behavioral symptoms in mice. *Cell*. (2019) 177:1600–18.e17. doi: 10.1016/j.cell.2019.05.004

89. Liu S, Li E, Sun Z, Fu D, Duan G, Jiang M, et al. Altered gut microbiota and short chain fatty acids in Chinese children with autism spectrum disorder. *Sci Rep*. (2019) 9:287. doi: 10.1038/s41598-018-36430-z

90. Hsiao EY, McBride SW, Hsien S, Sharon G, Hyde ER, McCue T, et al. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell*. (2013) 155:1451–63. doi: 10.1016/j.cell.2013.11.024

91. Iglesias-Vazquez L, Van Ginkel Riba G, Arija V, Canals J. Composition of gut microbiota in children with autism spectrum disorder: A systematic review and meta-analysis. *Nutrients*. (2020) 12. doi: 10.3390/nu12030792

92. Macfabe D. Autism: metabolism, mitochondria, and the microbiome. *Glob Adv Health Med*. (2013) 2:52–66. doi: 10.7453/gahmj.2013.089

93. Dogan M, Albayrak Y, Erbas O. Torasemide improves the propionic acid-induced autism in rats: A histopathological and imaging study. *Alpha Psychiatry*. (2023) 24:22–31. doi: 10.5152/alphapsychiatry.2023.22975

94. Nankova BB, Agarwal R, MacFabe DF, La Gamma EF. Enteric bacterial metabolites propionic and butyric acid modulate gene expression, including CREB-dependent catecholaminergic neurotransmission, in PC12 cells–possible relevance to autism spectrum disorders. *PLoS One*. (2014) 9:e103740. doi: 10.1371/journal.pone.0103740

95. Mirzaei R, Bouzari B, Hosseini-Fard SR, Mazaheri M, Ahmadyousefi Y, Abdi M, et al. Role of microbiota-derived short-chain fatty acids in nervous system disorders. *BioMed Pharmacother*. (2021) 139:111661. doi: 10.1016/j.biopha.2021.111661

96. Matta SM, Hill-Yardin EL, Crack PJ. The influence of neuroinflammation in Autism Spectrum Disorder. *Brain Behav Immun*. (2019) 79:75–90. doi: 10.1016/j.bbi.2019.04.037

97. de Magistris L, Familiari V, Pascotto A, Sapone A, Frolli A, Iardino P, et al. Alterations of the intestinal barrier in patients with autism spectrum disorders and in their first-degree relatives. *J Pediatr Gastroenterol Nutr*. (2010) 51:418–24. doi: 10.1097/MPG.0b013e3181dcc4a5

98. Cristiano C, Hoxha E, Lippiello P, Balbo I, Russo R, Tempia F, et al. Maternal treatment with sodium butyrate reduces the development of autism-like traits in mice offspring. *BioMed Pharmacother*. (2022) 156:113870. doi: 10.1016/j.biopha.2022.113870

99. Wang X, Sun Z, Yang T, Lin F, Ye S, Yan J, et al. Sodium butyrate facilitates CRHR2 expression to alleviate HPA axis hyperactivity in autism-like rats induced by prenatal lipopolysaccharides through histone deacetylase inhibition. *mSystems*. (2023) 8:e0041523. doi: 10.1128/msystems.00415–23

100. Kang DW, Adams JB, Gregory AC, Borody T, Chittick L, Fasano A, et al. Microbiota Transfer Therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. *Microbiome*. (2017) 5:10. doi: 10.1186/s40168–016-0225–7

101. Kang DW, Adams JB, Coleman DM, Pollard EL, Maldonado J, McDonough-Means S, et al. Long-term benefit of Microbiota Transfer Therapy on autism symptoms and gut microbiota. *Sci Rep*. (2019) 9:5821. doi: 10.1038/s41598–019-42183–0

102. Li N, Chen H, Cheng Y, Xu F, Ruan G, Ying S, et al. Fecal microbiota transplantation relieves gastrointestinal and autism symptoms by improving the gut microbiota in an open-label study. *Front Cell Infect Microbiol*. (2021) 11:759435. doi: 10.3389/fcimb.2021.759435

103. Dossaji Z, Khattak A, Tun KM, Hsu M, Batra K, Hong AS. Efficacy of fecal microbiota transplant on behavioral and gastrointestinal symptoms in pediatric autism: A systematic review. *Microorganisms*. (2023) 11. doi: 10.3390/microorganisms11030806

104. Iannone LF, Gomez-Eguilaz M, De Caro C. Gut microbiota manipulation as an epilepsy treatment. *Neurobiol Dis*. (2022) 174:105897. doi: 10.1016/j.nbd.2022.105897

105. Williams DM. Clinical pharmacology of corticosteroids. *Respir Care*. (2018) 63:655–70. doi: 10.4187/respcare.06314

106. Dabbah-Assadi F, Handel R, Shamir A. What we know about the role of corticosteroids in psychiatric disorders; evidence from animal and clinical studies. *J Psychiatr Res*. (2022) 155:363–70. doi: 10.1016/j.jpsychires.2022.09.032

107. Taylor JL, Corbett BA. A review of rhythm and responsiveness of cortisol in individuals with autism spectrum disorders. *Psychoneuroendocrinology*. (2014) 49:207–28. doi: 10.1016/j.psyneuen.2014.07.015

108. Malek M, Ashraf-Ganjouei A, Moradi K, Bagheri S, Mohammadi MR, Akhondzadeh S. Prednisolone as adjunctive treatment to risperidone in children with regressive type of autism spectrum disorder: A randomized, placebo-controlled trial. *Clin Neuropharmacol*. (2020) 43:39–45. doi: 10.1097/WNF.0000000000000382

109. Brito AR, Vairo GPT, Dias A, Olej B, Nascimento OJM, Vasconcelos MM. Effect of prednisolone on language function in children with autistic spectrum disorder: a randomized clinical trial. *J Pediatr (Rio J)*. (2021) 97:22–9. doi: 10.1016/j.jped.2019.10.012

110. Arumugam TV, Tang SC, Lathia JD, Cheng A, Mughal MR, Chigurupati S, et al. Intravenous immunoglobulin (IVIG) protects the brain against experimental stroke by preventing complement-mediated neuronal cell death. *Proc Natl Acad Sci U.S.A*. (2007) 104:14104–9. doi: 10.1073/pnas.0700506104

111. Bayry J, Ahmed EA, Toscano-Rivero D, Vonniessen N, Genest G, Cohen CG, et al. Intravenous immunoglobulin: mechanism of action in autoimmune and inflammatory conditions. *J Allergy Clin Immunol Pract*. (2023) 11:1688–97. doi: 10.1016/j.jaip.2023.04.002

112. Kaneko Y, Nimmerjahn F, Ravetch JV. Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. *Science*. (2006) 313:670–3. doi: 10.1126/science.1129594

113. Anthony RM, Wermeling F, Karlsson MC, Ravetch JV. Identification of a receptor required for the anti-inflammatory activity of IVIG. *Proc Natl Acad Sci U.S.A*. (2008) 105:19571–8. doi: 10.1073/pnas.0810163105

114. Franco A, Touma R, Song Y, Shimizu C, Tremoulet AH, Kanegaye JT, et al. Specificity of regulatory T cells that modulate vascular inflammation. *Autoimmunity*. (2014) 47:95–104. doi: 10.3109/08916934.2013.860524

115. Wang Z, Xie L, Ding G, Song S, Chen L, Li G, et al. Single-cell RNA sequencing of peripheral blood mononuclear cells from acute Kawasaki disease patients. *Nat Commun*. (2021) 12:5444. doi: 10.1038/s41467–021-25771–5

116. Maddur MS, Rabin M, Hegde P, Bolgert F, Guy M, Vallat JM, et al. Intravenous immunoglobulin exerts reciprocal regulation of Th1/Th17 cells and regulatory T cells in Guillain-Barre syndrome patients. *Immunol Res*. (2014) 60:320–9. doi: 10.1007/s12026–014-8580–6

117. Zhang G, Wang Q, Song Y, Cheng P, Xu R, Feng X, et al. Intravenous immunoglobulin promotes the proliferation of CD4(+)CD25(+) Foxp3(+) regulatory T cells and the cytokines secretion in patients with Guillain-Barre syndrome in vitro. *J Neuroimmunol*. (2019) 336:577042. doi: 10.1016/j.jneuroim.2019.577042

118. Hafizi S, Tabatabaei D, Lai MC. Review of clinical studies targeting inflammatory pathways for individuals with autism. *Front Psychiatry*. (2019) 10:849. doi: 10.3389/fpsyt.2019.00849

119. Rossignol DA, Frye RE. A systematic review and meta-analysis of immunoglobulin G abnormalities and the therapeutic use of intravenous immunoglobulins (IVIG) in autism spectrum disorder. *J Pers Med*. (2021) 11. doi: 10.3390/jpm11060488

120. Niederhofer H, Staffen W, Mair A. Immunoglobulins as an alternative strategy of psychopharmacological treatment of children with autistic disorder. *Neuropsychopharmacology*. (2003) 28:1014–5. doi: 10.1038/sj.npp.1300130

121. Connery K, Tippett M, Delhey LM, Rose S, Slattery JC, Kahler SG, et al. Intravenous immunoglobulin for the treatment of autoimmune encephalopathy in children with autism. *Transl Psychiatry*. (2018) 8:148. doi: 10.1038/s41398–018-0214–7

122. Hardy D. Autoimmune encephalitis in children. *Pediatr Neurol*. (2022) 132:56–66. doi: 10.1016/j.pediatrneurol.2022.05.004

123. Whiteley P, Marlow B, Kapoor RR, Blagojevic-Stokic N, Sala R. Autoimmune encephalitis and autism spectrum disorder. *Front Psychiatry*. (2021) 12:775017. doi: 10.3389/fpsyt.2021.775017

124. Smets I, Titulaer MJ. Antibody therapies in autoimmune encephalitis. *Neurotherapeutics*. (2022) 19:823–31. doi: 10.1007/s13311–021-01178–4

125. Abulayha A, Bredan A, El Enshasy H, Daniels I. Rituximab: modes of action, remaining dispute and future perspective. *Future Oncol*. (2014) 10:2481–92. doi: 10.2217/fon.14.146

126. Gresa-Arribas N, Titulaer MJ, Torrents A, Aguilar E, McCracken L, Leypoldt F, et al. Antibody titres at diagnosis and during follow-up of anti-NMDA receptor encephalitis: a retrospective study. *Lancet Neurol*. (2014) 13:167–77. doi: 10.1016/S1474–4422(13)70282–5

127. Ellwardt E, Ellwardt L, Bittner S, Zipp F. Monitoring B-cell repopulation after depletion therapy in neurologic patients. *Neurol Neuroimmunol Neuroinflamm*. (2018) 5:e463. doi: 10.1212/NXI.0000000000000463

128. Frankovich J, Swedo S, Murphy T, Dale RC, Agalliu D, Williams K, et al. Clinical management of pediatric acute-onset neuropsychiatric syndrome: part II-use of immunomodulatory therapies. *J Child Adolesc Psychopharmacol*. (2017) 27:574–93. doi: 10.1089/cap.2016.0148

129. Krouse A, Li H, Krenzer JA, Rose WN. Plasmapheresis, rituximab, and ceftriaxone provided lasting improvement for a 27-year-old adult male with pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS). *Case Rep Psychiatry*. (2021) 2021:8697902. doi: 10.1155/2021/8697902

130. Yang J, Liu X. Immunotherapy for refractory autoimmune encephalitis. *Front Immunol*. (2021) 12:790962. doi: 10.3389/fimmu.2021.790962

131. Kang S, Tanaka T, Kishimoto T. Therapeutic uses of anti-interleukin-6 receptor antibody. *Int Immunol*. (2015) 27:21–9. doi: 10.1093/intimm/dxu081

132. Lee WJ, Lee ST, Moon J, Sunwoo JS, Byun JI, Lim JA, et al. Tocilizumab in autoimmune encephalitis refractory to rituximab: an institutional cohort study. *Neurotherapeutics*. (2016) 13:824–32. doi: 10.1007/s13311–016-0442–6

133. Lee WJ, Lee ST, Shin YW, Lee HS, Shin HR, Kim DY, et al. Rituximab and tocilizumab (T-SIRT) in anti-NMDAR encephalitis. *Neurotherapeutics*. (2021) 18:474–87. doi: 10.1007/s13311–020-00921–7

134. Moltrasio C, Romagnuolo M, Marzano AV. NLRP3 inflammasome and NLRP3-related autoinflammatory diseases: From cryopyrin function to targeted therapies. *Front Immunol*. (2022) 13:1007705. doi: 10.3389/fimmu.2022.1007705

135. Soriano A, Soriano M, Espinosa G, Manna R, Emmi G, Cantarini L, et al. Current therapeutic options for the main monogenic autoinflammatory diseases and PFAPA syndrome: evidence-based approach and proposal of a practical guide. *Front Immunol*. (2020) 11:865. doi: 10.3389/fimmu.2020.00865

136. Reyes AZ, Hu KA, Teperman J, Wampler Muskardin TL, Tardif JC, Shah B, et al. Anti-inflammatory therapy for COVID-19 infection: the case for colchicine. *Ann Rheum Dis*. (2021) 80:550–7. doi: 10.1136/annrheumdis-2020–219174

137. Potere N, Del Buono MG, Caricchio R, Cremer PC, Vecchie A, Porreca E, et al. Interleukin-1 and the NLRP3 inflammasome in COVID-19: Pathogenetic and therapeutic implications. *EBioMedicine*. (2022) 85:104299. doi: 10.1016/j.ebiom.2022.104299

138. Bertoni A, Penco F, Mollica H, Bocca P, Prigione I, Corcione A, et al. Spontaneous NLRP3 inflammasome-driven IL-1-beta secretion is induced in severe COVID-19 patients and responds to anakinra treatment. *J Allergy Clin Immunol*. (2022) 150:796–805. doi: 10.1016/j.jaci.2022.05.029

139. Khani E, Shahrabi M, Rezaei H, Pourkarim F, Afsharirad H, Solduzian M. Current evidence on the use of anakinra in COVID-19. *Int Immunopharmacol*. (2022) 111:109075. doi: 10.1016/j.intimp.2022.109075

140. Sodeifian F, Nikfarjam M, Kian N, Mohamed K, Rezaei N. The role of type I interferon in the treatment of COVID-19. *J Med Virol*. (2022) 94:63–81. doi: 10.1002/jmv.27317

141. Martinez GJ, Celermajer DS, Patel S. The NLRP3 inflammasome and the emerging role of colchicine to inhibit atherosclerosis-associated inflammation. *Atherosclerosis*. (2018) 269:262–71. doi: 10.1016/j.atherosclerosis.2017.12.027

142. Slobodnick A, Shah B, Krasnokutsky S, Pillinger MH. Update on colchicine, 2017. *Rheumatol (Oxford)*. (2018) 57:i4–i11. doi: 10.1093/rheumatology/kex453

143. Shah B, Allen N, Harchandani B, Pillinger M, Katz S, Sedlis SP, et al. Effect of colchicine on platelet-platelet and platelet-leukocyte interactions: a pilot study in healthy subjects. *Inflammation*. (2016) 39:182–9. doi: 10.1007/s10753–015-0237–7

144. Deftereos SG, Giannopoulos G, Vrachatis DA, Siasos GD, Giotaki SG, Gargalianos P, et al. Effect of colchicine vs standard care on cardiac and inflammatory biomarkers and clinical outcomes in patients hospitalized with coronavirus disease 2019: the GRECCO-19 randomized clinical trial. *JAMA Netw Open*. (2020) 3:e2013136. doi: 10.1001/jamanetworkopen.2020.13136

145. Jyonouchi H, Geng L, Rossignol DA, Frye RE. Long COVID syndrome presenting as neuropsychiatric exacerbations in autism spectrum disorder: insights for treatment. *J Pers Med*. (2022) 12. doi: 10.3390/jpm12111815

146. Dinoto A, Ferrari S, Mariotto S. Treatment options in refractory autoimmune encephalitis. *CNS Drugs*. (2022) 36:919–31. doi: 10.1007/s40263-022-00943-z

147. Costagliola G, Depietri G, Michev A, Riva A, Foiadelli T, Savasta S, et al. Targeting inflammatory mediators in epilepsy: A systematic review of its molecular basis and clinical applications. *Front Neurol*. (2022) 13:741244. doi: 10.3389/fneur.2022.741244

148. Yamanaka G, Ishida Y, Kanou K, Suzuki S, Watanabe Y, Takamatsu T, et al. Towards a treatment for neuroinflammation in epilepsy: interleukin-1 receptor antagonist, anakinra, as a potential treatment in intractable epilepsy. *Int J Mol Sci*. (2021) 22. doi: 10.3390/ijms22126282

149. Ayash TA, Vancolen SY, Segura M, Allard MJ, Sebire G. Protective effects of interleukin-1 blockade on group B streptococcus-induced chorioamnionitis and subsequent neurobehavioral impairments of the offspring. *Front Endocrinol (Lausanne)*. (2022) 13:833121. doi: 10.3389/fendo.2022.833121

150. Petrasek T, Vojtechova I, Klovrza O, Tuckova K, Vejmola C, Rak J, et al. mTOR inhibitor improves autistic-like behaviors related to Tsc2 haploinsufficiency but not following developmental status epilepticus. *J Neurodev Disord*. (2021) 13:14. doi: 10.1186/s11689–021-09357–2

151. Xing X, Zhang J, Wu K, Cao B, Li X, Jiang F, et al. Suppression of Akt-mTOR pathway rescued the social behavior in Cntnap2-deficient mice. *Sci Rep*. (2019) 9:3041. doi: 10.1038/s41598–019-39434–5

152. Basile MS, Cavalli E, McCubrey J, Hernandez-Bello J, Munoz-Valle JF, Fagone P, et al. The PI3K/Akt/mTOR pathway: A potential pharmacological target in COVID-19. *Drug Discovery Today*. (2022) 27:848–56. doi: 10.1016/j.drudis.2021.11.002

153. Khalid T, Hasan A, Fatima JE, Faridi SA, Khan AF, Mir SS. Therapeutic role of mTOR inhibitors in control of SARS-CoV-2 viral replication. *Mol Biol Rep*. (2023) 50:2701–11. doi: 10.1007/s11033–022-08188–1

154. Pereira G, Leao A, Erustes AG, Morais IBM, Vrechi TAM, Zamarioli LDS, et al. Pharmacological modulators of autophagy as a potential strategy for the treatment of COVID-19. *Int J Mol Sci*. (2021) 22. doi: 10.3390/ijms22084067

155. Sharma A, Mehan S. Targeting PI3K-AKT/mTOR signaling in the prevention of autism. *Neurochem Int*. (2021) 147:105067. doi: 10.1016/j.neuint.2021.105067

156. Sharma A, Bhalla S, Mehan S. PI3K/AKT/mTOR signalling inhibitor chrysophanol ameliorates neurobehavioural and neurochemical defects in propionic acid-induced experimental model of autism in adult rats. *Metab Brain Dis*. (2022) 37:1909–29. doi: 10.1007/s11011–022-01026–0

157. Tercan H, Riksen NP, Joosten LAB, Netea MG, Bekkering S. Trained immunity: long-term adaptation in innate immune responses. *Arterioscler Thromb Vasc Biol*. (2021) 41:55–61. doi: 10.1161/ATVBAHA.120.314212

158. Jyonouchi H, Geng L. Associations between monocyte and T cell cytokine profiles in autism spectrum disorders: effects of dysregulated innate immune responses on adaptive responses to recall antigens in a subset of ASD children. *Int J Mol Sci*. (2019) 20. doi: 10.3390/ijms20194731

159. Jyonouchi H, Geng L, Rose S, Bennuri SC, Frye RE. Variations in mitochondrial respiration differ in IL-1ss/IL-10 ratio based subgroups in autism spectrum disorders. *Front Psychiatry*. (2019) 10:71. doi: 10.3389/fpsyt.2019.00071

160. Jyonouchi H, Geng L, Toruner GA, Rose S, Bennuri SC, Frye RE. Serum microRNAs in ASD: association with monocyte cytokine profiles and mitochondrial respiration. *Front Psychiatry*. (2019) 10:614. doi: 10.3389/fpsyt.2019.00614

161. Mehandru S, Merad M. Pathological sequelae of long-haul COVID. *Nat Immunol*. (2022) 23:194–202. doi: 10.1038/s41590-021-01104-y

162. d'Angelo DM, Di Filippo P, Breda L, Chiarelli F. Type I interferonopathies in children: an overview. *Front Pediatr*. (2021) 9:631329. doi: 10.3389/fped.2021.631329

163. Fernandez-Castaneda A, Lu P, Geraghty AC, Song E, Lee MH, Wood J, et al. Mild respiratory COVID can cause multi-lineage neural cell and myelin dysregulation. *Cell*. (2022) 185:2452–2468.e16. doi: 10.1016/j.cell.2022.06.008

164. Monje M, Iwasaki A. The neurobiology of long COVID. *Neuron*. (2022) 110:3484–96. doi: 10.1016/j.neuron.2022.10.006

165. Urban P, Italiani P, Boraschi D, Gioria S. The SARS-coV-2 nucleoprotein induces innate memory in human monocytes. *Front Immunol*. (2022) 13:963627. doi: 10.3389/fimmu.2022.963627

166. Nabizadeh F, Balabandian M, Sodeifian F, Rezaei N, Rostami MR, Naser Moghadasi A. Autoimmune encephalitis associated with COVID-19: A systematic review. *Mult Scler Relat Disord*. (2022) 62:103795. doi: 10.1016/j.msard.2022.103795

167. Payus AO, Jeffree MS, Ohn MH, Tan HJ, Ibrahim A, Chia YK, et al. Immune-mediated neurological syndrome in SARS-CoV-2 infection: a review of literature on autoimmune encephalitis in COVID-19. *Neurol Sci*. (2022) 43:1533–47. doi: 10.1007/s10072-021-05785-z

168. Yamaoka K, Oku K. JAK inhibitors in rheumatology. *Immunol Med*. (2023) 46:143–52. doi: 10.1080/25785826.2023.2172808

169. Gupta N, Papasotiriou S, Hanauer S. The evolving role of JAK inhibitors in the treatment of inflammatory bowel disease. *Expert Rev Clin Immunol*. (2023) 19:1075–89. doi: 10.1080/1744666X.2023.2214728

170. Jain NK, Tailang M, Jain HK, Chandrasekaran B, Sahoo BM, Subramanian A, et al. Therapeutic implications of current Janus kinase inhibitors as anti-COVID agents: A review. *Front Pharmacol*. (2023) 14:1135145. doi: 10.3389/fphar.2023.1135145

171. Mortezavi M, Martin DA, Schulze-Koops H. After 25 years of drug development, do we know JAK? *RMD Open*. (2022) 8. doi: 10.1136/rmdopen-2022–002409

172. Mella P, Schumacher RF, Cranston T, de Saint Basile G, Savoldi G, Notarangelo LD. Eleven novel JAK3 mutations in patients with severe combined immunodeficiency-including the first patients with mutations in the kinase domain. *Hum Mutat*. (2001) 18:355–6. doi: 10.1002/humu.1199

173. Xin P, Xu X, Deng C, Liu S, Wang Y, Zhou X, et al. The role of JAK/STAT signaling pathway and its inhibitors in diseases. *Int Immunopharmacol*. (2020) 80:106210. doi: 10.1016/j.intimp.2020.106210

174. Hoang TN, Pino M, Boddapati AK, Viox EG, Starke CE, Upadhyay AA, et al. Baricitinib treatment resolves lower-airway macrophage inflammation and neutrophil recruitment in SARS-CoV-2-infected rhesus macaques. *Cell*. (2021) 184:460–47. e21. doi: 10.1016/j.cell.2020.11.007

175. Wolfe CR, Tomashek KM, Patterson TF, Gomez CA, Marconi VC, Jain MK, et al. Baricitinib versus dexamethasone for adults hospitalised with COVID-19 (ACTT-4): a randomised, double-blind, double placebo-controlled trial. *Lancet Respir Med*. (2022) 10:888–99. doi: 10.1016/S2213–2600(22)00088–1

176. Yuan Y, Jiao B, Qu L, Yang D, Liu R. The development of COVID-19 treatment. *Front Immunol*. (2023) 14:1125246. doi: 10.3389/fimmu.2023.1125246

177. Guimaraes PO, Quirk D, Furtado RH, Maia LN, Saraiva JF, Antunes MO, et al. Tofacitinib in patients hospitalized with Covid-19 pneumonia. *N Engl J Med*. (2021) 385:406–15. doi: 10.1056/NEJMoa2101643

178. Maslennikov R, Ivashkin V, Vasilieva E, Chipurik M, Semikova P, Semenets V, et al. Tofacitinib reduces mortality in coronavirus disease 2019 Tofacitinib in COVID-19. *Pulm Pharmacol Ther*. (2021) 69:102039. doi: 10.1016/j.pupt.2021.102039

179. Cao Y, Wei J, Zou L, Jiang T, Wang G, Chen L, et al. Ruxolitinib in treatment of severe coronavirus disease 2019 (COVID-19): A multicenter, single-blind, randomized controlled trial. *J Allergy Clin Immunol*. (2020) 146:137–46.e3. doi: 10.1016/j.jaci.2020.05.019

180. Mohamed MF, Bhatnagar S, Parmentier JM, Nakasato P, Wung P. Upadacitinib: Mechanism of action, clinical, and translational science. *Clin Transl Sci*. (2024) 17:e13688. doi: 10.1111/cts.13688

181. Le AM, Gooderham M, Torres T. Abrocitinib for the treatment of atopic dermatitis. *Immunotherapy*. (2023) 15:1351–62. doi: 10.2217/imt-2023–0057

182. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature*. (2006) 441:235–8. doi: 10.1038/nature04753

183. Bunte K, Beikler T. Th17 cells and the IL-23/IL-17 axis in the pathogenesis of periodontitis and immune-mediated inflammatory diseases. *Int J Mol Sci*. (2019) 20. doi: 10.3390/ijms20143394

184. Schinocca C, Rizzo C, Fasano S, Grasso G, La Barbera L, Ciccia F, et al. Role of the IL-23/IL-17 pathway in rheumatic diseases: an overview. *Front Immunol*. (2021) 12:637829. doi: 10.3389/fimmu.2021.637829

185. Jung SM, Kim WU. Targeted immunotherapy for autoimmune disease. *Immune Netw*. (2022) 22:e9. doi: 10.4110/in.2022.22.e9

186. De Biasi S, Meschiari M, Gibellini L, Bellinazzi C, Borella R, Fidanza L, et al. Marked T cell activation, senescence, exhaustion and skewing towards TH17 in patients with COVID-19 pneumonia. *Nat Commun*. (2020) 11:3434. doi: 10.1038/s41467–020-17292–4

187. Wang J, Li Q, Qiu Y, Lu H. COVID-19: imbalanced cell-mediated immune response drives to immunopathology. *Emerg Microbes Infect*. (2022) 11:2393–404. doi: 10.1080/22221751.2022.2122579

188. Choto TA, Makupe I, Cakana AZ, Sibanda EN, Mduluza T. Excessive neutrophil recruitment promotes typical T-helper 17 responses in Coronavirus disease 2019 patients. *PloS One*. (2022) 17:e0273186. doi: 10.1371/journal.pone.0273186

189. Parackova Z, Bloomfield M, Klocperk A, Sediva A. Neutrophils mediate Th17 promotion in COVID-19 patients. *J Leukoc Biol*. (2021) 109:73–6. doi: 10.1002/JLB.4COVCRA0820-481RRR

190. Dhawan M, Rabaan AA, Alwarthan S, Alhajri M, Halwani MA, Alshengeti A, et al. Regulatory T cells (Tregs) and COVID-19: unveiling the mechanisms, and therapeutic potentialities with a special focus on long COVID. *Vaccines (Basel)*. (2023) 11. doi: 10.3390/vaccines11030699

191. Queiroz MAF, Neves P, Lima SS, Lopes JDC, Torres M, Vallinoto I, et al. Cytokine profiles associated with acute COVID-19 and long COVID-19 syndrome. *Front Cell Infect Microbiol*. (2022) 12:922422. doi: 10.3389/fcimb.2022.922422

192. Chen L, Ruan G, Cheng Y, Yi A, Chen D, Wei Y. The role of Th17 cells in inflammatory bowel disease and the research progress. *Front Immunol*. (2022) 13:1055914. doi: 10.3389/fimmu.2022.1055914

193. Singh R, Koppu S, Perche PO, Feldman SR. The cytokine mediated molecular pathophysiology of psoriasis and its clinical implications. *Int J Mol Sci*. (2021) 22. doi: 10.3390/ijms222312793

194. Radtke S, Grossberg AL, Wan J. Mental health comorbidity in youth with atopic dermatitis: A narrative review of possible mechanisms. *Pediatr Dermatol*. (2023) 40:977–82. doi: 10.1111/pde.15410

Glossary

4-EPS	4-ethylphenylsulfate
5-HT	5-hydroxytryptophan
AA	arachidonic acid
ABC	aberrant behavioral checklist
AE	autoimmune encephalitis
AHRs	aryl hydrocarbon receptors
ASD	autism spectrum disorder
BBB	blood brain barrier
BM	bone marrow
BT	butyrate
C3	complement 3
CAM	complementary alternative medicine
CCL5	chemokine C-C motif ligand 5
CIDP	chronic inflammatory demyelinating polyneuropathy
COVID-19	coronavirus disease-2019
COXs	cyclooxygenases
CNS	central nervous system
CS	corticosteroids
CSF	cerebrospinal fluid
DCs	dendritic cells
EMT	fecal microbiota transplant
EUA	emergency use authorization
GBS	Guillain-Barré
GI	gastrointestinal
GOF	gain of function
HPA	hypothalamic-pituitary-adrenal
IBD	inflammatory bowel disease
IDO	indoleamine 2,3-dioxygenase
IIM	innate immune memory
IFN	interferon
IL	interleukin
IL-1Ra	IL-1 receptor antagonist
IPA	indole-3-proprionic acid
IVIg	intravenous immunoglobulin
KYNA	kynurenic acid
LOF	loss of function
LOXs	lipoxygenases
LTs	leukotrienes
MIA	maternal immune activation
mTOR	mammalian target of rapamycin
NAC	N-acetylcysteine
NMDA	N-methyl-D-aspartate
PANDAS	pediatric acute neuropsychiatric disorders associated with Streptococcal infection
PANS	pediatric acute-onset neuropsychiatric syndrome
PGs	prostaglandins
QUIN	quinolinic acid
SCFA	short chain fatty acid
SERT	serotonin reuptake transporter
SRS	social responsiveness scores
SSRIs	selective serotonin reuptake inhibitors
TGF	transforming growth factor
Th	T-helper
TI	trained immunity
TNF	tumor necrosis factor
Treg cells	regulatory T cells
TSC	tuberous sclerosis complex

(Continued)

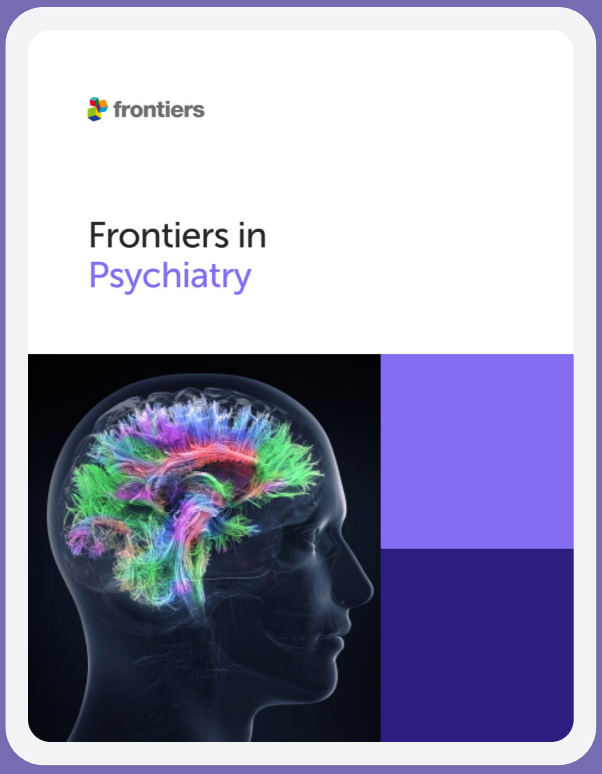
Frontiers in Psychiatry

Explores and communicates innovation in the field of psychiatry to improve patient outcomes

The third most-cited journal in its field, using translational approaches to improve therapeutic options for mental illness, communicate progress to clinicians and researchers, and consequently to improve patient treatment outcomes.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact