

REVIEW

Autism spectrum disorder: pathogenesis, biomarker, and intervention therapy

Hongbin Zhuang^{1,#}  | Zhiyuan Liang^{1,#} | Guanwei Ma¹ | Ayesha Qureshi¹ |
Xiaoqian Ran¹ | Chengyun Feng² | Xukun Liu¹ | Xi Yan¹ | Liming Shen^{1,3,*} 

¹College of Life Science and Oceanography, Shenzhen University, Shenzhen, P. R. China

²Maternal and Child Health Hospital of Baoan, Shenzhen, P. R. China

³Shenzhen-Hong Kong Institute of Brain Science-Shenzhen Fundamental Research Institutions, Shenzhen, P. R. China

*Correspondence

Liming Shen, College of Life Sciences and Oceanography, Shenzhen University, Shenzhen 518060, P. R. China.
Email: slm@szu.edu.cn

[Correction added on 2 April 2024, after first online publication: correction in changing the heading numbers]

Funding information

National Natural Science Foundation of China, Grant/Award Number: 31870825; Shenzhen-Hong Kong Institute of Brain Science-Shenzhen Fundamental Research Institutions, Grant/Award Number: 2023SHIBS0003; Shenzhen Bureau of Science, Technology, and Information, Grant/Award Number: JCYJ20170412110026229

Abstract

Autism spectrum disorder (ASD) has become a common neurodevelopmental disorder. The heterogeneity of ASD poses great challenges for its research and clinical translation. On the basis of reviewing the heterogeneity of ASD, this review systematically summarized the current status and progress of pathogenesis, diagnostic markers, and interventions for ASD. We provided an overview of the ASD molecular mechanisms identified by multi-omics studies and convergent mechanism in different genetic backgrounds. The comorbidities, mechanisms associated with important physiological and metabolic abnormalities (i.e., inflammation, immunity, oxidative stress, and mitochondrial dysfunction), and gut microbial disorder in ASD were reviewed. The non-targeted omics and targeting studies of diagnostic markers for ASD were also reviewed. Moreover, we summarized the progress and methods of behavioral and educational interventions, intervention methods related to technological devices, and research on medical interventions and potential drug targets. This review highlighted the application of high-throughput omics methods in ASD research and emphasized the importance of seeking homogeneity from heterogeneity and exploring the convergence of disease mechanisms, biomarkers, and intervention approaches, and proposes that taking into account individuality and commonality may be the key to achieve accurate diagnosis and treatment of ASD.

KEYWORDS

autism spectrum disorder, biomarker, intervention therapy, molecular mechanisms, multi-omics

[#]Hongbin Zhuang and Zhiyuan Liang have contributed equally to this study.

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2024 The Authors. *MedComm* published by Sichuan International Medical Exchange & Promotion Association (SCIMEA) and John Wiley & Sons Australia, Ltd.

1 | INTRODUCTION

Autism spectrum disorder (ASD) is a group of developmental neurological disorders characterized by early onset of abnormal social communication and restricted repetitive behaviors and interests. Since ASD was first discovered and defined, researchers have not stopped studying and exploring it (Figure S1).^{1–4} Currently, the percentage of children with ASD has steadily increased since the 1970s, when it was less than 0.4%. It is currently estimated to be between 1% and 2%.^{5–7} The rate of ASD in 8-year-old children in the United States has increased from one in 44 in 2018 to one in 36 in 2020.^{8,9} In China, the incidence of ASD in children aged 6–12 years is ~0.7%.^{10,11} As a result, ASD have attracted widespread societal attention.

The etiology of ASD is extremely complex. Twin studies suggest that genes play a key role in the pathogenesis of ASD, and its heritability estimates range from 64% to 91%.¹² In families with children with ASD, the average rate of ASD recurrence is estimated to be 15%–25% for male newborns and 5%–15% for female newborns.^{13,14} Besides, environmental factors are also implicated in the development of ASD, including prenatal/perinatal, microbial–gut–brain axis, and others. Prenatal/perinatal causes included maternal age >35 years, maternal characteristics of metabolic syndrome, use of antidepressant valproic acid (VPA) medications, and the effects of infection and inflammation.^{15,16} Environmental factors can directly influence specific susceptibility genes, prompting epigenetic modifications such as DNA methylation and histone changes (phosphorylation and acetylation), which increase the risk of developing ASD.¹⁷ ASD arises from a complex interplay of genetic and environmental factors, leading to changes in brain structure and function that manifest as behavioral abnormalities (Figure 1).

Moreover, the heterogeneity of ASD impedes both pinpointing underlying mechanisms and tailoring effective therapies. Interestingly, the previous studies have shown that the function of ASD-associated genes converges with the affected cell type^{18–23} and that the affected brain has a characteristic molecular pathology.²² ASD-specific molecular changes are mainly concentrated in central nervous system (CNS).^{18–23} Besides, individuals with ASD have different comorbidities, but all share the same social communication deficits and repetitive stereotyped behavioral phenotypes, implying a common underlying biological mechanism among them.²⁴ The heterogeneity of ASD does not preclude the possibility of finding common features or mechanisms that could lead to breakthroughs in the pathogenesis, diagnosis, and treatment of ASD. Efforts have been made to identify biomarkers, pathological mechanisms, and drug targets, and to explore the possibility of defining ASD subgroups by biological features.

In this review, we summarized the heterogeneity of ASD and explore its underlying disease mechanisms based on genes and multi-omics studies. We focused on searching convergent disease pathways under genetic backgrounds and comorbidities. In addition, the mechanisms associated with common physiological and metabolic abnormalities and the gut microbiota were reviewed. An overview of research advances in ASD biomarkers was provided, and its role in early diagnosis was emphasized. Advances in behavioral interventions and pharmacological studies of ASD were also reviewed.

2 | HETEROGENEITY OF ASD

Heterogeneity in etiology, phenotype, and outcome are hallmarks of ASD.²⁵ These factors contribute to a clinical heterogeneity, which manifest as diverse deficits or impairments in behavioral features and communicative functioning. The remarkable heterogeneity of ASD complicates and diversifies the clinical diagnosis and the individualization of treatment for ASD, which involves a combination of multiple genes, environmental factors, and mental health disorders. Heterogeneity of genes, comorbidity in ASD, and gender bias contribute to the heterogeneity of ASD.²⁵

2.1 | The challenge from heterogeneity of genes

With the application of genome-wide linkage and association analysis, copy number variant analysis, candidate gene resequencing and association analysis, and exome sequencing, many genes associated with ASD have been identified. Over 1200 genes have been recorded in the SFARI autism gene database (<https://www.sfari.org/>). More than 100 risk genes have been identified, including de novo mutations, genomic copy number variants, and single base mutations. Notably, children with ASD are genetic heterogeneous, with genetic variants detected in about 10%–20% of cases, but no single gene or mutation can cause more than 1% of cases,²⁶ and genetic testing is still not available to accurately predict or diagnose ASD.

2.2 | Comorbidity in ASD

In addition to core symptoms, children with ASD often have learning difficulties, intellectual disabilities (IDs), and other behavioral problems that may manifest as aggression, self-injurious behavior, impulsivity, irritability, hyperactivity, anxiety, and mood symptoms.²⁷ The severity of clinical symptoms and behavioral difficulties varies

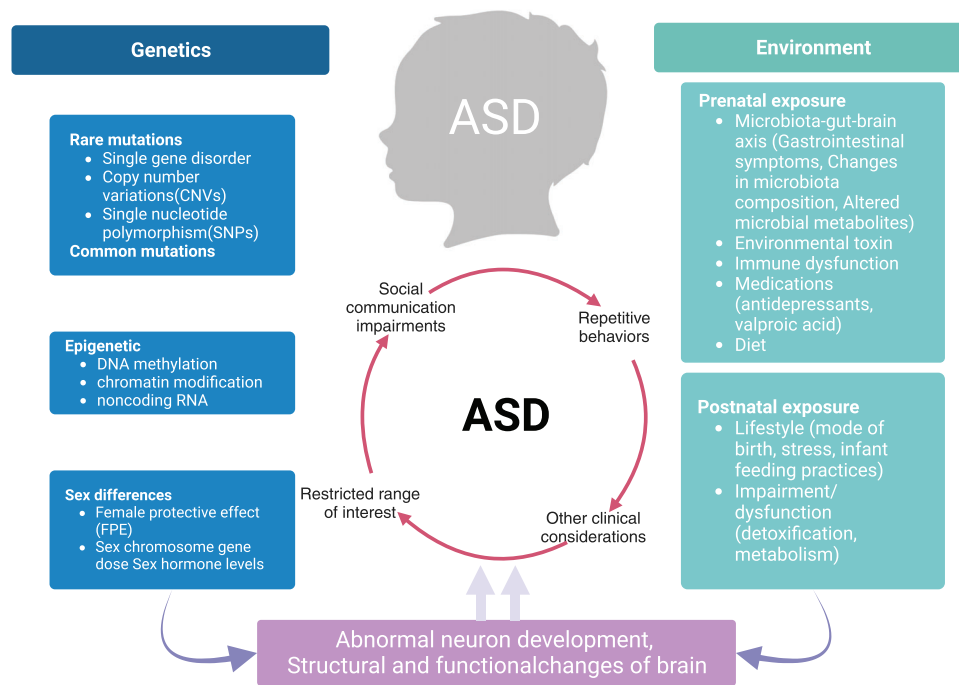


FIGURE 1 Potential influences of autism spectrum disorder (ASD). ASD is a heterogeneous group of neurodevelopmental disorders characterized by social communication impairments, repetitive behaviors, restricted range of interest, and other clinical considerations. ASD is a multifactorial disease that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

from person to person with autism and can have a severe or mild impact on daily life. Individuals with ASD are also more likely to have comorbid developmental and psychiatric problems such as attention deficit hyperactivity disorder (ADHD), anxiety and depression, ID, and specific disorders such as epilepsy, motor coordination, feeding difficulties, sleep disturbances, and gastrointestinal problems.²⁸ About 29% of individuals with ASD are likely to have savant skills.²⁹ The situation is complicated by changes in behavior and symptoms throughout development and maturity, as well as comorbidities that occur simultaneously.

2.3 | Gender bias in ASD

Male preponderance is a highly replicated finding in ASD despite striking heterogeneity in symptoms and severity. The ratio of male to female prevalence was 4:1.³⁰ In different studies, it has been reported that ASD is more prevalent in males possibly due to sex-specific single-nucleotide polymorphisms, single-nucleotide variants,

micro-deletions, copy number variants, and proteins.^{31–36} The findings of these studies have, however, not been consistently replicated in studies of the highly heterogeneous ASD.³⁷ ASD preponderance and severity differences between males and females are explained by the female protective effect (FPE) theory.²⁶ As part of the FPE, the greater variability model is included. Which asserts that males are more genetically variable, resulting in a higher incidence and decreased severity of ASD.^{38,39} Additionally, the FPE incorporates a liability threshold model, which is based on the hypothesis that females who fulfill diagnostic thresholds for autism are more likely to carry mutations than males, and relatives of females with ASD tend to be more affected than relatives of males with autism.⁴⁰ Other studies examining groups of people with ASDs and siblings of those with the disorder neither find an increase in the genetic burden of females with the disorder nor an increased incidence in female relatives of those with the disorder.^{37,41,42} It is possible that these differences can be attributed to the heterogeneity in the samples and the different methodologies employed. The future will require replication with larger groups.

3 | POTENTIAL PATHOGENESIS OF ASD

Here, we reviewed the underlying mechanisms with the association of ASD risk genes, omics studies, ASD occurrence in different genetic backgrounds, and its common mechanisms between ASD and its comorbidities. We also summarized the mechanisms associated with important physiological and metabolic abnormalities, as well as gut microbiota.

3.1 | Pathway networks associated with ASD risk genes based on SFARI database

Single gene mutations merely account for 1%–2% of autism cases and they act through distinct molecular pathways.^{43,44} We gathered the ASD risk genes from SFARI database and categorized them into three groups based on risk level. The Gene Ontology (GO) analysis was conducted on three groups, respectively. In the first set, most of risk genes were enriched in histone modification, cognition, as well as regulation of transporter activity pathway. Regulation of neurological system process, synapse organization, and social behavior pathways were placed in a prominent position within pathway network (Figure S2A). These results implicated that impairment of cognition is the most obvious character. Individuals with autism spectrum conditions or rare mutation related to ASD have profound impairments in the interpersonal social domain.^{45–48} In the second set, a majority of the risk genes exhibited enrichment in modulation of synaptic transmission, synapse organization, and learning or memory (Figure S2B). Additionally, some pathways involved human traits and actions were found, including learning or memory, social behavior, mating, circadian rhythm, sleep, and locomotory behavior. The change of these human action may be potential indication for ASD.^{49–53} In the third set, many risk genes were enriched in cellular response to peptide, regulation of cell growth, and modulation of synaptic transmission (Figure S2C).

3.2 | Multiple omics revealed pathological mechanism of ASD

Omics techniques allowed an in-depth study of ASD from a wide range of samples. The advantage of omics approaches is that they provide a complete overview of biological “features” (genes/transcripts/proteins/metabolites). It provided the most appropriate stratification of diseases or identification of new biomarkers. Meanwhile, multi-omics can integrate information across different populations,

validate them against each other, identify key genes, proteins and metabolic pathways, explore pathological mechanisms, and provide a scientific basis for the disease diagnosis and treatment. Here, we reviewed the omics studies related to ASD and the signaling pathways, in particular the convergent signaling pathways (Table S1) which associated with synaptic dysfunction, glutamatergic and GABAergic synapse imbalance, and postsynaptic density (PSD), as well as important physiological and metabolic abnormalities.

3.2.1 | The signaling pathways of synaptic dysfunction

The main signaling pathways involved in synaptic dysfunction include phosphatidylinositol 3-kinase/Protein kinase B/Mammalian target of rapamycin (PI3K/Akt/mTOR) signal and abnormal autophagy, extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) signal, Janus kinase and microtubule interacting protein 1 (JAK/MP1) pathway, and calcium signaling. Among them, dysregulation of the PI3K/Akt/mTOR pathway was considered as a point of convergence ASD.^{54–56} mTORC1 severed as a key role to tightly coordinates synaptic signaling pathways downstream of glutamate and neurotrophic receptors.⁵⁷ An unbiased proteomic showed that a brief repression of mTORC1 activity causes a significant remodeling of proteins resided in the PSD.⁵⁸ A rat fetal brain transcriptome demonstrated prominent maternal immune activation (MIA)-induced transcriptional dysregulation of mTOR and EIF4E-dependent signaling.⁵⁹ The significant proteins from S-nitrosylation proteomics could be enriched in mTORC1 upstream pathway in InsG3680(+/-) ASD mouse models.⁶⁰ DEPs from frontal cortex (FC) and hippocampus of Tsc1+/- mouse model were involved in myelination, dendrite, and oxidative stress, an up-regulation of ribosomal proteins and the mTOR kinase.⁶¹ In addition, a leukocyte transcriptomics identified a perturbed gene network involved with PI3K/AKT and its downstream pathways such as mTOR, autophagy, viral translation, and FC receptor signaling were enriched from 1–4-year-old male toddlers with ASD or typical development.⁶² Likewise, autophagy dysfunction mediated by PI3K/AKT/mTOR pathway is a causative factor for ASD.^{55,63,64}

Accumulating evidence suggested ERK/MAPK signaling as a downstream mediator of divergent genetic mutations linked to certain forms of autism.^{65–68} It also could be a converge on mTOR signaling pathway.⁶⁹ A global down-regulation of the MAPK/ERK pathway and decrease in phosphorylation level of ERK1/2 were

found in *Fmr1*-KO cell lines.^{70,71} NMDA NR1-knockdown mouse show the abnormalities of ERK signaling pathway in FC and hippocampus.⁷² MAPKAPK3 and MRPL33 in human blood were associated with a higher risk of ASD, and MAPK/ERK signaling pathways and mitochondrial dysfunction play key roles in the pathogenesis of ASD.⁷³

The alteration of JAKMIP1 could be found in individuals with distinct syndromic forms of ASD, fragile X syndrome, and 15q duplication syndrome.⁷⁴ A previous study found that CYFIP1 play a role in regulating two dysregulated genes, JAKMIP1 and GPR155 compared the mRNA expression profile in lymphoblastoid cells from autism.⁷⁵ An enriched network from interactome showed that JAKMIP1 interacted with proteins related to signaling and interaction, nervous system development and function, and protein synthesis. Notably, its loss affected neuronal translation and glutamatergic N-methyl-D-aspartate receptor (NMDAR) signaling.⁷⁴

Calcium signaling has a prominent effect on pathogenesis of ASD.⁷⁶ An action of calcium ion plays an essential role for neurodevelopment.⁷⁷ ERK signaling has also been found to be greatly linked to calcium channels to cause abnormal synaptic functions, chromatin remodeling, and ion channel activity.^{78,79} Ca^{2+} /calmodulin-dependent protein kinase II is considered as key node in synaptic plasticity of ASD.⁸⁰ Its interactome identified proteins related to NMDARs, synaptic scaffolds, myosins, tubulin and microtubules, actin cytoskeleton, ribosome and translation, mitochondria, and others.⁸¹ Synaptic fraction contained more CaMKII-associated proteins including scaffolding, microtubule organization, actin organization, ribosomal function, vesicle trafficking, and others.⁸¹ Activated CaMKII phosphorylates multiple substrates in the PSD, including scaffold protein PSD-95, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) receptor targeting subunit stargazing, and proteins involved in cytoskeleton rearrangement.⁸²

3.2.2 | Imbalance between glutamatergic and GABAergic synapse

Accumulating evidence supported a hypothesis that the imbalance between excitation and inhibition (E/I) caused by changes in the availability of glutamate and/or GABA signal transmission contribute to pathological synaptic transmission and neural circuits in ASD.^{83–87} A broad transcriptomics from postmortem samples with ASD demonstrated that both rare and common ASD-associated genetic variation converge within a down-regulated synaptic signaling.⁸⁸ Previous study found a decrease of AMPA-type glutamate receptors, glutamate transporters, and density

of GABAA receptors in the cerebellum and anterior cingulate cortex of ASD.⁸⁹ An orthogonal selected reaction monitoring assays validated the proteomics results in NMDA NR1-knockdown mouse to show the abnormalities of synaptic long-term potentiation and myelination in FC and hippocampus.⁷² Another proteomics study showed up-regulation of glutamatergic ion channels and down-regulation of neurofilament proteins in ASD brain.⁹⁰ Similarly, a cortical transcriptome of ASD exhibited analogous cortical-striatal hyperconnectivity at the protein level with mTOR or TSC2.⁹¹ A single-cell transcriptomics from *Chd8* heterozygote mice strengthen the E/I balance hypothesis of ASD in general.⁹²

Interestingly, previous metabolomics studies found that ASD often suffer from dysregulated amino acid metabolism and glutamate urinary level was lower compared with their unaffected siblings.^{93,94} The reduced pyridoxal phosphate in urine from ASD children implicated the dysregulation of biotransformation of glutamate into GABA.⁹⁵ Similarly, a strongly reduced glycine level would primarily affect NMDAR excitatory tone, overall impairing downstream glutamatergic, and GABAergic transmissions.⁹⁶

3.2.3 | Essential role of postsynaptic density in neural communication

The PSD of synapses is a wide range of scaffolding proteins, receptors, and signaling molecules that acts as a switchboard of neurotransmitter molecular and have strong association to ASD.^{97,98} Glutamate receptor levels could be regulated by endocytosis of PSD scaffolding proteins.⁹⁹ In general, E/I balance required the integrity of PSD to transmit signal between neurons.^{100–102} Several genes encoding PSD have been identified disruptive mutations in psychiatric disorder patients, including ASD.^{98,103}

Synaptic protein/pathways resource (SyPPRes) was identified as the prioritization of ASD risk factors across 41 in vivo interactome, which show a larger number of shared protein associations to *Psd95/Dlgap1/Shank3* indicating a role of core-PSD scaffolds interactions.¹⁰⁴

The alteration of macromolecular complex proteins such as SHANK3 can cause ASD.¹⁰⁵ To quantify the proteins in PSD fractions, the most altered levels of proteins exhibiting ionotropic glutamate receptor activity, cell-cell signaling, and cytoskeleton organization as the results of SHANK3 deficiency.¹⁰⁶ A zebrafish embryo model of ASD induced by VPA showed the significant decrease of *Shank3* in transcriptome.¹⁰⁷ Striatal regions of *Shank2*-mutant mice showed distinct patterns from transcriptomic including synapse, ribosome, mitochondria, spliceosome, and extracellular matrix.¹⁰⁸ The transcriptomic from hippocampal

showed strongly enriched GO terms associated with PSD, synapse, and postsynaptic membrane.¹⁰⁸ Other omics studies related to ASD risk genes have achieved similar results, such as SAP97 gene,¹⁰⁹ p140Cap gene,^{110–112} Pten gene,¹¹³ and nSR100 gene.¹¹⁴

3.2.4 | Others

Previous omics studies have also revealed that physiological and metabolic abnormalities such as mitochondrial dysfunction, oxidation, and inflammation are associated with ASD. The mitochondrial deficiency is expected to explain the underlying damage mechanism in ASD. ASD were described as mitochondrial diseases and its potential mechanism was identified through phosphoproteomics.¹¹⁵ The alternated pathways in brain of autistic subjects were associated with energy metabolism, synaptic vesicle regulation as well as myelination.¹¹⁶ The change of mitochondrial function, energy metabolism, EIF2 signaling, immune functions, ubiquitination, and DNA repair were found in global proteomics of peripheral blood-derived lymphoblasts with homozygous HERC2 variants.¹¹⁷ A transcriptome suggested that mitochondrial function, ribosome, and spliceosome components were down-regulated in postmortem brain of ASD.¹¹⁸

A metabolic profiling of lymphoblastoid cells revealed a decreased tryptophan metabolism in ASD and showed a reduced generation of nicotinamide adenine dinucleotide (NADH), a critical energy carrier in mitochondria.¹¹⁹ The metabolic clusters containing lactate or pyruvate, succinate, α -ketoglutarate, glycine, ornithine, and 4-hydroxyproline highlighted potential dysregulation in amino acid and energy metabolism in ASD plasma.¹²⁰ Importantly, a metabolomics in cerebrospinal fluid analysis from ASD showed that L-cysteine, adenine, and dodecanoic acid were important metabolites for ASD.¹²¹ Additionally, amino acid and energy metabolism pathways were most disrupted in all neurodevelopment disorders.¹²¹ A previous study performed proteomics and metabolomics on amniotic fluids from pregnant woman with male fetuses and premutation in FMR1 gene. The result showed the mitochondrial dysfunction induced by the deficits in prenatal serine biosynthesis underlie.¹²² A wide range of aberrant mitochondria-related pathways, including respiratory electron transport chain, cellular response to stress, regulation of neuron apoptotic process, and reactive oxygen species (ROS) metabolic process were triggered by SHANK3 mutation in mouse cortex.¹²³ Untargeted metabolomics revealed that key metabolic mitochondrial/extramitochondrial pathways was up-regulated in mecp2-deficient mouse cortex.¹²⁴ VPA-induced alterations in metabolites of serum, urine, and brain cortex were asso-

ciated with mitochondrial dysfunction metabolism and CNS disorders.¹²⁵

A mechanistic modeling based on transcriptome suggested a direct link between inflammation and ASD in neurons.¹¹⁸ Notedly, the MIA is a one of the common environmental risk factors of ASD pathology during pregnancy.^{126–128} The adaptive immune pathway was enriched in maternal blood from mothers of children later diagnosed with ASD by transcriptome.¹²⁹ Maternal inflammation with elevated kynurenine metabolites is related to the risk of abnormal brain development in ASD.¹³⁰ Similarly, the increased paternal age at conception has been associated with ASD.^{131,132}

In the metabolic profile, prostaglandin D2, which is a type of inflammatory mediators was increased in plasma of young boys with ASD and implicated with neuroinflammation.¹³³ In the liver of BTBR mouse model of autism, 12 differential metabolites suggested that bile acid-mediated activation of LXR α might contribute to metabolic dysfunction of lipid and leukotriene D4 produced by the activation of 5-LOX led to hepatic inflammation.¹³⁴ In ASD children brain, abnormal levels of N-acetyl-compounds, glutamate glutamine, creatine phosphocreatine (Cr), or choline-compounds (Cho) implicated that neuron or glial density, mitochondrial energetic metabolism, and/or inflammation contribute to ASD neuropathology.¹³⁵ The consistent appearance of inflammation regulation in proteomics from Mecp2-mutant mouse, cells generated from induced pluripotent stem cells (iPSC) in Rett syndrome (RTT), and RTT peripheral samples implied that it contributed to the destruction of the nervous system.¹³⁶

In summary, the above-mentioned signal pathways play a significant role in the typical neurodevelopment process, and their dysfunction can lead to downstream alterations, such as an imbalance in excitatory and inhibitory synapses. This can result in the transmission of erroneous signals within neural circuits, may be caused by inflammation and reoxidation. The maintenance of stable neural communication is contingent upon the integration of synapse construction, such as PSD, and the provision of sustainable energy from mitochondria. As a result, a series of aberrant signaling molecules, excitatory and inhibitory imbalances, PSD, mitochondrial dysfunction, and inflammation ultimately lead to neural immaturity and damage in ASD pathology (Figure 2).

3.3 | Studies on the pathogenesis of ASD in different genetic backgrounds

The search for “commonalities” among children with ASD has become a focus of current research and a breakthrough

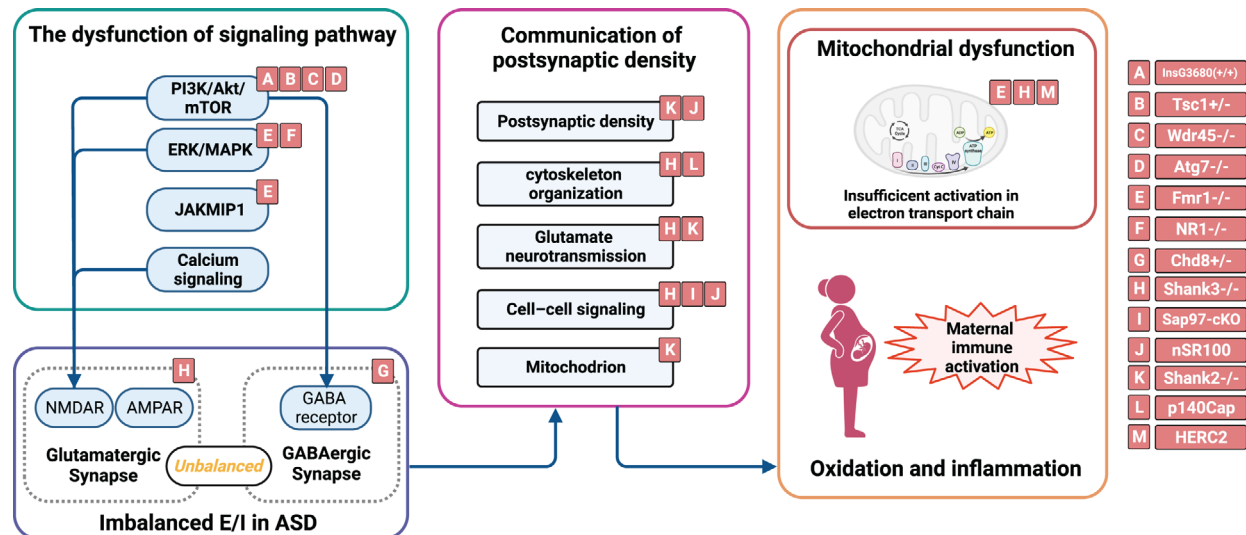


FIGURE 2 The graphical abstract of potential pathology of autism spectrum disorder (ASD) investigated by multi-omics methods. The dysregulation of signaling pathways in neuron lead to abnormal balance between excitatory and inhibition. Many genes mutation influenced the postsynaptic density including the cytoskeleton organization, glutamate neurotransmission, cell-cell signaling, and mitochondrial function. The accumulation of negative effect further impacts the downstream of synapse. The maternal immune activation and mitochondrial dysregulation are associated with oxidation and inflammation. The red labels indicates that the genes are associated with the process in the studies. E/I, excitation and inhibition.

point.^{137–140} ASD-related syndromes with a clear genetic cause for the autism phenotype offer the best opportunity to elucidate the underlying mechanisms of ASD and to identify possible therapeutic targets¹⁴¹ and diagnostic markers.¹⁴² In recent years, there has been notable advancement in the identification of genes closely linked to ASD. These genes exhibited distinct molecular functions but may share biological pathways. In the context of known genes, the research on genes and pathological mechanisms, diagnostic markers, and even imaging is conducive to finding the commonality between different genes (Table 1).

A recent study of iPSC-derived “brain-like organs” from children carrying three different ASD risk genes showed that although each gene acts through a unique underlying molecular mechanism, they have similar overall defects that affect similar aspects of neurogenesis and the same type of neurons.²⁴ Using iPSC, Pintacuda et al. constructed protein–protein interaction networks among 13 ASD-related genes in human excitatory neurons, revealing the neuron-specific biology associated with ASD.¹⁵⁰ Three animal experiments with known genetic backgrounds suggest that synapses play a key role in the pathogenesis of ASD.^{144,146} Among them, Jordan and coworkers compared the synaptic proteomes of five mouse models of autism revealing convergent molecular similarities, including defects in oxidative phosphorylation and Rho GTPase signaling.¹⁴⁶ They also compared synaptic proteomes of seven mouse models of autism revealing molec-

ular subtypes and defects in Rho GTPase signaling.¹⁴⁶ Another study investigated seven animal models of ASD and showed that there is great heterogeneity between models. However, high-dimensional measurements of synaptic protein networks may allow a promising avenue for subtype differentiation of ASD with common molecular pathology. Notably, this approach demonstrated convergence between the glutamate synapse protein interaction networks of the VPA and TSC2 mouse models, both converging on a putative “mTOR” cluster.¹⁴⁴

Similarly, a previous study identified distinct and overlapping phenotypes at the level of behavior, brain structure and circuitry by analyzing the function of 10 autism genes in zebrafish. The study observed that the forebrain contributed most to brain size differences between ASD genes, that brain activity phenotypes were concentrated in regions involved in sensory-motor control, that dopaminergic and microglia abnormalities were the confluence of two genes (SCN2A and DYRK1A), and implied that neuroimmune dysfunction was associated with autism biology.¹⁵¹ In addition, Willsey et al. employed parallel in vivo analyses and systems biology approaches to examine 10 genes linked to ASD by utilizing tropical African clawed toads. The results suggested that cortical neurogenesis served as a convergence vulnerability site in ASDs. Moreover, estrogen is a restorative factor for several different autism genes and they revealed a conserved role for estrogen in inhibiting sonic Hedgehog signaling.¹⁵²

TABLE 1 Studies on different genotypes of autism spectrum disorder (ASD).

No.	Author	Sample	Genotype	Method	Major finding
1	Ellegood et al. (2015) ¹⁴³	Mouse brain	15q11-13, 16p11, AndR, BALB/c, BTBR, CNTNAP2, En2, FMR1, GTF2i, ITGβ3, Mecp2, NLGN3, NRXN1α, SLC6A4, SHANK3, XO	MRI	26 different mouse models were examined, the parieto-temporal lobe, cerebellar cortex, frontal lobe, hypothalamus, and the striatum are the abnormal regions, unknown connections between Nrnx1α, En2, Fmr1, Nlgn3, BTBR, and Slc6A4 were identified.
2	Brown et al. (2018) ¹⁴⁴	Mouse FC, HC	CNTNAP2, FMR1, Shank3B, Shank3Δex4-9, TSC2, Ube3a2xTG,	QMI	A unique set of disrupted interactions was displayed by each model, but synaptic activity-related interactions were disrupted. Potential relationships among models and deficits in AKT signaling in Ube3a2xTG mice were confirmed.
3	Jin et al. (2020) ¹⁴⁵	Mouse CPN, CIN, AC, OC, MG	Adnp, Ank2, Arid1b, Ash1l, Asxl3, Chd2, Chd8, Cntnb1, Cul3, Ddx3x, Dscam, Dyrk1a, Fbxo1l, Gatad2b, Kdm5b, Lar4p4b, Mbd5, Med13l, Mill1, Myst4, Pogz, Pten, Qrich1, Satb2, Scn2a1, Setd2, Setd5, Spen, Stard9, Syngap1, Tcf20, Tcf712, Tnrc6b, Upf3b, Wac	In vivo Perturb-Seq	In vivo Perturb-Seq can serve as a tool to reveal cell-intrinsic functions at single-cell resolution in complex tissues, which can be applied across diverse and tissues in the intact organism.
4	Carbonell et al. (2021) ¹⁴⁶	Mouse HC	Anks1b, BTBR, Cntnap2, Cacna1c, Fmr1, Pten, Shank3	TMT, SDS-PAGE, LC-MS	Hippocampal synaptic proteomes from seven mouse models were identified, common altered cellular and molecular pathways at the synapse were also identified.
5	Zerbi et al. (2021) ¹⁴⁷	Mouse brain	16p11.2, BTBRT, CDKL5, CHD8, CNTNAP2, En2, FRM1.1, FRM1.2, Het, IL6, Mecp2, SGSH, SHANK3b, Syn2, TREM2	MRI	ASD-associated etiologies cause a broad spectrum of connectional abnormalities, etiological variability is a key determinant of connectivity heterogeneity in ASD, identification of etiologically relevant connectivity subtypes could improve diagnostic label accuracy in the non-syndromic ASD population.
6	Willsey et al. (2021) ¹⁴⁸	Xenopus tropicalis brain	ARID1B, ADNP, ANK2, CHD8, CHD2, DYRK1A, NRXN1, POGZ, SCN2A, SYNGAP1	LWI	Mutations lead to an increase in the ratio of neural progenitor cells to maturing neurons, systematic small molecule screening identifies that estrogen rescues the convergent phenotype and mitigate a broad range of ASD genetic risks.
7	Shen et al. (2022) ¹⁴⁹	Human blood	ASH1L, DDX3X, GIGYF2, NAA15, SCN2A	iTRAQ, LC-MS/MS, ELISA	The DEPs and differential metabolites of plasma could distinguish the cases from controls. Proteomic results highlighted complement, inflammation, immunity, mitochondrial dysfunction, proteasome, ubiquitin-mediated proteolysis, and ER stress in the pathogenesis of ASD.

(Continues)

TABLE 1 (Continued)

No.	Author	Sample	Genotype	Method	Major finding
8	Paulsen et al. (2022) ¹⁴⁴	Human CC	ARID1B, CHD8, SUV420H1	IC, WB	Cell-type-specific neurodevelopmental abnormalities that are shared across ASD risk genes and are modulated by human genomic context were uncovered, convergence in the neurobiological basis of how different risk genes contribute to ASD pathology were found.
9	Pintacuda et al. (2023) ¹⁵⁰	Human brain excitatory iNs	ARID1B, ANK2, ADNP, CTNBN1, CHD8, DYRK1A, GIGYF1, MED13L, PTEN, SCN2A, SYNGAP1, SHANK3, TLK2	WB, LC-MS/MS	The ASD-linked brain-specific isoform of ANK2 is important for its interactions with synaptic proteins and to characterize a PTEN-ANKAP8L interaction that influences neuronal growth, the IGF2BP1-3 complex emerged as a convergent point in the network that may regulate a transcriptional circuit of ASD-associated genes.
10	Carbonell et al. (2023) ¹⁴⁶	Mouse HC	Anks1b, BTBR, Cntnap2, Fmr1, Pten	TMT, LC-MS	Changes in oxidative phosphorylation and Rho family small GTPase signaling were revealed, the ANKS1B model displays altered Rac1 activity counter to that observed in other models was confirmed.
11	Mendes et al. (2023) ¹⁵¹	Zebrafish brain	CHD8, CNTNAP2, CUL3, DYRK1A, GRIN2B, KATNAL2, KDM5B, SCN2A, TBR1, POGZ	IC, CI, RNA-Seq	A global increase in microglia resulting from ASD gene loss of function in select mutants, implicates neuroimmune dysfunction as a key pathway relevant to ASD biology.

Abbreviations: AC, astrocytes; CC, cerebral cortex; CI, confocal imaging; CIN, cortical inhibitory neurons; CPN, cortical projection neurons; ELISA, Enzyme-linked immuno sorbent assay; ER, Endoplasmic reticulum; FC, frontal cortex; HC, hippocampus; IC, immunohistochemistry; iNs, induced neurons; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LWI, large-scale whole-brain imaging; MG, microglia; MRI, magnetic resonance imaging; OC, oligodendrocytes; QMI, quantitative multiplex co-immunoprecipitation; SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis; TMT, tandem mass tag system; WB, western blot.

In vivo Perturb-Seq technology based on CRISPR-Cas9 and single-cell RNA sequencing technology developed a high-throughput genetic screening method to study the function of numerous genes in complex tissues at single-cell resolution. Recently, Zhang and coworkers applied this method to analyze the effects of 35 ASD/ND risk genes on brain development in mice. The authors identified cell type specific and evolutionarily conserved gene modules from neuronal and glial cell categories.¹⁴⁵

These studies exemplify the examination of genetic heterogeneity in ASD by conducting studies of common features of ASD and controls based on known genetic backgrounds. The findings suggested that ASD-associated susceptibility genes ultimately converge on common signaling pathways and that these convergence sites are key to understand ASD pathology. Therefore, categorizing genes based on shared biology despite their heterogeneity

might represent a path toward precision medicine in ASD, bridging the gap between gene discovery and actionable biological mechanisms.¹⁵¹

Moreover, similar results have been obtained in imaging studies under different genetic backgrounds. Functional magnetic resonance imaging analysis of 16 mouse mutants with ASD-related mutations identified brain connectivity subtypes among the mutants despite the presence of distinct phenotypes.¹⁴⁷ Likewise, although mouse mutants with 26 ASD genes exhibited heterogeneous neuroanatomical phenotypes, clustering of these mutants by shared features allowed identification of gene subgroups.¹⁴³

Overall, these studies suggest that conducting research on the convergent mechanisms among ASD-related genes and elucidating the shared pathways could provide information to unravel the mechanisms of ASD and explore potential therapeutic targets and diagnostic biomarkers.

3.4 | Common mechanisms associated with ASD and its comorbidities

The comorbidities in most children with ASD is a notable attribute, contributing to its diverse and intricate nature.¹⁵³ Thus, investigating common mechanisms between ASD and comorbidities, as well as the specific genes and mechanisms that lead to their respective occurrence, is a topic of interest in the field of ASD research, and its study contributes to the diagnosis and treatment of ASD. Previous studies have shown some common mechanisms between these comorbidities and ASD.¹⁵³ For example, recent studies have highlighted points of convergence between ASD and neurodevelopmental disorders (NDD) genes.¹⁵⁴ Chromosomal microarray and sequencing studies have identified significant genetic overlap between ASD and other NDD and neurological disorders, including ID, epilepsy, and schizophrenia.^{155,156} Two meta-analyses of genome-wide associations have also shown that ASD shares a common genetic background in neuropsychiatric disorders.^{157,158} Genes involved in synaptic structure and function are implicated in a variety of disorders, including schizophrenia, ASDs, and other NDDs.^{159–161} The gene discovery can help to distinguish this complexity by analyzing the genetic structure and risk gene associations of different subtypes or comorbidities. In addition, several environmental factors have been found to be associated with ASD and its comorbidities, such as MIA in the prenatal environment, stress, drug exposure, and malnutrition,^{126,127,162–164} as well as gastrointestinal dysfunction and disruption of intestinal flora.^{165–167} These studies suggest that although the heterogeneity of ASD is complicated by the occurrence of comorbidities, common mechanisms may still be found between ASD and its comorbidities.

3.5 | Mechanisms associated with important physiological and metabolic abnormalities

As mentioned above, immune dysregulation, inflammation, oxidative stress, and mitochondrial dysfunction are closely associated with ASD and are important physiological and metabolic abnormalities in ASD.^{128,138,168–170} They may be the intersection of genetic and environmental factors and contribute to ASD.

Immunity and neuroinflammation play a key role in the development of ASD.^{171–173} Immune dysfunction in ASD involves a network of interactions between several cell types from the innate and adaptive immune response. Multiple immune factors mediate the effects

of CNS function. Some cytokines inhibit neurogenesis and promote neuronal death, whereas others promote the growth and proliferation of neurons and oligodendrocytes. Complement proteins and microglia can be involved in synaptic scaling and pruning, while brain-reactive autoantibodies can alter neuronal development or function.¹⁷² Active microglia and astrocytes have been observed in the brains of ASD. Activation of microglia in different brain regions was observed, including an increase in cell number or cell density, morphological changes, and phenotypic alterations.¹⁷⁴ Activation of microglia releases inflammatory cytokines and chemokines such as interleukin (IL)-6, IL-12, IL- β , and tumor necrosis factor- α (TNF- α). Excessive induction of nitric oxide synthase (NOS) and ROS affects synaptic plasticity and produces behavioral abnormalities associated with ASD.¹⁷⁵

Oxidative stress is associated with mitochondrial dysfunction. Decreased endogenous antioxidant capacity, particularly reduced total glutathione (tGSH) levels and altered glutathione peroxidase (GPx), superoxide dismutase, and catalase activities, have been reported in ASD, which is consistent with elevated oxidative stress indicators in children with ASD.¹⁷⁶ The prevalence of mitochondrial disease in ASD is 4%–5%, which is significantly higher than in the general population (about 0.01%).^{177,178} Mitochondrial abnormalities such as increased hydrogen peroxide, decreased NADH, and mitochondrial DNA over-replication have been observed in lymphocytes isolated from subjects with ASD.¹⁷⁹ Mitochondria produce adenosine triphosphate (ATP). Reduced ATP production and elevated levels of lactate and pyruvate in individuals with ASD may indicate mitochondrial dysfunction in autism.

These physiologic and pathologic processes interact with each other, and multiple mechanisms are interrelated.^{128,170,180,181} Oxidative stress can lead to mitochondrial dysfunction, and abnormal mitochondrial function leads to increased ROS metabolism and oxidative stress, creating a vicious cycle. The association between gut flora and MIA is also reflected in the pathogenesis of ASD.^{128,180} MIA induces an immune response in pregnant women, leading to further inflammation and oxidative stress, as well as mitochondrial dysfunction in the placenta and fetal brain. These negative factors lead to neurodevelopmental deficits in the developing fetal brain, which subsequently lead to symptoms of behavioral disorders in the offspring.¹²⁸ In summary, accumulating research into these common pathophysiologic mechanisms will enhance our comprehension of ASD diagnosis and treatment, while provide insight into general or subgroup-specific processes that may contribute to the development of ASD and other psychiatric disorders.¹⁶⁸

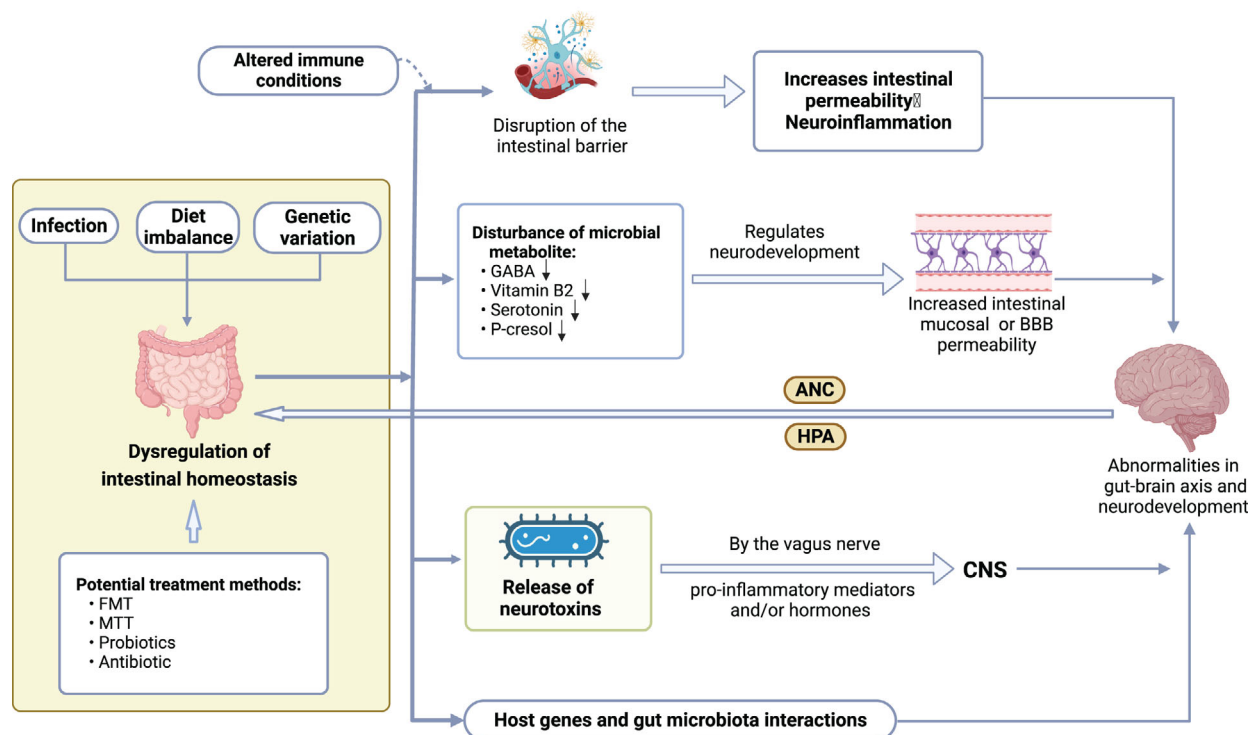


FIGURE 3 Potential mechanisms of gut microbiota imbalance and autism spectrum disorder (ASD) occurrence. The gut flora and brain can interact through immune, metabolic, and gut nervous system pathways and ultimately leading to abnormalities in gut brain axis and neural development. Gut flora alternation causes metabolism changes and its dysbiosis linked to greater intestinal mucosa and blood–brain barrier (BBB) permeability. Feedback regulation exists in gene expression, dietary preference, and gut flora. ANC, autonomic nervous system; CNS, central nervous system; FMT, fecal microbiota transplantation; HPA, hypothalamic–pituitary–adrenal; MTT, microbiota transfer therapy.

3.6 | Pathological mechanisms of ASD associated with gut microbiota

A series of studies have reported significant differences in the composition of gut microbiota between ASD cases and healthy controls (Figure 3). Changes in gut microbiota cause changes in metabolism. Several animal experiments have demonstrated an association between ASD and gut microbiota. Transplantation of gut flora from the individuals with ASD into germ-free mice leads to autism-like symptoms in the mice, which may be related to the regulation of tryptophan and 5-hydroxytryptaminergic synaptic metabolism¹⁸² or it may lead to alterations in neuroactive metabolites.¹⁸³ It has also been found that changes in the gut microbiota of children with ASD affect glutathione (GSH) synthesis¹⁸⁴ and degradation of organic toxins, lacking biosynthetic pathways for several neurotransmitters¹⁸⁵ or vitamins.¹⁸⁶ In addition, some bacterial metabolites may contribute to the development of autism-like behaviors, such as elevated acetaminophen sulfate levels.^{187,188} The presence of gut dysbiosis has also been linked to heightened permeability of the intestinal mucosa or the blood–

brain barrier. For example, abnormal metabolism of some short-chain fatty acids (SCFAs) affected tight junction proteins associated with blood–brain barrier permeability.¹⁸⁹ The neurotoxins are released by a variety of harmful bacteria that are delivered via the enteric vagus nerve to the CNS.¹⁸² The permitting pro-inflammatory mediators and/or hormones enter the circulation and to be transported from bloodstream to the brain, where they may ultimately affected the CNS neurodevelopment and/or function.¹⁸²

Studies on *Cntnap2* double knockout¹⁹⁰ and *CHD8* single knockout autism model mice¹⁹¹ have shown that the combined effects of host genes and gut flora in interacting with each other lead to behavioral abnormalities in autism. Genetic factors and dietary habits can alter the composition of the gut microbiota, while imbalances in the gut microbiota can also trigger aberrant gene expression and influence dietary preferences.¹⁹² Moreover, the nervous system can act on the gastrointestinal tract and its microbiota through specific pathways (e.g., the autonomic nervous system axis and the hypothalamic–pituitary–adrenal axis) to regulate intestinal motility and

secretion, and to influence gut microbial composition and function.

Furthermore, studies of gut microbiota have also revealed that pathological inflammation of ASD occurs not only in the CNS and periphery, but also in the gut. The damaged, inflamed, and permeable epithelia are the predominant routes utilized by commensal bacteria to migrate to the bloodstream.¹⁹³ The microbial metabolites are likely the most significant contributors to systemic inflammation and subsequent neuroinflammation.^{193,194} The occurrence of abnormal oxidation or unsuitable activation of immune led to subsequent inflammation and neuroinflammation in CNS, periphery, and gut of ASD.

Taken together, dysbiosis of the gut microbiota may be an important contributor to ASD, leading to disruptions in gut–brain axis connectivity and neurodevelopment caused by bacterial metabolites, the enteric nervous system, and the systemic immune system. An in-depth exploration of the possible molecular mechanisms by which gut microbes influence behavioral changes in ASD offers great potential for intervention, diagnosis, and therapeutic evaluation in ASD. Notably, to date, the relationship between the gut microbiota and autism symptom severity is difficult to determine, and no specific bacterial group could be identified as being solely responsible.¹⁹⁵

4 | STUDY ON DIAGNOSTIC BIOMARKERS OF ASD

A widely accepted consensus in clinical practice is that timely identification and diagnosis play a crucial role in facilitating early intervention and prognostic outcomes. To achieve this goal, the American Academy of Pediatrics recommends that all children should be screened for autism for the first time at 9 months of age and at routine developmental monitoring centers at 18, 24, and 30 months of age.¹⁹⁶ In China, there are similar consensus or norms.¹⁹⁷ Therefore, it is necessary to identify early behavioral features of ASD that can be used for early diagnosis. Moreover, there is a need to investigate biomarkers for objective diagnosis. Measurable laboratory biomarkers may be an opportunity to identify risks that not only provide an earlier and more reliable diagnosis, but further differentiate the autism spectrum based on common pathophysiological features, allowing for individualized treatment and response monitoring, and increasing the chances of success of future drug development programs.¹⁹⁸ To date, some consensus has raised on the early behavioral features of ASD.^{28,196} Although progress has been made in the study of diagnostic markers, most biomarkers have not yet been validated and further research is required.

A recent study conducted a systematic review of diagnostic molecular markers for ASD.¹⁹⁹ The majority of these markers are measured peripherally via blood, and although there is considerable variation between and within individual biomarkers, two major groups are apparent, one consisting of cytokines and growth factors (e.g., IL-6, brain-derived neurotrophic factor) and the other consisting of amino acids, neurotransmitters (e.g., cysteine, serotonin, GABA), and hormones (e.g., vitamin D). In between these two groups are molecules related to reduction/oxidation (redox), including GSH, which is the most frequently detected molecule. Most papers also report an association between molecular markers and ASD diagnostic status.

In this section, we provide an overview in terms of non-targeted omics and targeted research of ASD diagnostic markers, as well as research on diagnostic markers associated with important physiological and metabolic abnormalities of ASD, and gut microbiota.

4.1 | The identification of potential biomarkers by non-targeted omics

Genetic testing, proteomics, and metabolomics were employed in previous study to screen a number of genes, proteins, peptides, and metabolites that have the potential to be diagnostic markers for ASD.¹⁸ Protein and metabolite-based tests provided the highest diagnostic accuracy for ASD, which combined with multiple features may further improve diagnostic accuracy.²⁰⁰

There have been several reports and reviews on the proteomics of ASD protein diagnostic markers, mainly including blood, urine, and saliva studies. Overall, candidate proteins obtained from proteomic studies have little or no reproducibility in independent cohorts.²⁰¹ However, bioinformatics analysis showed that the majority of proteins in different studies were associated with complement and coagulation cascades, focal adhesion, platelet activation, vitamin digestion and absorption, immune response, inflammatory response, cholesterol metabolism, lipid metabolism, oxidative stress, and energy metabolism. These mechanisms are evidently prevalent in individuals with ASD, thus indicating a convergence of protein-associated mechanisms that hold promise as potential diagnostic markers.^{201–205}

Metabolism-based analyses have the advantage of being sensitive to the interactions between genomic, gut microbiome, dietary, and environmental factors. The metabolite differences between disease and normal states has received increasing attention in recent years. Studies of blood and urine metabolomics in children with

autism versus controls have shown that although fewer metabolisms show consistent changes across studies, the mechanisms by which they are associated are convergent and correlate with common pathogenesis and pathophysiological changes in ASD. Changes in blood metabolites are mainly associated with mitochondrial dysfunction, oxidative stress, fatty acid metabolism, energy metabolism, cholesterol metabolism, neurotransmitters, and mammalian–microbial co-metabolism pathway.^{18,206} Most of the changes in urinary metabolites are related to amino acid metabolism, energy metabolism, oxidative stress, intestinal flora, and neurotransmission. The metabolism of some amino acids (e.g., tryptophan and branched-chain amino acids) and neurotransmitters (e.g., glutamate, ROS, and lipids) may play an important role in the pathogenesis of ASD.^{18,206}

A recent study analyzed blood and urine metabolites from the same group of children with autism and found decreased urinary taurine and catechol levels and increased plasma taurine and catechol levels.²⁰⁷ Another urine metabolomics study in twins found that phenylpyruvate and taurine were elevated in the autistic group, while carnitine was decreased, and arginine and proline metabolic pathways were enriched. In twins, there was a significant positive correlation between indole-3-acetate and autistic traits.²⁰⁸ In addition, in some recent omics studies,^{209–212} machine learning methods have been used to screen diagnostic markers from omics data.

The combined multi-omics approach has been reported in several studies of diagnostic markers for ASD.^{142,213,214} For example, using metabolomic and transcriptomic approaches, Dai et al. revealed that blood uric acid levels were significantly lower in children with ASD and the expression levels of some genes related to purine metabolism differed between children with ASD and controls.²¹³ Integrated proteome and metabolome analysis, another study found that six signaling pathways were significantly enriched in ASD, three of which were correlated with impaired neuroinflammation (GSH metabolism, metabolism of xenobiotics by cytochrome P450, and transendothelial migration of leukocyte).²¹⁴ Although further validation is needed, in combination with proteomic and metabolomic data, a previous study suggests that glycerophospholipid metabolism and N-glycan biosynthesis may play a key role in the pathogenesis of ASD.¹⁴²

Moreover, to explore the effect of ASD gene heterogeneity on the study and application of diagnostic markers, Shen et al. preliminarily detected five children with ASD carrying risk genes for ASD from 126 cases through gene-targeted testing, proteomic, and metabolomics in plasma and peripheral blood mononuclear cells (PBMCs) com-

pared to healthy controls.¹⁴² The results showed that although the children with ASD differed in their expression patterns of total proteins and metabolites, the differential proteins and metabolites identified were still able to distinguish cases from controls well, and the mechanisms of association were consistent with those reported in previous studies.¹⁸ Based on this, they added the group of children clinically diagnosed with ASD but not detected as carrying risk genes to further the study and obtained similar conclusions.²¹⁵ These findings support that, despite the presence of genetic heterogeneity, it is possible to identify markers for diagnosis among children with different genetic backgrounds.

4.2 | Targeted research and application of diagnostic markers

The targeted validation and detection of diagnostic markers, especially using some high-throughput methods (e.g., targeted proteomics, metabolomics), is convenient and important. This is primarily due to the utilization of multiple markers in the combined diagnosis of multifactorial diseases, which typically results in enhanced diagnostic accuracy and specificity compared to single diagnostic marker. Here, we focus on targeted proteomics and metabolomics studies. However, in reality, any study that addresses the common pathophysiological mechanisms associated with ASD is also a targeted study, such as studies that have selected a panel of cytokines for peripheral blood testing based on literature reports.^{216,217} Studies targeting a particular class of biomarkers related to oxidative stress, mitochondria, gut microbiota, etc., are also in line with this idea. They are reviewed in Sections 4.3 and 4.4. Indeed, genetic testing with a panel consisting of known ASD-related genes should also be included.^{161,218,219}

4.2.1 | Targeted proteomics research

Applying targeted proteomics multiple reaction monitoring technology, we have previously performed targeting studies on the proteins of ASD plasma complement and coagulation cascades, and combined with machine learning methods, we obtained a set of 12 differential protein combinations with diagnostic potential.²¹² The complement system composed of more than 40 proteins served as an important component of the human immune system. The expression of complement or complement and coagulation cascade-related proteins has been frequently reported alteration in the peripheral blood of ASD since the first proteomic studies on peripheral

blood in ASD,^{18,142,211,220–222} while changes in the brain have also been reported.^{221,222} The association of complement with neuropsychiatric disorders has recently attracted attention.^{221,222} The correlation between alterations of complement proteins in brain and periphery of children with ASD remains unclear, and the underlying mechanisms are not comprehensively understood, thus necessitating further research.

4.2.2 | Targeted metabolomics studies

Metabolomics is capable of identifying biochemical imbalances that are frequently present in children with ASD, primarily involving amino acids, reactive oxidative stress, neurotransmitters, and the microbial–gut–brain axis,^{206,223} and their changes further support the association of these mechanisms with ASD. Studies on the targeted metabolomics of ASD are progressing rapidly, including those on the targeted metabolomics of body fluids such as blood and urine. We have summarized them in Table 2.

At present, targeted detection of metabolites altered in blood include amino acids (tyrosine, tryptophan, arginine, proline, methionine, cysteine, and taurine), lipids (phospholipids, sphingolipids, and fatty acids), and metabolites in the urea cycle and xenobiotics metabolism.^{142,235} The metabolites associated with branched-chain amino acid (BCAA) metabolism,²³⁶ fatty acid metabolism (free carnitine, short- and long-chain acylcarnitine),²²⁷ tricarboxylic acid (TCA) cycle, fatty acids, oxidative phosphorylation, mitochondrial dysfunction, gut microbiome metabolism,^{142,237} and neurotransmitter metabolism²³⁸ in the plasma of ASD are also involved.

Similarly, in targeted metabolomics studies of urine, previous studies have targeted the abnormalities of reactive oxidative stress, gut bacteria metabolism,²³⁹ amino acid (tyrosine, tryptophan, arginine, proline, methionine, cysteine, and taurine), lipid (phospholipid, sphingolipid, and fatty acid), urea cycle, xenobiotics metabolism,^{239,240} TCA cycle, and glutamate metabolism²⁴⁰ in urine of ASD. Additional studies have also observed abnormalities of ornithine (urea) cycle, methionine, lysine, reactive oxidative stress, and tryptophan–serotonin metabolism in urine of children with ASD.²³⁹ Of interest, a prior study applied a targeted metabolomics approach to examine markers of oxidative stress and gut microbiota dysbiosis reported in previous studies and determined that levels of methylguanidine and n-acetylarginine, which are associated with oxidative stress, and the gut bacterial metabolites indolol sulfate and indole-3-acetic acid were elevated in the urine of children with ASD.²⁴¹

4.3 | Study of biomarkers associated with important physiological and metabolic abnormalities in ASD

4.3.1 | Biomarkers associated with immunity/inflammation

The mounting evidence of altered central and peripheral immune system function supports to the notion that a subgroup of ASD may exhibited some form of immune system dysregulation.²⁴² The levels of different cytokines in the peripheral blood of ASD have been extensively investigated, and several meta-analyses have reviewed the relationship.^{243–246} A systematic review and meta-analysis showed that the pro-inflammatory cytokines interferon (IFN)- γ , IL-1 β , and IL-6 were elevated in blood of children with ASD, while the anti-inflammatory cytokine transforming growth factor- β 1 was decreased. Levels of several chemokines associated with recruitment of inflammatory cells, including eotaxin, IL-8, and monocyte chemotactic protein-1 (MCP-1), were elevated. Another meta-analysis showed that individuals with autism had lower levels of the anti-inflammatory cytokines IL-10 and IL-1Ra, and higher concentrations of the pro-inflammatory cytokines IFN- γ , IL-1 β , IL-6, and TNF- α than controls.²⁴⁵ Also, meta-regression analyses point to the interaction of latitude, age, and gender with peripheral alterations of associated pro-inflammatory cytokines.²⁴⁴ A recent meta-analysis found that the levels of peripheral IL-6, IL-1b, IL-12p70, MIF, eotaxin-1, MCP-1, IL-8, IL-7, IL-2, IL-12, TNF- α , IL-17, and IL-4 were significantly changed in ASD compared with controls. These findings reinforce the clinical evidence that ASD is associated with an abnormal inflammatory response. These cytokines may be a series of potential biomarkers in the peripheral blood of ASD.²⁴⁶ Besides, previous studies have reported that levels of some pro- and anti-inflammatory cytokines and chemokines are associated with severity of abnormal behavior and impaired developmental and adaptive functioning.^{247–249} For example, IL-6 has been extensively studied and its levels are elevated in ASD and correlate with severity.¹⁹⁹ Indeed, cytokine changes have also been reported in post-mortem brain tissue^{250,251} and PBMCs.²⁴⁸ The changes in mRNA expression of some cytokines were found in whole blood from subjects with ASD.²⁵²

4.3.2 | Oxidative stress-related biomarkers

In terms of markers associated with oxidative stress, a recent meta-analysis showed that blood levels of oxidized glutathione (GSSG), malondialdehyde, homocysteine,

TABLE 2 Research on potential biomarkers of autism spectrum disorder (ASD) based on targeted metabolomics.

No.	Author	Sample	Method	Related metabolites	Metabolic process involved
1	West et al. (2014) ²²⁴	Blood	GC-MS, LC-HRMS	Decreased ^a : homocitrulline, citric acid, lactic acid, heptadecanoic acid, myristic acid Increased ^a : aspartic acid, serine, glutamic acid, glutaric acid, soleucine acid, 2-hydroxyvaleric, 3-aminoisobutyric acid, 5-hydroxynorvaline	Mitochondrial dysfunction, abnormal gut microbiome metabolism
2	Anwar et al. (2018) ²²⁵	Blood	LC-MS/MS	Decreased ^b : FL, G-HI, NFK Increased ^b : CMA, AASA, GSA, arginine, glutamic	Abnormal protein glycosylation, protein oxidative metabolism
3	Delaye et al. (2018) ²²⁶	Blood	Ion exchange chromatography	Decreased ^b : glutamate, serine, ornithine, proline	Glutamate neurotransmission, gastrointestinal abnormalities
4	Lv et al. (2018) ²²⁷	Blood	MS/MS	Decreased ^a : free carnitine, glutaric carnitine, octyl carnitine, 24 carbonyl carnitine, carnosyl carnitine	Mitochondrial dysfunction, abnormal fatty acid metabolism
5	Smith et al. (2019) ²²⁸	Blood	LC-MS/MS, MRM	Decreased ^a : leucine, isoleucine, valine Increased ^a : glutamine, glycine, ornithine	Protein synthesis, neurotransmission, AA/BCAA metabolism
6	Brister et al. (2022) ²²⁹	Blood	LC-MS/MS	Decreased ^b : Nε-fructosyl-lysine Increased ^b : Nω-carboxymethylarginine, Nε-(1-carboxyethyl) lysine, glutamic semialdehyde, 3-nitrotyrosineα-amino adipic semialdehyde	Energy metabolism, amino acid neurotransmitter metabolism, branched-chain amino acid metabolism, nicotinamide metabolism, aminoacyl tRNA biosynthesis
7	Shen et al. (2022) ¹⁴⁹	Blood	LC-MS/MS	Decreased ^b : L-glutamate, pyridoxamine, O-phospho-4-hydroxy-L-threonine, L-aspartate, 4-pyridoxate, phosphatidylethanolamine, 2-oxoglutarate Increased ^b : L-glutamine, creatineacetyl glycine, serylserine, 1-acyl-sn-glycero-3-phosphocholine, ornithine, phosphatidylserine	Mitochondrial dysfunction, oxidative stress, energy metabolism, amino acid, vitamin, lipid metabolism
8	Kaluzna-Czaplinska et al. (2010) ²³⁰	Urine	GC-MS	Increased ^a : urine homovanillic acid, vanilla mandelic acid	Neurotransmitter metabolism, visual perception/memory, repetitive behavior, emotional disorders
9	Mavel et al. (2013) ²³¹	Urine	¹ H- ¹³ C NMR	Decreased ^b : creatine, 3-methylhistidine Increased ^b : glycine, taurine, succinate, β-alanine	Taurine and succinic acid
10	Emond et al. (2013) ²³²	Urine	GC-MS	Decreased ^b : 1H-indole-3-acetate, phosphate, palmitate, stearate, 3-methyladipate, hippurate, vanillylhydracrylate, 4-hydroxyphenyl-2-hydroxyacetate, 3-hydroxyphenylacetate Increased ^b : succinate, glycolate	Intestinal bacteria microbial pathways
11	Nadal-Desbarats et al. (2014) ²³³	Urine	¹ H-NMR, ¹ H- ¹³ C HSQC-NMR	Decreased ^b : glutamate, creatine, 3-methylhistidine Increased ^b : succinate	Energy metabolism disorder, mitochondrial dysfunction, amino acid metabolism of gut microbiota

(Continues)

TABLE 2 (Continued)

No.	Author	Sample	Method	Related metabolites	Metabolic process involved
12	Liu et al. (2019) ²³⁴	Urine	LC-MS/MS	Decreased ^a : Lys, Thr, Car, Pro, EtN, Hcy, Aad, Cit, Ans, 5Ava, Asp Increased ^a : MetS, Harg, 3MHis, Cr, Arg, 5HT, Hyp	Oxidative stress, abnormal ornithine cycle, abnormal lysine metabolism, abnormal 5HT metabolism, E/I balance

Abbreviations: 3MHis, 3-methyl-histidine; 5Ava, 5-aminovaleric acid; 5HT, 5-hydroxytryptamine; Aad, α -amino adipic acid; AA/BCAA, amino acids/branched-chain amino acid; AASA, α -amino adipic semialdehyde; Ans, anserine; Arg, arginine; Asp, aspartic acid; Car, carnosine; Cit, citrulline; CMA, N ω -carboxymethylarginine; Cr, creatinine; E/I, excitation and inhibition; EtN, ethanolamine; FL, N ϵ -fructosyl-lysine; GC-MS, gas chromatography-mass spectrometry; G-HI, hydroimidazol one; GSA, glutamic semialdehyde; Harg, homoarginine; Hcy, homocysteine; HSQC-NMR, heteronuclear singular quantum correlation-nuclear magnetic resonance; Hyp, 4-hydroxyproline; LC-HRMS, liquid chromatography-tandem high-resolution mass spectrometry; LC-MS/MS, liquid chromatograph-tandem mass spectrometry; Lys, lysine; MetS, methionine sulfoxide; MRM, multiple reaction monitoring; NFK, N-formylkynurenine; Pro, proline; TD, typically developing; Thr, threonine.

^aASD compared to TD.

^bASD compared to Ctrl.

S-adenosylhomocysteine, nitric oxide, and copper were higher in children with ASD than in healthy controls, whereas GSH, tGSH, GSH/GSSG, tGSH/GSSG, methionine, cysteine, vitamin B9, vitamin D, vitamin B12, vitamin E, S-adenosylmethionine/S-adenosylhomocysteine, and calcium concentrations were decreased.²⁵³ Given the consistent and large effective size, **GSH metabolism biomarkers have the potential to inform early diagnosis of ASD.**²⁵³

Biomarkers of oxidative stress associated with ASD have recently been reviewed.²⁵⁴ GSH is an important antioxidant in the human body, it is converted to GSSG by GPx and reduced back to GSH by GSH reductase. Elevated levels of oxidative stress in ASD cause increased GSH depletion, which disrupts the dynamic balance between GSH and GSSG. The increased GSH/GSSG ratio is consistent with various pertinent studies, indicating that its efficacy as a reliable indicator of oxidative stress.²⁵⁴ In addition, blood levels of vitamin B9 and B12 were significantly lower in children with autism than in controls,^{253,255,256} and this deficiency resulted in decreased homocysteine remethylation and increased homocysteine levels. Vitamin B12 deficiency may lead to hypomethylation and affect brain development.²⁵⁷ Vitamin deficiencies in children with ASD may be due to poor nutrition, poor digestion, and absorption, or dysbiosis of the intestinal flora.^{95,254} These results clarified blood oxidative stress profile in children with ASD, strengthening clinical evidence of increased oxidative stress implicating in pathogenesis of ASD.

4.3.3 | Mitochondria-related diagnostic markers

A meta-analysis showed that the regulation of mitochondrial biomarkers (including lactate, pyruvate, carnitine, and ubiquinone) was decreased in ASD, and that some of these markers correlated with ASD severity.¹⁷⁷

4.4 | Biomarkers associated with gut microbiota

Changes in the gut microbiota and metabolites may lead to changes in metabolites in blood and urine, providing an opportunity to develop diagnostic tests for early detection of ASD. For example, studies have shown that combining Veillonella and Enterobacteriaceae and 17 bacterial metabolic functions to create diagnostic models can effectively differentiate between ASD and healthy children.²⁵⁸ Several studies have shown that high levels of p-cresol are detected in stool, blood, and urine of children with ASD.^{18,224,259–262} Of interest, p-cresol is only produced in the gastrointestinal tract and correlates with autistic behavior and ASD severity.²⁶³ In addition, other gut microbial metabolites including SCFAs, free amino acids, indoles, and lipopolysaccharides, have been detected in the blood and urine from children with ASD.^{263,264} The analysis of gut microbes and the detection of microbial-derived metabolites in stool, as well as the detection of gut microbial-derived metabolites in blood and urine, may provide an alternative method for the early diagnosis of ASD and is worthy of initiating research (Table S2).

Overall, current research on diagnostic biomarkers for ASD suggests that despite the presence of heterogeneity in ASD, it is still possible to find diagnostic biomarkers. The mechanisms involved in the candidate diagnostic biomarkers identified in the existing studies are convergent. In the high-throughput screening stage, there is still a lack of unified research methods, especially unified experimental conditions, and some studies need to overcome the shortage of small sample sizes. The targeted detection methods is beneficial for the practical application and translation of potential diagnostic biomarkers. It may be a panel composed of biomarkers involved in different mechanisms, or biomarkers related to a certain type of important physiological and metabolic changes.

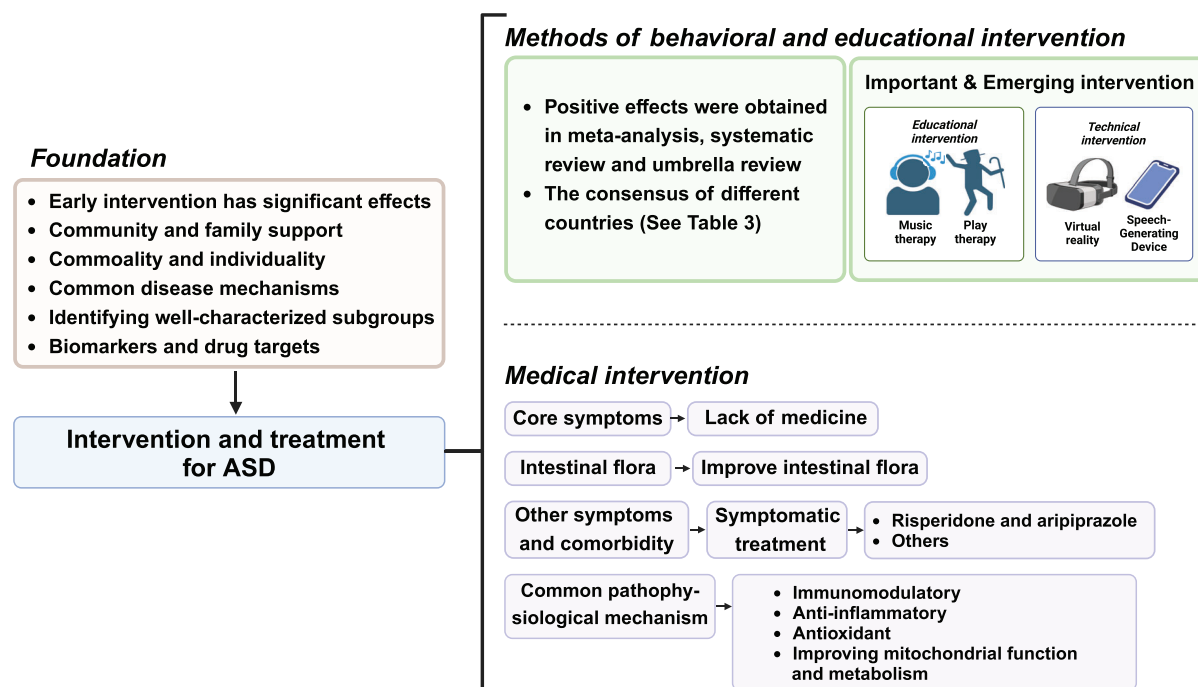


FIGURE 4 The summary of intervention therapy in autism spectrum disorder (ASD). Interventions for ASD mainly include behavioral and educational interventions, and we provide an overview of recent meta-analyses, reviews, and consensus on them, as well as other important and emerging interventions. In terms of pharmacologic interventions, there are still no medications that target the core symptoms, and drug treatment is mainly for other abnormal symptoms or neuropsychiatric comorbidities of ASD. Treatments for common pathophysiology and gut flora are under investigation. Overall, early intervention has a significant effect, with community and family support being important. Given the characteristics of ASD, intervention and treatment need to take into account both commonalities and individuality. Finding common disease mechanisms and identifying well-characterized subgroups will provide the basis for disease diagnosis and treatment, and disease markers and drug targets can influence and inform each other.

5 | INTERVENTION AND TREATMENT OF ASD

Early detection and early intervention are effective for ASD. To date, more than 100 interventions for ASD have been developed, but there is a lack of interventions that target their core symptoms (Figure 4). The goal of ASD treatment is to improve the individual's functioning and well-being. Intervention therapy is more effective in improving ASD-related symptoms (e.g., effective use of language) than ASD characteristics. Early interventions based on mature behavior analysis can help ASD acquire specific skills to address problem behaviors. Here, we reviewed recent meta-analyses, reviews, and consensus on intervention approaches, focusing on approaches that are evidence based and have positive outcomes in some respects (Table 3). In addition, there are many ASD interventions that overlap with each other in terms of operationalization, and there is a tendency for interventions to learn from and integrate with each other, and for each class of approaches to be divided into different "subcategories," as well as some important or emerging approaches (Figure 4). We also made a review in this section.

5.1 | Advances in behavioral and educational interventions

A recent review summarized evidence-supported intervention approaches, including behavioral approaches (e.g., early intensive behavioral intervention [EIBI], discrete trial training), developmental approaches (e.g., developmental, individual differences, relationship-based/Floortime model, preschool autism communication trial [PACT]), naturalistic developmental behavioral intervention (NDBI) (e.g., Early Start Denver Model [ESDM], pivotal response treatment [PRT], JASPER, Project ImPACT), treatment and education of autistic and related communication children (TEACCH), psychotherapy (cognitive behavioral therapy [CBT]), and group social skills interventions.²⁸ In this review, the authors highlight NDBI, parent-mediated interventions, CBT, and the fact that school-aged children with ASD can often receive behavioral, speech, integration, and physical therapy in the school setting.²⁸

A recent umbrella review identified several psychosocial interventions that are expected to improve symptoms associated with ASD at different stages of life, such as

TABLE 3 Recommended behavioral and educational interventions for autism spectrum disorder.

No.	Author	Title	Recommended interventions
1	Xu et al. (2017) ²⁶⁵	Expert consensus on early identification, screening and early intervention of children with autism spectrum disorders	① ABA ② TEACCH ③ ESDM ④ PRT ⑤ PACT ⑥ RIT ⑦ JA
2	Howes et al. (2018) ²⁶⁶	Autism spectrum disorder: consensus guidelines on assessment, treatment, and research from the British Association for Psychopharmacology	① Psychological approaches: social learning program, behavioral and life-skills interventions, cognitive-behavioral interventions, facilitated communication ② Pharmacological treatment: serotonergic agents, glutamatergic agents, GABAergic agents, dopamine receptor blockers ③ Non-pharmacological approaches: social-communication interventions, behavioral interventions, alternative interventions
3	Sandbank et al. (2020) ²⁶⁷	Project aim: autism intervention meta-analysis for studies of young children	① Behavioral approaches: EIBI, DTI, PECS, PBS, ABA ② Developmental approaches: DF, HM ③ NDBI: ESDM, EMT, PRT, JA, SP, EG, RG ④ TEACCH ⑤ Sensory-based interventions ⑥ Animal-assisted interventions: EAAT ⑦ Technology-based interventions: CAI, TTDVD
4	Hyman et al. (2020) ²⁶⁸	Identification, evaluation, and management of children with autism spectrum disorder	① ABA ② Developmental relationship-focus intervention ③ NDBI ④ Parent-mediated treatment or training ⑤ Educational interventions: LEAP, TEACCH ⑥ Other therapeutic interventions: SLI, MT, HM
5	Gosling et al. (2022) ²⁶⁹	Efficacy of psychosocial interventions for autism spectrum disorder: an umbrella review	① PMI ② TECH ③ SSG ④ DEV ⑤ CBT ⑥ NDBI ⑦ TEACCH
6	Hirota et al. (2023) ²⁸	Autism spectrum disorder: a review	① Behavioral approaches: EIBI, DTI ② Developmental approaches: FM, PACT ③ NDBI: ESDM, PRT, JASPER, PI ④ TEACCH ⑤ CBT ⑥ GSSIs ⑦ Pharmacological interventions: aripiprazole, risperidone, methylphenidate, atomoxetine, extended-release guanfacine, melatonin, oxytocin

Abbreviations: ABA, applied behavior analysis; CAI, computer-assisted instruction; CBT, cognitive behavioral therapy; DEV, developmental interventions; DF, DIR/Floortime; DTI, discrete tracking instruction; EAAT, equine-assisted activities and therapy; EG, engagement; EIBI, early intensive behavioral intervention; EMT, enhanced milieu teaching; ESDM, Early Start Denver Model; FM, Floortime model; GSSI, group social skills intervention; HM, Hanen models; JA, joint attention; JASPER, Joint Attention, Symbolic Play, Engagement and Regulation; LEAP, learning experiences and alternative programs for preschoolers and their parents; MT, motor therapies; NDBI, naturalistic developmental behavioral intervention; PACT, preschool autism communication trial; PBS, positive behavioral supports; PECS, picture exchange communication system; PI, parent involvement; PMI, parent-mediated interventions; PRT, pivotal response treatment; RG, regulation; RIT, reciprocal imitation training; SLI, speech and language interventions; SP, symbolic play; SSG, social skill groups; TEACCH, treatment and education of autistic and related communication children; TECH, technology-mediated interventions; TTDVD, the transporters™ DVD series.

early reinforcement behavioral interventions, developmental interventions, natural developmental behavioral interventions, and parent-mediated interventions that improve social communication deficits, overall cognitive abilities, and adaptive behaviors in children with ASD in preschool-age children. The effectiveness of social skills groups in improving social communication deficits and overall ASD symptoms in school-aged children and adolescents is supported by suggestive evidence.²⁶⁹ Another umbrella review identified positive therapeutic effects of behavioral interventions, developmental interventions, NDBI, technology-based interventions, and CBT for several child and family outcomes.²⁷⁰

Moreover, a recent systematic review and meta-analysis summarized the effects of seven early intervention types (behavioral, developmental, NDBI, TEACCH, sensory based, animal assisted, and technology based, aged between 0 and 8 years).²⁶⁷ Of these, significant positive effects were found for behavioral, developmental, and NDBI intervention types. When effect size estimates were limited to studies with a randomized controlled trial (RCT) design, there was evidence of positive summary effects for developmental and NDBI intervention types only. When effect estimates were limited to RCT designs and outcomes without detectable risk of bias, no intervention type showed a significant effect on any outcome.²⁶⁷ Together, despite the availability of multiple intervention models for children with ASD, many have still failed to demonstrate effectiveness in clinical trials. More well-designed RCTs are still needed to gain a clearer understanding of the efficacy of these interventions^{269,271} (Table 4).

When developing a consensus, Chinese experts selected and recommended methods that are supported by randomized controlled studies, have a high level of evidence-based medical evidence, and have a recommendation rating of “strongly recommended” for children with ASD under the age of 3 years and are eligible for implementation in China. The early intervention methods that are supported by randomized controlled studies have evidence-based ratings and “strongly recommended” ratings for children with ASD under 3 years of age and are eligible for implementation in China, including ESDM, PRT, PACT, reciprocal imitation training, and joint attention (JA) training.²⁶⁵

Furthermore, it is also worth mentioning a recent report by the Lancet Commission, which states that individualized, stepped care strategies can meet an individual’s needs throughout the life course, leading to effective assessment and care. The importance of community and family supports in lifelong intervention and treatment for individuals with autism. It further describes the broad spectrum of autism and introduces the concept of “profound autism”; that is, “profound autism” should be paid attention to.²⁸⁵

5.2 | Methods of behavioral intervention

5.2.1 | Applied behavior analysis

Over the past decades, applied behavior analysis (ABA) has been at the forefront of these interventions and has been recommended as a scientifically validated intervention in different countries.²⁸⁶ Due to its high level of acceptance, ABA interventions have also become the benchmark for existing and subsequently developed interventions. In most studies, this approach has shown positive improvements in cognition, language development, social skills and communication, and adaptive behavior in children with ASD, along with reductions in problem behaviors.²⁸⁷

EIBI was the first intensive ABA therapy proposed for ASD, focusing on eliminating atypical behaviors and building learning capacity. Since then, treatments for ASD have weakened structural features while focusing on more complex cognitive and social skills.^{288,289} The EIBI model relies heavily on discrete tracking instruction (DTI), which focuses on reducing extraneous details and teaching skills and learning content in a repetitive and streamlined manner. Ongoing data collection and analysis are key components of DTI,^{290,291} and these data are an important reference for determining how quickly children progress and whether program modifications are needed. In general, DTI is more appropriate for developing JA, play, or imitation skills in children around 2 years of age,²⁹² and may also be of shorter duration as conditions improve to address more complex social behaviors.²⁹³

One of the earliest alternative forms of ABA for ASD was the Natural Language Paradigm (NLP), the earliest natural language training strategy,²⁹⁴ whose main advantage was the integration of therapy into natural, ongoing social, and play activities. PRT²⁹⁵ and ESDM are the naturalistic language strategies with the most empirical evidence to support their effectiveness. As an extension of NLP, the training goals of PRT focus on motivation to interact with others, self-management, self-regulation, and response to multiple cues.²⁹⁶ Its validity has been supported by several studies.

5.2.2 | Physical exercise

Studies have found that children with ASD spend significantly less time per day participating in moderate to vigorous physical activity compared to normally growing children.²⁹⁷ Physical activity of appropriate intensity is a remedy to reduce physical-motor deficits, stereotypic and aggressive behaviors, and improve cognitive functioning in individuals with ASD.^{298–300} In recent years, there has been an explosion of systematic evaluations and

TABLE 4 Related clinical trials related to interventions for autism spectrum disorders (ASD).

No.	Author	Study type	Clinical trial number	Sample size	Conclusion
1	Gabriels et al. (2015) ²⁷²	Retrospective case	NCT 02301195	116	The study further establishes the evidence base supporting EAAT as a viable therapeutic option for children and adolescents with ASD. Further research is needed to examine the joint attention and movement experiences are key THR mechanisms to observe behavioral and social communication improvements in the ASD population.
2	Bearss et al. (2015) ²⁷³	Retrospective case	NCT 01233414	30	Significant improvement (>12 units) in two patients and minor improvement (8–12 units) in eight patients.
3	Bieleninik et al. (2017) ²⁷⁴	Retrospective case	ISRCTN 78923965	167	CBT was efficacious for children with ASD and interfering anxiety, an adapted CBT approach showed additional advantages. CBT can be considered as a professional reference for psychological treatment of autistic children.
4	Sharda et al. (2018) ²⁷⁵	Retrospective case	ISRCTN 26821793	51	The study provides the first evidence that 8–12 weeks of individual music intervention can indeed improve social communication and function brain connectivity.
5	Grimaldi et al. (2018) ²⁷⁶	Retrospective case	NCT 02720900	61	After 1 week of medication, all patients had significant improvements in abnormal behavior and irritability scores, with the risperidone group showing significant improvement at each assessment period.
6	DeVane et al. (2019) ²⁷⁷	Retrospective case	NCT 01333072	364	ASD children who underwent improvisational music therapy and enhanced standard care showed improvement in scale assessment results, but compared with the two methods there was no significant difference in symptom severity based on the ADOS social affect domain over 5 months, indicating that the effect of using improvisational music therapy to reduce symptoms in ASD children was not significant.
7	Voss et al. (2019) ²⁷⁸	Retrospective case	NCT 03569176	71	In terms of socialization, children who received the wearable intervention improved significantly than those who received only standard-of-care behavioral treatments, indicating potential for digital home therapy.
8	Malow et al. (2020) ²⁷⁹	Retrospective case	NCT 01906866	80	Nightly pediatric prolonged-release melatonin at optimal dose of 2, 5, or 10 mg is safe and effective for long-term treatment in children and adolescents with ASD and insomnia, which has no detrimental effects on children's growth and pubertal development.
8	Wood et al. (2020) ²⁸⁰	Retrospective case	NCT 02028247	150	A whole-plant extract BOL-DP-O-01-W which contains CBD and THC in a 20:1 ratio improved disruptive behaviors on one primary outcome measures and on a secondary outcome, an index of ASD core symptoms, with acceptable adverse events.
9	Sikich et al. (2021) ²⁸¹	Retrospective case	NCT 01944046	277	In this trial involving children and adolescents with ASD, 24 weeks of daily intranasal oxytocin treatment, as compared with placebo, did not improve social interaction or other measures of social function related to ASD.

(Continues)

TABLE 4 (Continued)

No.	Author	Study type	Clinical trial number	Sample size	Conclusion
10	Aran et al. (2021) ²⁸²	Retrospective case	NCT 02956226	30	Children on exclusion diets were less likely to report gastrointestinal abnormalities and had lower abundance of the Bifidobacterium and Veillonellaceae families but higher presence of Faecalibacterium and Bacteroidetes. A combined dietary approach resulted in significant changes in gut microbiota composition and metabolism.
11	Scahill et al. (2022) ²⁸³	Retrospective case	NCT 02483910	83	On CELF, DI + TAU did not meet the prespecified difference from TAU. When adjusted for IQ, DI + TAU was superior to TAU on CELF at end point. DI + TAU was superior to TAU on CGI-I.
12	Chu et al. (2023) ²⁸⁴	Retrospective case	ChiCTR 2100053165	78	Potentially positive effects of nonwearable digital therapy plus LSP on core symptoms associated with ASD were found in the study, which leading to a modest improvement in the function of sensory, motor and response inhibition, while reducing impulsivity and hyperactivity in preschoolers with both ASD and ADHD, and VR-CBT was found to be an effective and feasible adjunctive digital tool.

Abbreviations: ADHD, attention deficit hyperactivity disorder; ADOS, autism diagnostic observation schedule; CBD, cannabidiol; CBT, cognitive behavioral therapy; CELF, clinical evaluation of language fundamentals; CGI-I, clinical global impressions-improvement scale; DI, direct instruction language for learning; EAAT, equine-assisted activities and therapies; LSP, learning style profile; TAU, treatment as usual; THC, tetrahydrocannabinol; THR, therapeutic horseback riding; VR-CBT, virtual reality-incorporated cognitive behavioral therapy.

meta-analyses of exercise interventions on stereotypic behaviors, executive functions, and cognitive abilities in children and adolescents with ASD.^{298,299,301–303} Stereotypical behavior patterns of individuals with ASD are alleviated through exercise intervention.^{304,305} It is also beneficial to enhance overall cognitive flexibility and inhibitory control,³⁰¹ and reduce the deficits of social interaction.²⁹⁹ Different types of exercise all play a positive role in alleviating stereotypical behaviors in people with ASD.^{306–318} Although the molecular mechanisms involved in the beneficial effects of exercise on ASD remission are still unknown, the thesis that cytokines released after exercise play an important role in regulating neuronal metabolism, neuroinflammation, and neuroplasticity has been confirmed,^{316,319,320} which may be related to the improvement of symptoms in children with ASD and associated comorbidities.³¹⁶

5.3 | Methods of educational intervention

5.3.1 | Music therapy intervention

There is a long history of using music or music therapy services for non-musical goals (including social skills) for people with ASD.³²¹ Currently, most music therapy applications for ASD are focused on children and adoles-

cents; they are thought to have positive effects on social skills, including engagement behaviors,³²² increased emotional involvement,³²³ improved social interactions,^{324,325} increased social greeting routines,³²⁶ JA behaviors,^{327,328} peer interactions,³²⁹ communication skills,^{274,330,331} and cognitive social skills.³³²

There are also differences in the effectiveness of different types of music therapy for people with ASD. Improvisational music therapy (IMT) is one of the most studied music therapies for children with ASD.^{327,333–336} Family-centered music therapy as an important variant of IMT improves social interactions in families, communities, and parent–child relationships.³³⁷ However, studies have also reported contradictory results or no improvement in some areas.^{338,339} Nevertheless, the feasibility of music therapy interventions for children with ASD has received preliminary support, at least in terms of improving social interaction, verbal communication, initiating behavior, and social–emotional reciprocity.

5.3.2 | Play therapy intervention

Providing children with ASD the opportunity to engage in play activities can strengthen their connections with others and improve social interaction deficits. Patient-centered play therapy is considered an effective evidence-based intervention to improve core issues related to ASD, such

as social skills, communication, emotion regulation, and JA,^{340–343} while a reduction in repetitive behaviors is a strong reason for the validation of the effectiveness of play therapy.³⁴⁴

5.3.3 | Family involved intervention

Research has shown that involving parents in interventions reinforces the effectiveness of the intervention and the prevalence of skills outside of the school setting.³⁴⁵ Family-school partnerships (FSPs) are a child-centered approach, where families and school collaborate and coordinate to produce positive student outcomes in the social, emotional, behavioral, and academic domains.^{346,347} Active parental involvement in education and intervention can have a significant impact on children's learning and development, children's cognitive and language skills,³⁴⁸ school participation, academic achievement,³⁴⁹ and children's problem-solving skills can be improved and enhanced.³⁵⁰ In addition, parental involvement can lead to positive outcomes in prosocial behavior,³⁵¹ peer interaction, and self-regulatory skills.³⁵² In addition to the FSPs mentioned above, parent involvement (PI) is also applicable to family-level interventions and education for children with ASD. Unlike FSP, PI focuses more on the structure and process of activities.^{353,354} Numerous studies have shown that in children with ASD, improvements in social communication and reductions in restrictive and repetitive behaviors occur after interventions using the PI model.^{355–357}

5.4 | The interventions derived from technical devices for ASD

With the rapid development of modern technology, a number of assistive devices for the rehabilitation of people with autism have been developed and put into use. These devices have shown some effectiveness in ASD interventions and deserve further study and evaluation.

5.4.1 | Speech-generating device intervention application

Speech-generating device (SGD) is a portable electronic device that displays various graphic symbols or written language and generates digital or synthetic speech.^{356,357} For children with ASD, whose communication skills are severely lacking, the motor skills tolerance of SGD, the popularity of the output language, and the large storage space make it more socially acceptable.^{358,359} At the same time, the SGD's ability to request, tag, comment,

and answer questions extends its scope of application.³⁶⁰ Previous studies have shown that SGD can improve participants' communication skills,^{358,361,362} while the acquisition of communication skills is a top priority in early intervention programs for ASD.

5.4.2 | Virtual reality technology application

Virtual reality (VR) is a realistic and immersive three-dimensional virtual environment created by interactive software and hardware and is a product of multidisciplinary integration. With the increasing sophistication of VR technology, researchers have successfully applied it to the treatment of people with autism.^{363–368} On this basis, immersive virtual reality has been developed, which is able to reproduce real objects and scenes to a higher degree.^{369–372} However, VR still has some shortcomings, such as the current VR technologies used in ASD treatment are homogeneous and usually can target only one characteristic, and VR simulation scenes are still different from reality. It is expected that VR technology will continue to overcome its limitations and meet the individual needs of people with autism.

5.4.3 | Social bots' application

In contrast to VR, another more tangible technological development, humanoid robot, is also being used for the treatment of ASD. There is growing evidence that robotic assistance has a positive effect on the improvement of the condition of individuals with ASD.^{373–377} Unlike humans, robots operating in predictable and legitimate systems provide a highly structured learning environment for people with ASD, enabling structured and standardized interventions that will help them focus on relevant stimuli, and certain social behaviors may be simulated in the standardized social contexts created by such structured interactions.^{378,379} In the field of autism, there are precedents for the use of robots to assist in the diagnostic process, improve eye contact and spontaneous interaction, turn-taking activities, mimicry, emotion recognition, JA, triadic interactions, etc.^{373,376,380} The results of an induction training for people with ASD involving android robots are also encouraging and make other approaches to intervention using robots worth trying.³⁸¹

6 | MEDICAL INTERVENTION AND POTENTIAL DRUG TARGET

Currently, there is still a lack of drugs to treat the core symptoms of ASD, and research on them is difficult.^{382,383}

Here, we provide an overview of existing pharmacologic therapies for ASD as well as those that target its common pathophysiology and gut microbiota. With the rapid growth of genomics and systems neuroscience, a variety of new molecular targets are surfacing.³⁸⁴

6.1 | Drug treatment for ASD

There are currently no medications available worldwide that specifically target the core symptoms of ASD. More commonly, existing antipsychotics are used to alleviate anxiety,³⁸⁵ depression,^{386,387} or obsessive-compulsive disorder³⁸⁸ in order to ameliorate certain symptoms of ASD, such as ADHD.^{389,390} The US Food and Drug Administration (FDA) has approved two medications, risperidone and aripiprazole, for the pharmacologic treatment of ASD-related irritability and aggression.³⁹¹ However, while there are medications that can alleviate several specific conditions of ASD, the side effects should not be underestimated. For example, aripiprazole can cause side effects, such as drowsiness/sedation, increased sleep duration, and weight gain.³⁹² In addition, selective 5-hydroxytryptamine reuptake inhibitors have been approved by the FDA for a wide range of other disorders, and as a result they are frequently and increasingly used in the treatment of ASD.³⁹³ A recent review based on RCTs suggests that the following medications improve at least one core symptom area compared to placebo: aripiprazole, atoxetine, bumetanide, and risperidone for children/adolescents, and fluoxetine, fluvoxamine, oxytocin, and risperidone for adults.^{394,395}

Consequently, finding common mechanisms to screen for access to targeted drugs remains important and possible.^{396,397} For example, and clinical trials are attempting to use the GABAergic system as a therapeutic strategy for ASD,³⁹⁶ and a recent study showed that a clinically relevant selective ERK pathway inhibitor reverses the core deficits in a mouse model of autism.³⁹⁷

6.2 | Interventions and treatments related to common pathological mechanisms

Given that inflammation and immunity, oxidative stress, and mitochondrial dysfunction are common pathophysiological mechanisms of ASD, there are a number of proposals and studies targeting them, including antioxidant, anti-inflammatory, immunomodulatory, and improving mitochondrial function and metabolism.^{398–400}

Several clinical studies on antioxidant therapy for ASD have been reported, including radicicol,⁴⁰¹ resveratrol,⁴⁰² coenzyme Q10,⁴⁰³ N-acetylcysteine (NAC),⁴⁰⁴ omega-3

fatty acids,⁴⁰⁵ arachidonic acid, and docosahexaenoic acid (DHA),⁴⁰⁶ all of which showed beneficial effects except for resveratrol, whose role is uncertain. Of these, NAC appears to be the most effective antioxidant therapy.⁴⁰⁰ In addition, some studies have demonstrated that supplementation with micronutrients related to redox metabolism (e.g., methyl B12) can be helpful for children with autism.⁴⁰⁷ Other studies have evaluated antioxidant-rich foods, including broccoli,⁴⁰⁸ camel's milk,⁴⁰⁹ and dark chocolate.⁴¹⁰ Notably, there are antioxidants, such as radicicol, resveratrol, naringenin, curcumin, and guanidinium that are not only antioxidants, but also activators of Nrf2, a transcription factor involved in immune dysregulation, inflammation, oxidative stress, and mitochondrial dysfunction.⁴¹¹

However, many of the oxidative stress treatment groups in the study showed strong individual differences, reflecting the heterogeneity of ASD.⁴¹² Therefore, assessing and identifying physiological changes associated with ASD and taking targeted and personalized interventions are more likely to produce positive treatment outcomes.^{398,412} As mentioned in a recent review,³⁹⁸ folic acid supplementation has a positive effect in individuals with ASD identified by autoantibodies to the folate receptor,⁴¹³ whereas methylcobalamin has significant clinical utility when impaired methylation capacity.^{414,415} Mitochondrial regulatory cofactors should be considered when mitochondrial dysfunction is evident. Multivitamin/multimineral formulas, as well as biotin, appear to be appropriate when metabolic abnormalities have been identified, as well as the use of low-dose suramin antipurinergic therapy.⁴¹⁶

In addition, many antioxidant molecules available in nature show anti-inflammatory activity.⁴¹⁷ Some natural antioxidants have been carried out in human studies, such as GSH, vitamin C, NAC, flavonoids, luteolin, quercetin, rutin,^{418,419} palmitoylethanolamide and luteolin,⁴²⁰ DHA, eicosapentaenoic acid (EPA),⁴²¹ and Ginkgo biloba extract 761.⁴²² The most common of the inflammatory signaling pathways is nuclear factor- κ B (NF- κ B), MAPK, and JAK-STAT pathways.⁴²³ Several preclinical studies have been initiated targeting these pathways, including resveratrol,⁴²⁴ palmitoylethanolamide, and luteolin⁴²⁰ against the NF- κ B pathway, and luteolin, diosmine,⁴²⁵ and quercetin⁴²⁶ against the janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway, as well as IL-17A antibody against ERK/MAPK pathway.^{427,428}

Marchezan et al. classified immune and inflammatory interventions for ASD into two broad categories: (1) using radicicol, celecoxib, lenalidomide, hexacosanolate, spironolactone, flavonoid lignoceronin, corticosteroids, oral immunoglobulins, intravenous immunoglobulins, and cellular therapy. (2) Other ASD therapies that have been used or are being studied that are initially

characterized as neither anti-inflammatory nor immunomodulatory at first, but exhibit immunomodulatory capabilities throughout the course of treatment: risperidone, vitamin D, omega-3, ginkgo biloba, l-creatinine, n-acetylcysteine, and microbiome recovery.⁴²⁹ Another narrative review of randomized controlled placebo trials summarizes how immunomodulatory/anti-inflammatory therapeutic agents such as prednisolone, pregnenolone, celecoxib, minocycline, n-acetylcysteine, radicicic acid, and/or omega-3 fatty acids may be useful in the core management of (e.g., stereotypic behaviors) and related (e.g., irritability, hyperactivity, lethargy) symptoms in individuals with autism.⁴³⁰ Likewise, a review based on RCTs concluded that myostatin, haloperidol, folinic acid, guanfacine, omega-3 fatty acids, probiotics, radicicic acid, sodium alginate, and sodium valproate showed some signs of improvement, but were imprecise and unreliable.⁴³¹

Overall, among the several intervention approaches described above, attention needs to be paid to individualization, targeting interventions to subgroups of ASDs with associations with these pathophysiological mechanisms, and improving the efficacy of interventions.^{412,432} The literature on intervention efficacy is limited, and large-scale RCTs are still needed to provide strong evidence as well as the use of biomarkers.⁴³⁰

6.3 | Interventions for ASD targeting the microbiota-gut-brain axis

As research into the mechanisms associated with gut microbial imbalance and the development of ASD has intensified, probiotics, prebiotics, fecal microbiota transplantation (FMT), microbiota transfer therapy, antibiotics, and diet dietary adjustment methods received considerable attention.⁴³³ The beneficial effects of probiotics in improving mood and regulating host behavior have been explored, with specific probiotic therapy reducing the severity of ASD symptoms and developing strategies to manage typical social impairment, communication disorders, perceptual impairment, and behavioral limitations.^{434–439} FMT has been shown to be an established and effective treatment for recurrent *Clostridium difficile* infection.⁴⁴⁰ It has also been proposed as a safe and effective strategy to modulate the symbiosis of the gastrointestinal microbiota and improve behavioral symptoms in children with ASD.^{440,441} Recently, a study showed that FMT improved VPA-induced ASD mice by modulating 5-hydroxytryptaminergic and glutamatergic synaptic signaling pathways.⁴⁴² Modified FMT therapy for children with ASD resulted in significant improvements in gastrointestinal symptoms and ASD symptoms, and follow-up of these individuals after 2 years showed that most of the

improvements in gastrointestinal symptoms were maintained, including significant increases in bacterial diversity and relative abundance of beneficial bacteria such as bifidobacterial.^{440,443} However, due to the complexity of the intestinal microbiota, FMT therapies are still highly heterogeneous with respect to donor selection, material preparation, ideal dosing regimen, and cost-effectiveness, and are still a long way from clinical application. In addition, although these therapeutic modalities have been shown to be safe and effective for short-term supplementation, the safety over long periods of time remains uncertain needs to be validated by additional studies.¹⁹⁵ Overall, autism interventions targeting the gut–brain axis have the potential to be an effective treatment for ASD and are expected to have a positive effect on the improvement of ASD symptoms.⁴⁴⁴

7 | CONCLUSION AND PERSPECTIVES

ASDs have become a common neurological developmental disorder in children. Early detection and early intervention are highly effective. Heterogeneity is a distinctive feature of children with ASD. In addition to the core symptoms, children with ASD are accompanied by different behavioral abnormalities and comorbidities with varying degrees of severity, which exacerbates its complexity and poses challenges for its research and clinical translation.

In this paper, based on the review of the pathological mechanisms of ASD, the progress of its diagnostic markers and intervention methods are reviewed. ASD is caused by genetic and environmental factors and their interactions, its signaling pathways, and mechanisms are convergent. Oxidative stress, inflammation and immunity, mitochondrial dysfunction, and intestinal flora dysregulation are common pathophysiological mechanisms, and they are interrelated. There are also common mechanisms between ASD and comorbidities. These provide the basis for the diagnosis and treatment, at least on a stratified or subclass-based basis. Stratified biomarkers are objective measures used to define subgroups of individuals with common biological characteristics. The treatment and management of children with ASD often involves the management of associated medical problems and psychopathological comorbidities. Therefore, it is important to consider both commonalities and individuality in diagnosis, treatment, and intervention of ASD. Priority is given to personalized diagnosis and treatment for different individuals to improve the precision and efficacy of ASD diagnosis, treatment, and rehabilitation. With the application of high-throughput omics, such as genomics, proteomics, metabolomics, transcriptomics, as well as in-depth mechanism studies, it is expected to find common mechanisms

among individuals with ASD subjects and find specific early diagnostic biomarkers and drug therapeutic targets, which is a key research direction in the future. In terms of ASD intervention and treatment, large-scale RCT-based clinical studies need to be strengthened. In this context, maximizing the sample pools, designing studies with more diverse populations, increasing the number of subjects in RCTs, and defining more accurate patient codifies using gold standard diagnostic instruments are common themes for future developments in the field.

In summary, ASD is a highly heterogeneous, and it is particularly important to improve the understanding of the biological basis of the inherent heterogeneity of ASD, to search for potential common or convergent mechanisms, and to explore the “homogeneity” within the “heterogeneity.” On this basis, the development of diagnosis, intervention and treatment is an effective way to achieve accurate diagnosis and treatment of ASD.

AUTHOR CONTRIBUTIONS

All authors were involved in the conceptualization and design of this paper. Preparation of relevant materials, data collection, and analysis were also performed by all authors. The first draft of the manuscript was written by H.Z., Z.L., G.M., and A.Q. L.S., X.R., X.L., X.Y., and C.F. undertook the revision of the manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGMENTS

We are grateful to the BioRender website (<https://www.biorender.com/>) and PowerPoint for providing the image materials. We also thank the Instrument Analysis Center of Shenzhen University. This work was jointly supported by the National Natural Science Foundation of China (No. 31870825), the Shenzhen-Hong Kong Institute of Brain Science-Shenzhen Fundamental Research Institutions (No. 2023SHIBS0003), and the Shenzhen Bureau of Science, Technology, and Information (No. JCYJ20170412110026229).

CONFLICT OF INTEREST STATEMENT

All authors read and approved the final manuscript and declare they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data availability is not applicable to this review as no new data were created or analyzed in this study.

ETHICS STATEMENT

Not applicable.

ORCID

Hongbin Zhuang  <https://orcid.org/0000-0003-2661-3182>
Liming Shen  <https://orcid.org/0000-0003-0074-8556>

REFERENCES

1. Kanner L. Autistic disturbances of affective contact. *Acta Paedopsychiatr.* 1968;35(4):100-136.
2. Asperger H. Die “Autistischen psychopathen” im Kindesalter. *Arch Psychiat Nervenkrankheiten.* 1944;117(1):76-136.
3. Wing L, Gould J. Systematic recording of behaviors and skills of retarded and psychotic children. *J Autism Child Schizophr.* 1978;8(1):79-97.
4. APA. Diagnostic and Statistical Manual of Mental Disorders. *American Psychiatric Association;* 2013.
5. Fombonne E. Editorial: the rising prevalence of autism. *J Child Psychol Psychiatry.* 2018;59(7):717-720.
6. Lyall K, Croen L, Daniels J, et al. The changing epidemiology of autism spectrum disorders. *Annu Rev Public Health.* 2017;38:81-102.
7. Zeidan J, Fombonne E, Scorch J, et al. Global prevalence of autism: a systematic review update. *Autism Res.* 2022;15(5):778-790.
8. Maenner MJ, Shaw KA, Bakian AV, et al. Prevalence and characteristics of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 Sites, United States, 2018. *MMWR Surveill Summ.* 2021;70(11):1-16.
9. Maenner MJ, Warren Z, Williams AR, et al. Prevalence and characteristics of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 Sites, United States, 2020. *MMWR Surveill Summ.* 2023;72(2):1-14.
10. Zhou H, Xu X, Yan W, et al. Prevalence of autism spectrum disorder in China: a nationwide multi-center population-based study among children aged 6 to 12 years. *Neurosci Bull.* 2020;36(9):961-971.
11. Zhang ZC, Han J. The first national prevalence of autism spectrum disorder in China. *Neurosci Bull.* 2020;36(9):959-960.
12. Tick B, Bolton P, Happé F, Rutter M, Rijdsdijk F. Heritability of autism spectrum disorders: a meta-analysis of twin studies. *J Child Psychol Psychiatry.* 2016;57(5):585-595.
13. Constantino JN, Zhang Y, Frazier T, Abbacchi AM, Law P. Sibling recurrence and the genetic epidemiology of autism. *Am J Psychiatry.* 2010;167(11):1349-1356.
14. Palmer N, Beam A, Agniel D, et al. Association of sex with recurrence of autism spectrum disorder among siblings. *JAMA Pediatr.* 2017;171(11):1107-1112.
15. Miles JH, Takahashi TN, Bagby S, et al. Essential versus complex autism: definition of fundamental prognostic subtypes. *Am J Med Genet A.* 2005;135(2):171-180.
16. Cohen D, Pichard N, Tordjman S, et al. Specific genetic disorders and autism: clinical contribution towards their identification. *J Autism Dev Disord.* 2005;35(1):103-116.
17. Fakhoury M. Autistic spectrum disorders: a review of clinical features, theories and diagnosis. *Int J Dev Neurosci.* 2015;43:70-77.
18. Shen L, Liu X, Zhang H, Lin J, Feng C, Iqbal J. Biomarkers in autism spectrum disorders: current progress. *Clin Chim Acta.* 2020;502:41-54.
19. Won H, Mah W, Kim E. Autism spectrum disorder causes, mechanisms, and treatments: focus on neuronal synapses. *Front Mol Neurosci.* 2013;6:19.

20. Voineagu I, Wang X, Johnston P, et al. Transcriptomic analysis of autistic brain reveals convergent molecular pathology. *Nature*. 2011;474(7351):380-384.
21. Parikshak NN, Swarup V, Belgard TG, et al. Genome-wide changes in lncRNA, splicing, and regional gene expression patterns in autism. *Nature*. 2016;540(7633):423-427.
22. Velmeshev D, Magistri M, Mazza EMC, et al. Cell-type-specific analysis of molecular pathology in autism identifies common genes and pathways affected across neocortical regions. *Mol Neurobiol*. 2020;57(5):2279-2289.
23. Velmeshev D, Schirmer L, Jung D, et al. Single-cell genomics identifies cell type-specific molecular changes in autism. *Science*. 2019;364(6441):685-689.
24. Paulsen B, Velasco S, Kedaigle AJ, et al. Autism genes converge on asynchronous development of shared neuron classes. *Nature*. 2022;602(7896):268-273.
25. Masi A, DeMayo MM, Glozier N, Guastella AJ. An overview of autism spectrum disorder, heterogeneity and treatment options. *Neurosci Bull*. 2017;33(2):183-193.
26. Jeste SS, Geschwind DH. Disentangling the heterogeneity of autism spectrum disorder through genetic findings. *Nat Rev Neurol*. 2014;10(2):74-81.
27. Rosen TE, Mazefsky CA, Vasa RA, Lerner MD. Co-occurring psychiatric conditions in autism spectrum disorder. *Int Rev Psychiatry*. 2018;30(1):40-61.
28. Hirota T, King BH. Autism spectrum disorder: a review. *JAMA*. 2023;329(2):157-168.
29. Howlin P, Goode S, Hutton J, Rutter M. Savant skills in autism: psychometric approaches and parental reports. *Philos Trans R Soc Lond B Biol Sci*. 2009;364(1522):1359-1367.
30. Geschwind DH. Advances in autism. *Annu Rev Med*. 2009;60:367-380.
31. Werling DM, Geschwind DH. Sex differences in autism spectrum disorders. *Curr Opin Neurol*. 2013;26(2):146-153.
32. Carayol J, Schellenberg GD, Dombroski B, Genin E, Rousseau F, Dawson G. Autism risk assessment in siblings of affected children using sex-specific genetic scores. *Mol Autism*. 2011;2(1):17.
33. Steeb H, Ramsey JM, Guest PC, et al. Serum proteomic analysis identifies sex-specific differences in lipid metabolism and inflammation profiles in adults diagnosed with Asperger syndrome. *Mol Autism*. 2014;5(1):4.
34. Sato D, Lionel AC, Leblond CS, et al. SHANK1 deletions in males with autism spectrum disorder. *Am J Hum Genet*. 2012;90(5):879-887.
35. Tropeano M, Ahn JW, Dobson RJ, et al. Male-biased autosomal effect of 16p13.11 copy number variation in neurodevelopmental disorders. *PLoS One*. 2013;8(4):e61365.
36. Tropeano M, Howley D, Gazzellone MJ, et al. Microduplications at the pseudoautosomal SHOX locus in autism spectrum disorders and related neurodevelopmental conditions. *J Med Genet*. 2016;53(8):536-547.
37. Mitra I, Tsang K, Ladd-Acosta C, et al. Pleiotropic mechanisms indicated for sex differences in autism. *PLoS Genet*. 2016;12(11):e1006425.
38. Wing L. Sex ratios in early childhood autism and related conditions. *Psychiatry Res*. 1981;5(2):129-137.
39. Kreiser NL, White SW. ASD in females: are we overstating the gender difference in diagnosis? *Clin Child Fam Psychol Rev*. 2014;17(1):67-84.
40. Tsai L, Stewart MA, August G. Implication of sex differences in the familial transmission of infantile autism. *J Autism Dev Disord*. 1981;11(2):165-173.
41. Goin-Kochel RP, Abbacchi A, Constantino JN. Lack of evidence for increased genetic loading for autism among families of affected females: a replication from family history data in two large samples. *Autism*. 2007;11(3):279-286.
42. Messinger DS, Young GS, Webb SJ, et al. Early sex differences are not autism-specific: a Baby Siblings Research Consortium (BSRC) study. *Mol Autism*. 2015;6:32.
43. Gazzellone MJ, Zhou X, Lionel AC, et al. Copy number variation in Han Chinese individuals with autism spectrum disorder. *J Neurodev Disord*. 2014;6(1):34.
44. Rosti RO, Sadek AA, Vaux KK, Gleeson JG. The genetic landscape of autism spectrum disorders. *Dev Med Child Neurol*. 2014;56(1):12-18.
45. Lombardo MV, Barnes JL, Wheelwright SJ, Baron-Cohen S. Self-referential cognition and empathy in autism. *PLoS One*. 2007;2(9):e883.
46. Damaj L, Lupien-Meilleur A, Lortie A, et al. CACNA1A haploinsufficiency causes cognitive impairment, autism and epileptic encephalopathy with mild cerebellar symptoms. *Eur J Hum Genet*. 2015;23(11):1505-1512.
47. Fernandes JM, Cajão R, Lopes R, Jerónimo R, Barahona-Corrêa JB. Social cognition in schizophrenia and autism spectrum disorders: a systematic review and meta-analysis of direct comparisons. *Front Psychiatry*. 2018;9:504.
48. Chen G, Yu B, Tan S, et al. GIGYF1 disruption associates with autism and impaired IGF-1R signaling. *J Clin Invest*. 2022;132(19):e159806.
49. Goh S, Peterson BS. Imaging evidence for disturbances in multiple learning and memory systems in persons with autism spectrum disorders. *Dev Med Child Neurol*. 2012;54(3):208-213.
50. Connolly S, Anney R, Gallagher L, Heron EA. Evidence of assortative mating in autism spectrum disorder. *Biol Psychiatry*. 2019;86(4):286-293.
51. Ballester P, Richdale AL, Baker EK, Peiró AM. Sleep in autism: a biomolecular approach to aetiology and treatment. *Sleep Med Rev*. 2020;54:101357.
52. Abdul F, Sreenivas N, Kommu JVS, et al. Disruption of circadian rhythm and risk of autism spectrum disorder: role of immune-inflammatory, oxidative stress, metabolic and neurotransmitter pathways. *Rev Neurosci*. 2022;33(1):93-109.
53. Yenen AS, Çak HT. Melatonin and circadian rhythm in autism spectrum disorders. *Türk Psikiyatri Derg*. 2020;31(3):201-211.
54. Ganesan H, Balasubramanian V, Iyer M, et al. mTOR signalling pathway—a root cause for idiopathic autism? *BMB Rep*. 2019;52(7):424-433.
55. Zhang J, Zhang JX, Zhang QL. PI3K/AKT/mTOR-mediated autophagy in the development of autism spectrum disorder. *Brain Res Bull*. 2016;125:152-158.
56. Yeung KS, Tso WWY, Ip JJK, et al. Identification of mutations in the PI3K-AKT-mTOR signalling pathway in patients with macrocephaly and developmental delay and/or autism. *Mol Autism*. 2017;8:66.

57. Costa-Mattioli M, Monteggia LM. mTOR complexes in neurodevelopmental and neuropsychiatric disorders. *Nat Neurosci*. 2013;16(11):1537-1543.
58. Niere F, Namjoshi S, Song E, et al. Analysis of proteins that rapidly change upon mechanistic/mammalian target of rapamycin complex 1 (mTORC1) repression identifies Parkinson protein 7 (PARK7) as a novel protein aberrantly expressed in tuberous sclerosis complex (TSC). *Mol Cell Proteomics*. 2016;15(2):426-444.
59. Lombardo MV, Moon HM, Su J, Palmer TD, Courchesne E, Pramparo T. Maternal immune activation dysregulation of the fetal brain transcriptome and relevance to the pathophysiology of autism spectrum disorder. *Mol Psychiatry*. 2018;23(4):1001-1013.
60. Mencer S, Kartawy M, Lendenfeld F, et al. Proteomics of autism and Alzheimer's mouse models reveal common alterations in mTOR signaling pathway. *Transl Psychiatry*. 2021;11(1):480.
61. Wesseling H, Elgersma Y, Bahn S. A brain proteomic investigation of rapamycin effects in the Tsc1(+/-) mouse model. *Mol Autism*. 2017;8:41.
62. Gazestani VH, Pramparo T, Nalabolu S, et al. A perturbed gene network containing PI3K-AKT, RAS-ERK and WNT- β -catenin pathways in leukocytes is linked to ASD genetics and symptom severity. *Nat Neurosci*. 2019;22(10):1624-1634.
63. Wan H, Wang Q, Chen X, et al. WDR45 contributes to neurodegeneration through regulation of ER homeostasis and neuronal death. *Autophagy*. 2020;16(3):531-547.
64. Tang G, Gudsnek K, Kuo SH, et al. Loss of mTOR-dependent macroautophagy causes autistic-like synaptic pruning deficits. *Neuron*. 2014;83(5):1131-1143.
65. Pucilowska J, Vithayathil J, Pagani M, et al. Pharmacological inhibition of ERK signaling rescues pathophysiology and behavioral phenotype associated with 16p11.2 chromosomal deletion in mice. *J Neurosci*. 2018;38(30):6640-6652.
66. Eichler EE, Zimmerman AW. A hot spot of genetic instability in autism. *N Engl J Med*. 2008;358(7):737-739.
67. Murari K, Abushaibah A, Rho JM, Turner RW, Cheng N. A clinically relevant selective ERK-pathway inhibitor reverses core deficits in a mouse model of autism. *EBioMedicine*. 2023;91:104565.
68. Li Q, Shi Y, Li X, et al. Proteomic-based approach reveals the involvement of apolipoprotein A-I in related phenotypes of autism spectrum disorder in the BTBR mouse model. *Int J Mol Sci*. 2022;23(23):15290.
69. Gilman SR, Iossifov I, Levy D, Ronemus M, Wigler M, Vitkup D. Rare de novo variants associated with autism implicate a large functional network of genes involved in formation and function of synapses. *Neuron*. 2011;70(5):898-907.
70. Matic K, Eninger T, Bardoni B, Davidovic L, Macek B. Quantitative phosphoproteomics of murine Fmr1-KO cell lines provides new insights into FMRP-dependent signal transduction mechanisms. *J Proteome Res*. 2014;13(10):4388-4397.
71. D'Incal C, Broos J, Torfs T, Kooy RF, Vanden Berghe W. Towards kinase inhibitor therapies for fragile X syndrome: tweaking twists in the autism spectrum kinase signaling network. *Cells*. 2022;11(8):1325.
72. Wesseling H, Guest PC, Lee CM, Wong EH, Rahmoune H, Bahn S. Integrative proteomic analysis of the NMDA NR1 knockdown mouse model reveals effects on central and peripheral pathways associated with schizophrenia and autism spectrum disorders. *Mol Autism*. 2014;5:38.
73. Yang J, He X, Qian L, et al. Association between plasma proteome and childhood neurodevelopmental disorders: a two-sample Mendelian randomization analysis. *EBioMedicine*. 2022;78:103948.
74. Berg JM, Lee C, Chen L, et al. JAKMIP1, a novel regulator of neuronal translation, modulates synaptic function and autistic-like behaviors in mouse. *Neuron*. 2015;88(6):1173-1191.
75. Nishimura Y, Martin CL, Vazquez-Lopez A, et al. Genome-wide expression profiling of lymphoblastoid cell lines distinguishes different forms of autism and reveals shared pathways. *Hum Mol Genet*. 2007;16(14):1682-1698.
76. Pourtavakoli A, Ghafouri-Fard S. Calcium signaling in neurodevelopment and pathophysiology of autism spectrum disorders. *Mol Biol Rep*. 2022;49(11):10811-10823.
77. Hutchins BI, Li L, Kalil K. Wnt-induced calcium signaling mediates axon growth and guidance in the developing corpus callosum. *Sci Signal*. 2012;5(206):pt1.
78. Reilly J, Gallagher L, Leader G, Shen S. Coupling of autism genes to tissue-wide expression and dysfunction of synapse, calcium signalling and transcriptional regulation. *PLoS One*. 2020;15(12):e0242773.
79. Wen Y, Alshikho MJ, Herbert MR. Pathway network analyses for autism reveal multisystem involvement, major overlaps with other diseases and convergence upon MAPK and calcium signaling. *PLoS One*. 2016;11(4):e0153329.
80. Daroles L, Gribaudo S, Doulazmi M, et al. Fragile X mental retardation protein and dendritic local translation of the alpha subunit of the calcium/calmodulin-dependent kinase II messenger RNA are required for the structural plasticity underlying olfactory learning. *Biol Psychiatry*. 2016;80(2):149-159.
81. Baucum AJ 2nd, Shonesy BC, Rose KL, Colbran RJ. Quantitative proteomics analysis of CaMKII phosphorylation and the CaMKII interactome in the mouse forebrain. *ACS Chem Neurosci*. 2015;6(4):615-631.
82. Bezprozvanny I, Hiesinger PR. The synaptic maintenance problem: membrane recycling, Ca²⁺ homeostasis and late onset degeneration. *Mol Neurodegener*. 2013;8:23.
83. Guimarães-Souza EM, Joselevitch C, Britto LRG, Chiavegatto S. Retinal alterations in a pre-clinical model of an autism spectrum disorder. *Mol Autism*. 2019;10:19.
84. Rubinstein M, Westenbroek RE, Yu FH, Jones CJ, Scheuer T, Catterall WA. Genetic background modulates impaired excitability of inhibitory neurons in a mouse model of Dravet syndrome. *Neurobiol Dis*. 2015;73:106-117.
85. Rubenstein JL, Merzenich MM. Model of autism: increased ratio of excitation/inhibition in key neural systems. *Genes Brain Behav*. 2003;2(5):255-267.
86. Nelson SB, Valakh V. Excitatory/inhibitory balance and circuit homeostasis in autism spectrum disorders. *Neuron*. 2015;87(4):684-698.
87. Han S, Tai C, Westenbroek RE, et al. Autistic-like behaviour in Scn1a+/- mice and rescue by enhanced GABA-mediated neurotransmission. *Nature*. 2012;489(7416):385-390.
88. Gandal MJ, Haney JR, Wamsley B, et al. Broad transcriptomic dysregulation occurs across the cerebral cortex in ASD. *Nature*. 2022;611(7936):532-539.

89. Purcell AE, Jeon OH, Zimmerman AW, Blue ME, Pevsner J. Postmortem brain abnormalities of the glutamate neurotransmitter system in autism. *Neurology*. 2001;57(9):1618-1628.
90. Abraham JR, Szoko N, Barnard J, et al. Proteomic investigations of autism brain identify known and novel pathogenetic processes. *Sci Rep*. 2019;9(1):13118.
91. Pagani M, Barsotti N, Bertero A, et al. mTOR-related synaptic pathology causes autism spectrum disorder-associated functional hyperconnectivity. *Nat Commun*. 2021;12(1):6084.
92. Hoffmann A, Spengler D. Single-cell transcriptomics supports a role of CHD8 in autism. *Int J Mol Sci*. 2021;22(6):3261.
93. Lussu M, Noto A, Masili A, et al. The urinary (1) H-NMR metabolomics profile of an Italian autistic children population and their unaffected siblings. *Autism Res*. 2017;10(6):1058-1066.
94. Bitar T, Mavel S, Emond P, et al. Identification of metabolic pathway disturbances using multimodal metabolomics in autistic disorders in a Middle Eastern population. *J Pharm Biomed Anal*. 2018;152:57-65.
95. Gevi F, Belardo A, Zolla L. A metabolomics approach to investigate urine levels of neurotransmitters and related metabolites in autistic children. *Biochim Biophys Acta Mol Basis Dis*. 2020;1866(10):165859.
96. Gagliano A, Murgia F, Capodiferro AM, et al. (1)H-NMR-based metabolomics in autism spectrum disorder and pediatric acute-onset neuropsychiatric syndrome. *J Clin Med*. 2022;11(21):6493.
97. Droogers WJ, MacGillavry HD. Plasticity of postsynaptic nanostructure. *Mol Cell Neurosci*. 2023;124:103819.
98. Jung S, Park M. Shank postsynaptic scaffolding proteins in autism spectrum disorder: mouse models and their dysfunctions in behaviors, synapses, and molecules. *Pharmacol Res*. 2022;182:106340.
99. Wise A, Schatoff E, Flores J, et al. Drosophila-Cdhl (Rap/Fzr) a regulatory subunit of APC/C is required for synaptic morphology, synaptic transmission and locomotion. *Int J Dev Neurosci*. 2013;31(7):624-633.
100. Schmid A, Qin G, Wichmann C, et al. Non-NMDA-type glutamate receptors are essential for maturation but not for initial assembly of synapses at Drosophila neuromuscular junctions. *J Neurosci*. 2006;26(44):11267-11277.
101. Oliva C, Escobedo P, Astorga C, Molina C, Sierralta J. Role of the MAGUK protein family in synapse formation and function. *Dev Neurobiol*. 2012;72(1):57-72.
102. Davenport EC, Szulc BR, Drew J, et al. Autism and schizophrenia-associated CYFIP1 regulates the balance of synaptic excitation and inhibition. *Cell Rep*. 2019;26(8):2037-2051.
103. Iossifov I, O'Roak BJ, Sanders SJ, et al. The contribution of de novo coding mutations to autism spectrum disorder. *Nature*. 2014;515(7526):216-221.
104. Li J, Zhang W, Yang H, et al. Spatiotemporal profile of postsynaptic interactomes integrates components of complex brain disorders. *Nat Neurosci*. 2017;20(8):1150-1161.
105. Jin C, Lee Y, Kang H, et al. Increased ribosomal protein levels and protein synthesis in the striatal synaptosome of Shank3-overexpressing transgenic mice. *Mol Brain*. 2021;14(1):39.
106. Reim D, Distler U, Halbedl S, et al. Proteomic analysis of post-synaptic density fractions from Shank3 mutant mice reveals brain region specific changes relevant to autism spectrum disorder. *Front Mol Neurosci*. 2017;10:26.
107. Lee S, Chun HS, Lee J, et al. Plausibility of the zebrafish embryos/larvae as an alternative animal model for autism: a comparison study of transcriptome changes. *PLoS One*. 2018;13(9):e0203543.
108. Yoo YE, Yoo T, Kang H, Kim E. Brain region and gene dosage-differential transcriptomic changes in Shank2-mutant mice. *Front Mol Neurosci*. 2022;15:977305.
109. Gupta P, Uner OE, Nayak S, Grant GR, Kalb RG. SAP97 regulates behavior and expression of schizophrenia risk enriched gene sets in mouse hippocampus. *PLoS One*. 2018;13(7):e0200477.
110. Jaworski J, Kapitein LC, Gouveia SM, et al. Dynamic microtubules regulate dendritic spine morphology and synaptic plasticity. *Neuron*. 2009;61(1):85-100.
111. Alfieri A, Sorokina O, Adrait A, et al. Synaptic interactome mining reveals p140Cap as a new hub for PSD proteins involved in psychiatric and neurological disorders. *Front Mol Neurosci*. 2017;10:212.
112. Repetto D, Camera P, Melani R, et al. p140Cap regulates memory and synaptic plasticity through Src-mediated and citron-N-mediated actin reorganization. *J Neurosci*. 2014;34(4):1542-1553.
113. Thacker S, Eng C. Transcriptome-(phospho)proteome characterization of brain of a germline model of cytoplasmic-predominant Pten expression with autism-like phenotypes. *NPJ Genom Med*. 2021;6(1):42.
114. Quesnel-Vallières M, Dargaei Z, Irimia M, et al. Misregulation of an activity-dependent splicing network as a common mechanism underlying autism spectrum disorders. *Mol Cell*. 2016;64(6):1023-1034.
115. Walter C, Marada A, Suhm T, et al. Global kinome profiling reveals DYRK1A as critical activator of the human mitochondrial import machinery. *Nat Commun*. 2021;12(1):4284.
116. Broek JA, Guest PC, Rahmoune H, Bahn S. Proteomic analysis of post mortem brain tissue from autism patients: evidence for opposite changes in prefrontal cortex and cerebellum in synaptic connectivity-related proteins. *Mol Autism*. 2014;5:41.
117. Abraham JR, Barnard J, Wang H, et al. Proteomic investigations of human HERC2 mutants: insights into the pathobiology of a neurodevelopmental disorder. *Biochem Biophys Res Commun*. 2019;512(2):421-427.
118. Zhang P, Omanska A, Ander BP, Gandal MJ, Stamova B, Schumann CM. Neuron-specific transcriptomic signatures indicate neuroinflammation and altered neuronal activity in ASD temporal cortex. *Proc Natl Acad Sci U S A*. 2023;120(10):e2206758120.
119. Boccuto L, Chen CF, Pittman AR, et al. Decreased tryptophan metabolism in patients with autism spectrum disorders. *Mol Autism*. 2013;4(1):16.
120. Smith AM, Natowicz MR, Braas D, et al. A metabolomics approach to screening for autism risk in the children's autism metabolome project. *Autism Res*. 2020;13(8):1270-1285.
121. Brister D, Werner BA, Gideon G, et al. Central nervous system metabolism in autism, epilepsy and developmental delays: a cerebrospinal fluid analysis. *Metabolites*. 2022;12(5):371.
122. Nolin SL, Napoli E, Flores A, Hagerman RJ, Giulivi C. Deficits in prenatal serine biosynthesis underlie the mitochondrial

- dysfunction associated with the autism-linked FMR1 gene. *Int J Mol Sci.* 2021;22(11):5886.
123. Kartawy M, Khaliulin I, Amal H. Systems biology reveals S-nitrosylation-dependent regulation of mitochondrial functions in mice with Shank3 mutation associated with autism spectrum disorder. *Brain Sci.* 2021;11(6):677.
 124. Golubiani G, Lagani V, Solomon R, Müller M. Metabolomic fingerprint of Mecp2-deficient mouse cortex: evidence for a pronounced multi-faceted metabolic component in Rett syndrome. *Cells.* 2021;10(9):2494.
 125. Kim HY, Lee YJ, Kim SJ, et al. Metabolomics profiling of valproic acid-induced symptoms resembling autism spectrum disorders using 1H NMR spectral analysis in rat model. *J Toxicol Environ Health A.* 2022;85(1):1-13.
 126. Meyer U. Neurodevelopmental resilience and susceptibility to maternal immune activation. *Trends Neurosci.* 2019;42(11):793-806.
 127. Estes ML, McAllister AK. Maternal immune activation: implications for neuropsychiatric disorders. *Science.* 2016;353(6301):772-777.
 128. Usui N, Kobayashi H, Shimada S. Neuroinflammation and oxidative stress in the pathogenesis of autism spectrum disorder. *Int J Mol Sci.* 2023;24(6):5487.
 129. Zhu Y, Mordaunt CE, Durbin-Johnson BP, et al. Expression changes in epigenetic gene pathways associated with one-carbon nutritional metabolites in maternal blood from pregnancies resulting in autism and non-typical neurodevelopment. *Autism Res.* 2021;14(1):11-28.
 130. Murakami Y, Imamura Y, Kasahara Y, et al. Maternal inflammation with elevated kynurenine metabolites is related to the risk of abnormal brain development and behavioral changes in autism spectrum disorder. *Cells.* 2023;12(7):1087.
 131. Nevalainen T, Kananen L, Marttila S, et al. Increased paternal age at conception is associated with transcriptomic changes involved in mitochondrial function in elderly individuals. *PLoS One.* 2016;11(11):e0167028.
 132. Buizer-Voskamp JE, Laan W, Staal WG, et al. Paternal age and psychiatric disorders: findings from a Dutch population registry. *Schizophr Res.* 2011;129(2-3):128-132.
 133. Wang L, Zheng R, Xu Y, et al. Altered metabolic characteristics in plasma of young boys with autism spectrum disorder. *J Autism Dev Disord.* 2022;52(11):4897-4907.
 134. Cao C, Wang D, Zou M, Sun C, Wu L. Untargeted metabolomics reveals hepatic metabolic disorder in the BTBR mouse model of autism and the significant role of liver in autism. *Cell Biochem Funct.* 2023;41(5):553-563.
 135. O'Neill J, Bansal R, Goh S, Rodie M, Sawardekar S, Peterson BS. Parsing the heterogeneity of brain metabolic disturbances in autism spectrum disorder. *Biol Psychiatry.* 2020;87(2):174-184.
 136. Marballi K, MacDonald JL. Proteomic and transcriptional changes associated with MeCP2 dysfunction reveal nodes for therapeutic intervention in Rett syndrome. *Neurochem Int.* 2021;148:105076.
 137. Ayhan F, Konopka G. Regulatory genes and pathways disrupted in autism spectrum disorders. *Prog Neuropsychopharmacol Biol Psychiatry.* 2019;89:57-64.
 138. Wang L, Wang B, Wu C, Wang J, Sun M. Autism spectrum disorder: neurodevelopmental risk factors, biological mechanism, and precision therapy. *Int J Mol Sci.* 2023;24(3):1819.
 139. Guo H, Wang T, Wu H, et al. Inherited and multiple de novo mutations in autism/developmental delay risk genes suggest a multifactorial model. *Mol Autism.* 2018;9:64.
 140. Xie Y, Xu Z, Xia M, et al. Alterations in connectome dynamics in autism spectrum disorder: a harmonized mega- and meta-analysis study using the autism brain imaging data exchange dataset. *Biol Psychiatry.* 2022;91(11):945-955.
 141. Sztainberg Y, Zoghbi HY. Lessons learned from studying syndromic autism spectrum disorders. *Nat Neurosci.* 2016;19(11):1408-1417.
 142. Shen L, Zhang H, Lin J, et al. A combined proteomics and metabolomics profiling to investigate the genetic heterogeneity of autistic children. *Mol Neurobiol.* 2022;59(6):3529-3545.
 143. Ellegood J, Anagnostou E, Babineau BA, et al. Clustering autism: using neuroanatomical differences in 26 mouse models to gain insight into the heterogeneity. *Mol Psychiatry.* 2015;20(1):118-125.
 144. Brown EA, Lautz JD, Davis TR, et al. Clustering the autisms using glutamate synapse protein interaction networks from cortical and hippocampal tissue of seven mouse models. *Mol Autism.* 2018;9:48.
 145. Jin X, Simmons SK, Guo A, et al. In vivo Perturb-Seq reveals neuronal and glial abnormalities associated with autism risk genes. *Science.* 2020;370(6520):eaaz6063.
 146. Carbonell AU, Freire-Cobo C, Deyneko IV, et al. Comparing synaptic proteomes across five mouse models for autism reveals converging molecular similarities including deficits in oxidative phosphorylation and Rho GTPase signaling. *Front Aging Neurosci.* 2023;15:1152562.
 147. Zerbi V, Pagani M, Markicevic M, et al. Brain mapping across 16 autism mouse models reveals a spectrum of functional connectivity subtypes. *Mol Psychiatry.* 2021;26(12):7610-7620.
 148. Willsey HR, Exner CRT, Xu Y, et al. Parallel in vivo analysis of large-effect autism genes implicates cortical neurogenesis and estrogen in risk and resilience. *Neuron.* 2021;109(5):e8.
 149. Shen L, Zhang H, Lin J, et al. A combined proteomics and metabolomics profiling to investigate the genetic heterogeneity of autistic children. *Mol Neurobiol.* 2022;59(6):3529-3545.
 150. Pintacuda G, Hsu Y-HH, Tsafou K, et al. Protein interaction studies in human induced neurons indicate convergent biology underlying autism spectrum disorders. *Cell Genomics.* 2023;3(3):100250.
 151. Weinschutz Mendes H, Neelakantan U, Liu Y, et al. High-throughput functional analysis of autism genes in zebrafish identifies convergence in dopaminergic and neuroimmune pathways. *Cell Rep.* 2023;42(3):112243.
 152. Willsey HR, Exner CRT, Xu Y, et al. Parallel in vivo analysis of large-effect autism genes implicates cortical neurogenesis and estrogen in risk and resilience. *Neuron.* 2021;109(8):1409.
 153. Peng J, Zhou Y, Wang K. Multiplex gene and phenotype network to characterize shared genetic pathways of epilepsy and autism. *Sci Rep.* 2021;11(1):952.
 154. Moyses-Oliveira M, Yadav R, Erdin S, Talkowski ME. New gene discoveries highlight functional convergence in autism and related neurodevelopmental disorders. *Curr Opin Genet Dev.* 2020;65:195-206.
 155. Woodbury-Smith M, Bilder DA, Morgan J, et al. Combined genome-wide linkage and targeted association analysis of

- head circumference in autism spectrum disorder families. *J Neurodev Disord.* 2017;9:5.
156. De Rubeis S, Buxbaum JD. Genetics and genomics of autism spectrum disorder: embracing complexity. *Hum Mol Genet.* 2015;24(R1):R24-R31.
 157. Cross-Disorder Group of the Psychiatric Genomics Consortium. Genomic relationships, novel loci, and pleiotropic mechanisms across eight psychiatric disorders. *Cell.* 2019;179(7):1469-1482.e11.
 158. Grove J, Ripke S, Als TD, et al. Identification of common genetic risk variants for autism spectrum disorder. *Nat Genet.* 2019;51(3):431-444.
 159. Yu D, Sul JH, Tsetos F, et al. Interrogating the genetic determinants of Tourette's syndrome and other tic disorders through genome-wide association studies. *Am J Psychiatry.* 2019;176(3):217-227.
 160. Willsey HR, Willsey AJ, Wang B, State MW. Genomics, convergent neuroscience and progress in understanding autism spectrum disorder. *Nat Rev Neurosci.* 2022;23(6):323-341.
 161. Choi L, An JY. Genetic architecture of autism spectrum disorder: lessons from large-scale genomic studies. *Neurosci Biobehav Rev.* 2021;128:244-257.
 162. Bokobza C, Van Steenwinckel J, Mani S, Mezger V, Fleiss B, Gressens P. Neuroinflammation in preterm babies and autism spectrum disorders. *Pediatr Res.* 2019;85(2):155-165.
 163. Rudolph MD, Graham AM, Feczko E, et al. Maternal IL-6 during pregnancy can be estimated from newborn brain connectivity and predicts future working memory in offspring. *Nat Neurosci.* 2018;21(5):765-772.
 164. Han VX, Patel S, Jones HF, et al. Maternal acute and chronic inflammation in pregnancy is associated with common neurodevelopmental disorders: a systematic review. *Transl Psychiatry.* 2021;11(1):71.
 165. Bundgaard-Nielsen C, Lauritsen MB, Knudsen JK, et al. Children and adolescents with attention deficit hyperactivity disorder and autism spectrum disorder share distinct microbiota compositions. *Gut Microbes.* 2023;15(1):2211923.
 166. Mayer EA, Padua D, Tillisch K. Altered brain-gut axis in autism: comorbidity or causative mechanisms? *Bioessays.* 2014;36(10):933-939.
 167. Zhang M, Chu Y, Meng Q, et al. A quasi-paired cohort strategy reveals the impaired detoxifying function of microbes in the gut of autistic children. *Sci Adv.* 2020;6(43):eaba3760.
 168. Rossignol DA, Frye RE. A review of research trends in physiological abnormalities in autism spectrum disorders: immune dysregulation, inflammation, oxidative stress, mitochondrial dysfunction and environmental toxicant exposures. *Mol Psychiatry.* 2012;17(4):389-401.
 169. Gevezova M, Shirkov Y, Sarafian V, Plaimas K, Suratanee A, Maes M. Autistic spectrum disorder (ASD)—gene, molecular and pathway signatures linking systemic inflammation, mitochondrial dysfunction, transsynaptic signalling, and neurodevelopment. *Brain Behav Immun Health.* 2023;30:100646.
 170. Pacheva I, Ivanov I. Targeted biomedical treatment for autism spectrum disorders. *Curr Pharm Des.* 2019;25(41):4430-4453.
 171. Hughes HK, Moreno RJ, Ashwood P. Innate immune dysfunction and neuroinflammation in autism spectrum disorder (ASD). *Brain Behav Immun.* 2023;108:245-254.
 172. Meltzer A, Van de Water J. The role of the immune system in autism spectrum disorder. *Neuropsychopharmacology.* 2017;42(1):284-298.
 173. Onore C, Careaga M, Ashwood P. The role of immune dysfunction in the pathophysiology of autism. *Brain Behav Immun.* 2012;26(3):383-392.
 174. Liao X, Yang J, Wang H, Li Y. Microglia mediated neuroinflammation in autism spectrum disorder. *J Psychiatr Res.* 2020;130:167-176.
 175. Majhi S, Kumar S, Singh LA. Review on autism spectrum disorder: pathogenesis, biomarkers, pharmacological and non-pharmacological interventions. *CNS Neurol Disord Drug Targets.* 2023;22(5):659-677.
 176. Bjørklund G, Meguid NA, El-Bana MA, et al. Oxidative stress in autism spectrum disorder. *Mol Neurobiol.* 2020;57(5):2314-2332.
 177. Rossignol DA, Frye RE. Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis. *Mol Psychiatry.* 2012;17(3):290-314.
 178. Singh K, Singh IN, Diggins E, et al. Developmental regression and mitochondrial function in children with autism. *Ann Clin Transl Neurol.* 2020;7(5):683-694.
 179. Giulivi C, Zhang Y-F, Omanska-Klusek A, et al. Mitochondrial dysfunction in autism. *JAMA.* 2010;304(21):2389-2396.
 180. Srikantha P, Mohajeri MH. The possible role of the microbiota-gut-brain-axis in autism spectrum disorder. *Int J Mol Sci.* 2019;20(9):2115.
 181. Gevezova M, Sarafian V, Anderson G, Maes M. Inflammation and mitochondrial dysfunction in autism spectrum disorder. *CNS Neurol Disord Drug Targets.* 2020;19(5):320-333.
 182. Xiao L, Yan J, Yang T, et al. Fecal microbiome transplantation from children with autism spectrum disorder modulates tryptophan and serotonergic synapse metabolism and induces altered behaviors in germ-free mice. *Msystems.* 2021;6(2):e01343-20.
 183. Sharon G, Cruz NJ, Kang D-W, et al. Human gut microbiota from autism spectrum disorder promote behavioral symptoms in mice. *Cell.* 2019;177(6):1600-1618.
 184. Zhang M, Chu Y, Meng Q, et al. A quasi-paired cohort strategy reveals the impaired detoxifying function of microbes in the gut of autistic children. *Sci Adv.* 2020;6(43):eaba3760.
 185. Vuong HE, Hsiao EY. Emerging roles for the gut microbiome in autism spectrum disorder. *Biol Psychiatry.* 2017;81(5):411-423.
 186. Zhu J, Hua X, Yang T, et al. Alterations in gut vitamin and amino acid metabolism are associated with symptoms and neurodevelopment in children with autism spectrum disorder. *J Autism Dev Disord.* 2022;52(7):3116-3128.
 187. Sarkar A, Lehto SM, Harty S, Dinan TG, Cryan JF, Burnet PJ. Psychobiotics and the manipulation of bacteria-gut-brain signals. *Trends Neurosci.* 2016;39(11):763-781.
 188. Hsiao EY, McBride SW, Hsien S, et al. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell.* 2013;155(7):1451-1463.
 189. Bjørklund G, Pivina L, Dadar M, et al. Gastrointestinal alterations in autism spectrum disorder: what do we know? *Neurosci Biobehav Rev.* 2020;118:111-120.
 190. Buffington SA, Dooling SW, Sgritta M, et al. Dissecting the contribution of host genetics and the microbiome in complex behaviors. *Cell.* 2021;184(7):1740-1756.e16.

191. Yu Y, Zhang B, Ji P, et al. Changes to gut amino acid transporters and microbiome associated with increased E/I ratio in Chd8(+/-) mouse model of ASD-like behavior. *Nat Commun.* 2022;13(1):1151.
192. Liu X. The interaction of gut microbiota, genetic variation, and diet in autism spectrum disorder. *mLife.* 2022;1(3):241-244.
193. Panelli S, Capelli E, Lupo GFD, et al. Comparative study of salivary, duodenal, and fecal microbiota composition across adult celiac disease. *J Clin Med.* 2020;9(4):1109.
194. Bostick JW, Schonhoff AM, Mazmanian SK. Gut microbiome-mediated regulation of neuroinflammation. *Curr Opin Immunol.* 2022;76:102177.
195. Liu F, Li J, Wu F, Zheng H, Peng Q, Zhou H. Altered composition and function of intestinal microbiota in autism spectrum disorders: a systematic review. *Transl Psychiatry.* 2019;9(1):43.
196. Hyman SL, Levy SE, Myers SM. Identification, evaluation, and management of children with autism spectrum disorder. *Pediatrics.* 2020;145(1):e20193447.
197. General Office of the National Health Commission. *Notice of the General Office of the National Health Commission on exploring the Implementation of Special Services for the Prevention and Treatment of Depression and Senile Dementia.*
198. Cortese S, McGinn K, Højlund M, et al. The future of child and adolescent clinical psychopharmacology: a systematic review of phase 2, 3, or 4 randomized controlled trials of pharmacologic agents without regulatory approval or for unapproved indications. *Neurosci Biobehav Rev.* 2023;149:105149.
199. Parellada M, Andreu-Bernabeu Á, Burdeus M, et al. In search of biomarkers to guide interventions in autism spectrum disorder: a systematic review. *Am J Psychiatry.* 2023;180(1):23-40.
200. Ansel A, Posen Y, Ellis R, Deutsch L, Zisman PD, Gesundheit B. Biomarkers for autism spectrum disorders (ASD): a meta-analysis. *Rambam Maimonides Med J.* 2019;10(4):e0021.
201. Alharbi MG. Protein biomarkers in autistic children: a review. *Asian J Biochem Genet Mol Biol.* 2022;12(1):1-17.
202. Feng C, Chen Y, Pan J, et al. Redox proteomic identification of carbonylated proteins in autism plasma: insight into oxidative stress and its related biomarkers in autism. *Clin Proteomics.* 2017;14:2.
203. Cortelazzo A, De Felice C, Guerranti R, et al. Expression and oxidative modifications of plasma proteins in autism spectrum disorders: interplay between inflammatory response and lipid peroxidation. *Proteomics Clin Appl.* 2016;10(11):1103-1112.
204. Shen L, Zhao Y, Zhang H, et al. Advances in biomarker studies in autism spectrum disorders. *Adv Exp Med Biol.* 2019;1118:207-233.
205. Abraham J, Szoko N, Natowicz MR. Proteomic investigations of autism spectrum disorder: past findings, current challenges, and future prospects. *Adv Exp Med Biol.* 2019;1118:235-252.
206. Likhitweerawong N, Thonusin C, Boonchooduang N, et al. Profiles of urine and blood metabolomics in autism spectrum disorders. *Metab Brain Dis.* 2021;36(7):1641-1671.
207. Xu XJ, Cai XE, Meng FC, et al. Comparison of the metabolic profiles in the plasma and urine samples between autistic and typically developing boys: a preliminary study. *Front Psychiatry.* 2021;12:657105.
208. Arora A, Mastropasqua F, Bölte S, Tammimies K. Urine metabolomic profiles of autism and autistic traits—a twin study. *medRxiv.* 2023. 2023.04.24.23289030.
209. Al-Otaish H, Al-Ayadhi L, Bjørklund G, Chirumbolo S, Urbina MA, El-Ansary A. Relationship between absolute and relative ratios of glutamate, glutamine and GABA and severity of autism spectrum disorder. *Metab Brain Dis.* 2018;33(3):843-854.
210. El-Ansary A, Cannell JJ, Bjørklund G, et al. In the search for reliable biomarkers for the early diagnosis of autism spectrum disorder: the role of vitamin D. *Metab Brain Dis.* 2018;33(3):917-931.
211. Zhang H, Tang X, Feng C, et al. The use of data independent acquisition based proteomic analysis and machine learning to reveal potential biomarkers for autism spectrum disorder. *J Proteomics.* 2023;278:104872.
212. Cao X, Tang X, Feng C, et al. A systematic investigation of complement and coagulation-related protein in autism spectrum disorder using multiple reaction monitoring technology. *Neurosci Bull.* 2023;39(11):1623-1637.
213. Dai S, Lin J, Hou Y, Luo X, Shen Y, Ou J. Purine signaling pathway dysfunction in autism spectrum disorders: evidence from multiple omics data. *Front Mol Neurosci.* 2023;16:1089871.
214. Liu W, Li L, Xia X, et al. Integration of urine proteomic and metabolomic profiling reveals novel insights into neuroinflammation in autism spectrum disorder. *Front Psychiatry.* 2022;13:780747.
215. Tang X, Feng C, Zhao Y, et al. A study of genetic heterogeneity in autism spectrum disorders based on plasma proteomic and metabolomic analysis: multiomics study of autism heterogeneity. *Med Comm (2020).* 2023;4(5):e380.
216. Kordulewska NK, Kostyra E, Piskorz-Ogórek K, et al. Serum cytokine levels in children with spectrum autism disorder: differences in pro- and anti-inflammatory balance. *J Neuroimmunol.* 2019;337:577066.
217. Hu CC, Xu X, Xiong GL, et al. Alterations in plasma cytokine levels in Chinese children with autism spectrum disorder. *Autism Res.* 2018;11(7):989-999.
218. Hoang N, Buchanan JA, Scherer SW. Heterogeneity in clinical sequencing tests marketed for autism spectrum disorders. *NPJ Genom Med.* 2018;3:27.
219. Wang T, Guo H, Xiong B, et al. De novo genic mutations among a Chinese autism spectrum disorder cohort. *Nat Commun.* 2016;7:13316.
220. Corbett BA, Kantor AB, Schulman H, et al. A proteomic study of serum from children with autism showing differential expression of apolipoproteins and complement proteins. *Mol Psychiatry.* 2007;12(3):292-306.
221. Magdalon J, Mansur F, Teles ESAL, de Goes VA, Reiner O, Sertié AL. Complement system in brain architecture and neurodevelopmental disorders. *Front Neurosci.* 2020;14:23.
222. Druart M, Le Magueresse C. Emerging roles of complement in psychiatric disorders. *Front Psychiatry.* 2019;10:573.
223. Garcia-Gutierrez E, Narbad A, Rodríguez JM. Autism spectrum disorder associated with gut microbiota at immune, metabolomic, and neuroactive level. *Front Neurosci.* 2020;14:578666.
224. West PR, Amaral DG, Bais P, et al. Metabolomics as a tool for discovery of biomarkers of autism spectrum disorder in the blood plasma of children. *PLoS One.* 2014;9(11):e112445.
225. Anwar A, Abruzzo PM, Pasha S, et al. Advanced glycation endproducts, dityrosine and arginine transporter dysfunction

- in autism—a source of biomarkers for clinical diagnosis. *Mol Autism*. 2018;9(1):1-16.
226. Delaye J-B, Patin F, Lagrue E, et al. Post hoc analysis of plasma amino acid profiles: towards a specific pattern in autism spectrum disorder and intellectual disability. *Ann Clin Biochem*. 2018;55(5):543-552.
 227. Lv Q-Q, You C, Zou X-B, Deng H-Z. Acyl-carnitine, C5DC, and C26 as potential biomarkers for diagnosis of autism spectrum disorder in children. *Psychiatry Res*. 2018;267:277-280.
 228. Smith AM, King JJ, West PR, et al. Amino acid dysregulation metabolotypes: potential biomarkers for diagnosis and individualized treatment for subtypes of autism spectrum disorder. *Biol Psychiatry*. 2019;85(4):345-354.
 229. Brister D, Rose S, Delhey L, et al. Metabolomic signatures of autism spectrum disorder. *J Personalized Med*. 2022;12(10):1727.
 230. Kaluzna-Czaplinska J, Socha E, Rynkowski J. Determination of homovanillic acid and vanillylmandelic acid in urine of autistic children by gas chromatography/mass spectrometry. *Med Sci Monit*. 2010;16(9):CR445-CR450.
 231. Mavel S, Nadal-Desbarats L, Blasco H, et al. ^1H - ^{13}C NMR-based urine metabolic profiling in autism spectrum disorders. *Talanta*. 2013;114:95-102.
 232. Emond P, Mavel S, Aidoud N, et al. GC-MS-based urine metabolic profiling of autism spectrum disorders. *Anal Bioanal Chem*. 2013;405:5291-5300.
 233. Nadal-Desbarats L, Aidoud N, Emond P, et al. Combined ^1H -NMR and ^1H - ^{13}C HSQC-NMR to improve urinary screening in autism spectrum disorders. *Analyst*. 2014;139(13):3460-3468.
 234. Liu A, Zhou W, Qu L, et al. Altered urinary amino acids in children with autism spectrum disorders. *Front Cell Neurosci*. 2019;13:7.
 235. Delaye JB, Patin F, Lagrue E, et al. Post hoc analysis of plasma amino acid profiles: towards a specific pattern in autism spectrum disorder and intellectual disability. *Ann Clin Biochem*. 2018;55(5):543-552.
 236. Smith AM, King JJ, West PR, et al. Amino acid dysregulation metabolotypes: potential biomarkers for diagnosis and individualized treatment for subtypes of autism spectrum disorder. *Biol Psychiatry*. 2019;85(4):345-354.
 237. Wang L, Christophersen CT, Sorich MJ, Gerber JP, Angley MT, Conlon MA. Elevated fecal short chain fatty acid and ammonia concentrations in children with autism spectrum disorder. *Dig Dis Sci*. 2012;57(8):2096-2102.
 238. Brister D, Rose S, Delhey L, et al. Metabolomic signatures of autism spectrum disorder. *J Pers Med*. 2022;12(10):1727.
 239. Liu A, Zhou W, Qu L, et al. Altered urinary amino acids in children with autism spectrum disorders. *Front Cell Neurosci*. 2019;13:7.
 240. Nadal-Desbarats L, Aidoud N, Emond P, et al. Combined ^1H -NMR and ^1H - ^{13}C HSQC-NMR to improve urinary screening in autism spectrum disorders. *Analyst*. 2014;139(13):3460-3468.
 241. Olesova D, Galba J, Piastansky J, et al. A novel UHPLC-MS method targeting urinary metabolomic markers for autism spectrum disorder. *Metabolites*. 2020;10(11):443.
 242. Mead J, Ashwood P. Evidence supporting an altered immune response in ASD. *Immunol Lett*. 2015;163(1):49-55.
 243. Masi A, Quintana DS, Glozier N, Lloyd AR, Hickie IB, Guastella AJ. Cytokine aberrations in autism spectrum disorder: a systematic review and meta-analysis. *Mol Psychiatry*. 2015;20(4):440-446.
 244. Saghaazadeh A, Ataenia B, Keynejad K, Abdolalizadeh A, Hirbod-Mobarakeh A, Rezaei N. A meta-analysis of pro-inflammatory cytokines in autism spectrum disorders: effects of age, gender, and latitude. *J Psychiatr Res*. 2019;115:90-102.
 245. Saghaazadeh A, Ataenia B, Keynejad K, Abdolalizadeh A, Hirbod-Mobarakeh A, Rezaei N. Anti-inflammatory cytokines in autism spectrum disorders: a systematic review and meta-analysis. *Cytokine*. 2019;123:154740.
 246. Zhao H, Zhang H, Liu S, Luo W, Jiang Y, Gao J. Association of peripheral blood levels of cytokines with autism spectrum disorder: a meta-analysis. *Front Psychiatry*. 2021;12:670200.
 247. Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah IN, Van de Water J. Associations of impaired behaviors with elevated plasma chemokines in autism spectrum disorders. *J Neuroimmunol*. 2011;232(1-2):196-199.
 248. Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah I, Van de Water J. Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. *Brain Behav Immun*. 2011;25(1):40-45.
 249. Napolioni V, Ober-Reynolds B, Szelinger S, et al. Plasma cytokine profiling in sibling pairs discordant for autism spectrum disorder. *J Neuroinflammation*. 2013;10:38.
 250. Li X, Chauhan A, Sheikh AM, et al. Elevated immune response in the brain of autistic patients. *J Neuroimmunol*. 2009;207(1-2):111-116.
 251. Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. *Ann Neurol*. 2005;57(1):67-81.
 252. Eftekharian MM, Ghafouri-Fard S, Noroozi R, et al. Cytokine profile in autistic patients. *Cytokine*. 2018;108:120-126.
 253. Chen L, Shi XJ, Liu H, et al. Oxidative stress marker aberrations in children with autism spectrum disorder: a systematic review and meta-analysis of 87 studies ($N = 9109$). *Transl Psychiatry*. 2021;11(1):15.
 254. Liu X, Lin J, Zhang H, et al. Oxidative stress in autism spectrum disorder—current progress of mechanisms and biomarkers. *Front Psychiatry*. 2022;13:813304.
 255. Bala KA, Doğan M, Kaba S, Mutluer T, Aslan O, Doğan SZ. Hormone disorder and vitamin deficiency in attention deficit hyperactivity disorder (ADHD) and autism spectrum disorders (ASDs). *J Pediatr Endocrinol Metab*. 2016;29(9):1077-1082.
 256. Fuentes-Albero M, Cauli O. Homocysteine levels in autism spectrum disorder: a clinical update. *Endocr Metab Immune Disord Drug Targets*. 2018;18(4):289-296.
 257. Zhang Y, Hodgson NW, Trivedi MS, et al. Decreased brain levels of vitamin B12 in aging, autism and schizophrenia. *PLoS One*. 2016;11(1):e0146797.
 258. Lou M, Cao A, Jin C, et al. Deviated and early unsustainable stunted development of gut microbiota in children with autism spectrum disorder. *Gut*. 2022;71(8):1588-1599.
 259. De Angelis M, Piccolo M, Vannini L, et al. Fecal microbiota and metabolome of children with autism and pervasive developmental disorder not otherwise specified. *PLoS One*. 2013;8(10):e76993.

260. Gevi F, Zolla L, Gabriele S, Persico AM. Urinary metabolomics of young Italian autistic children supports abnormal tryptophan and purine metabolism. *Mol Autism*. 2016;7:47.
261. Chen Q, Qiao Y, Xu X-j, You X, Tao Y. Urine organic acids as potential biomarkers for autism-spectrum disorder in Chinese children. *Front Cellular Neurosci*. 2019;13:150.
262. Kang D-W, Adams JB, Vargason T, Santiago M, Hahn J, Krajmalnik-Brown R. Distinct fecal and plasma metabolites in children with autism spectrum disorders and their modulation after microbiota transfer therapy. *Mosphere*. 2020;5(5):e00314-e00320.
263. Fattorusso A, Di Genova L, Dell'Isola GB, Mencaroni E, Esposito S. Autism spectrum disorders and the gut microbiota. *Nutrients*. 2019;11(3):521.
264. Mohamadkhani A. Gut microbiota and fecal metabolome perturbation in children with autism spectrum disorder. *Middle East J Dig Dis*. 2018;10(4):205-212.
265. Xu X, Zou X, Li T. Expert consensus on early identification, screening and early intervention of children with autism spectrum disorders. *Chin J Pediatr*. 2017;55(12):890-897.
266. Howes OD, Rogdaki M, Findon JL, et al. Autism spectrum disorder: consensus guidelines on assessment, treatment and research from the British Association for Psychopharmacology. *J Psychopharmacol*. 2018;32(1):3-29.
267. Sandbank M, Bottema-Beutel K, Crowley S, et al. Project AIM: autism intervention meta-analysis for studies of young children. *Psychol Bull*. 2020;146(1):1.
268. Hyman SL, Levy SE, Myers SM, Council on Children With Disabilities, Section on Developmental and Behavioral Pediatrics. Identification, evaluation, and management of children with autism spectrum disorder. *Pediatrics*. 2020;145(1):e20193447.
269. Gosling CJ, Cartigny A, Mellier BC, Solanes A, Radua J, Delorme R. Efficacy of psychosocial interventions for Autism spectrum disorder: an umbrella review. *Mol Psychiatry*. 2022;27(9):3647-3656.
270. Trembath D, Varcin K, Waddington H, et al. Non-pharmacological interventions for autistic children: an umbrella review. *Autism*. 2023;27(2):275-295.
271. French L, Kennedy EM. Annual research review: early intervention for infants and young children with, or at-risk of, autism spectrum disorder: a systematic review. *J Child Psychol Psychiatry*. 2018;59(4):444-456.
272. Gabriels RL, Pan Z, Dechant B, Agnew JA, Brim N, Mesibov G. Randomized controlled trial of therapeutic horseback riding in children and adolescents with autism spectrum disorder. *J Am Acad Child Adolesc Psychiatry*. 2015;54(7):541-549.
273. Bearss K, Johnson C, Smith T, et al. Effect of parent training vs parent education on behavioral problems in children with autism spectrum disorder: a randomized clinical trial. *JAMA*. 2015;313(15):1524-1533.
274. Bieleninik Ł, Geretsegger M, Mössler K, et al. Effects of improvisational music therapy vs enhanced standard care on symptom severity among children with autism spectrum disorder: the TIME—a randomized clinical trial. *JAMA*. 2017;318(6):525-535.
275. Sharda M, Tuerk C, Chowdhury R, et al. Music improves social communication and auditory-motor connectivity in children with autism. *Transl Psychiatry*. 2018;8(1):231.
276. Grimaldi R, Gibson GR, Vulevic J, et al. A prebiotic intervention study in children with autism spectrum disorders (ASDs). *Microbiome*. 2018;6(1):133.
277. DeVane CL, Charles JM, Abramson RK, et al. Pharmacotherapy of autism spectrum disorder: results from the randomized BAART clinical trial. *Pharmacother J Human Pharmacol Drug Therapy*. 2019;39(6):626-635.
278. Voss C, Schwartz J, Daniels J, et al. Effect of wearable digital intervention for improving socialization in children with autism spectrum disorder: a randomized clinical trial. *JAMA Pediatr*. 2019;173(5):446-454.
279. Malow BA, Findling RL, Schroder CM, et al. Sleep, growth, and puberty after 2 years of prolonged-release melatonin in children with autism spectrum disorder. *J Am Acad Child Adolesc Psychiatry*. 2021;60(2):252-261.e3.
280. Wood JJ, Kendall PC, Wood KS, et al. Cognitive behavioral treatments for anxiety in children with autism spectrum disorder: a randomized clinical trial. *JAMA Psychiatry*. 2020;77(5):474-483.
281. Sikich L, Kolevzon A, King BH, et al. Intranasal oxytocin in children and adolescents with autism spectrum disorder. *N Engl J Med*. 2021;385(16):1462-1473.
282. Aran A, Harel M, Cassuto H, et al. Cannabinoid treatment for autism: a proof-of-concept randomized trial. *Mol Autism*. 2021;12(1):6.
283. Scahill L, Shillingsburg MA, Ousley O, et al. A randomized trial of direct instruction language for learning in children with autism spectrum disorder. *J Am Acad Child Adolesc Psychiatry*. 2022;61(6):772-781.
284. Chu L, Shen L, Ma C, et al. Effects of a nonwearable digital therapeutic intervention on preschoolers with autism spectrum disorder in China: open-label randomized controlled trial. *J Med Internet Res*. 2023;25:e45836.
285. Lord C, Charman T, Havdahl A, et al. The Lancet Commission on the future of care and clinical research in autism. *Lancet North Am Ed*. 2022;399(10321):271-334.
286. Kux L. *Food and Drug Administration [Docket No. FDA-2012-D-0146]: Guidance for Industry on Irritable Bowel Syndrome-Clinical Evaluation of Drugs for Treatment; Availability; Correction*. Federal Register. Vol 77. Department of Health and Human Services. 2012:32124-32125.
287. Dawson G, Bernier R. A quarter century of progress on the early detection and treatment of autism spectrum disorder. *Dev Psychopathol*. 2013;25(4pt2):1455-1472.
288. Lovaas OI. Behavioral treatment and normal educational and intellectual functioning in young autistic children. *J Consult Clin Psychol*. 1987;55(1):3.
289. Smith T, Iadarola S. Evidence base update for autism spectrum disorder. *J Clin Child Adolesc Psychol*. 2015;44(6):897-922.
290. Cummings AR, Carr JE. Evaluating progress in behavioral programs for children with autism spectrum disorders via continuous and discontinuous measurement. *J Appl Behav Anal*. 2009;42(1):57-71.
291. Lerman DC, Dittlinger LH, Fentress G, Lanagan T. A comparison of methods for collecting data on performance during discrete trial teaching. *Behav Anal Pract*. 2011;4:53-62.
292. Myers SM, Johnson CP, American Academy of Pediatrics Council on Children With Disabilities. Management

- of children with autism spectrum disorders. *Pediatrics*. 2007;120(5):1162-1182.
293. LeBlanc LA, Coates AM, Daneshvar S, Charlop-Christy MH, Morris C, Lancaster BM. Using video modeling and reinforcement to teach perspective-taking skills to children with autism. *J Appl Behav Anal*. 2003;36(2):253-257.
 294. Koegel RL, O'dell MC, Koegel LK. A natural language teaching paradigm for nonverbal autistic children. *J Autism Dev Disord*. 1987;17(2):187-200.
 295. Hardan AY, Gengoux GW, Berquist KL, et al. A randomized controlled trial of pivotal response treatment group for parents of children with autism. *J Child Psychol Psychiatry*. 2015;56(8):884-892.
 296. Mohammadzahari F, Koegel LK, Rezaee M, Rafiee SM. A randomized clinical trial comparison between pivotal response treatment (PRT) and structured applied behavior analysis (ABA) intervention for children with autism. *J Autism Dev Disord*. 2014;44:2769-2777.
 297. Batey C, Missiuna C, Timmons B, Hay J, Faught B, Cairney J. Self-efficacy toward physical activity and the physical activity behavior of children with and without developmental coordination disorder. *Hum Mov Sci*. 2014;36:258-271.
 298. Tan BW, Pooley JA, Speelman CP. A meta-analytic review of the efficacy of physical exercise interventions on cognition in individuals with autism spectrum disorder and ADHD. *J Autism Dev Disord*. 2016;46:3126-3143.
 299. Bremer E, Crozier M, Lloyd M. A systematic review of the behavioural outcomes following exercise interventions for children and youth with autism spectrum disorder. *Autism*. 2016;20(8):899-915.
 300. Oriel KN, George CL, Peckus R, Semon A. The effects of aerobic exercise on academic engagement in young children with autism spectrum disorder. *Pediatric Phys Ther*. 2011;23(2):187-193.
 301. Liang X, Li R, Wong SH, et al. The effects of exercise interventions on executive functions in children and adolescents with autism spectrum disorder: a systematic review and meta-analysis. *Sports Med*. 2022;52(1):75-88.
 302. Teh EJ, Vijayakumar R, Tan TXJ, Yap MJ. Effects of physical exercise interventions on stereotyped motor behaviours in children with ASD: a meta-analysis. *J Autism Dev Disord*. 2022;52(7):2934-2957.
 303. Sam K-L, Chow B-C, Tong K-K. Effectiveness of exercise-based interventions for children with autism: a systematic review and meta-analysis. *Int J Learn Teach*. 2015;1(2):98-103.
 304. Petrus C, Adamson SR, Block L, Einarson SJ, Sharifnejad M, Harris SR. Effects of exercise interventions on stereotypic behaviours in children with autism spectrum disorder. *Physiother Can*. 2008;60(2):134-145.
 305. Toscano CV, Carvalho HM, Ferreira JP. Exercise effects for children with autism spectrum disorder: metabolic health, autistic traits, and quality of life. *Percept Mot Skills*. 2018;125(1):126-146.
 306. Bahrami F, Movahedi A, Marandi SM, Sorensen C. The effect of karate techniques training on communication deficit of children with autism spectrum disorders. *J Autism Dev Disord*. 2016;46:978-986.
 307. Gabriels RL, Agnew JA, Holt KD, et al. Pilot study measuring the effects of therapeutic horseback riding on school-age children and adolescents with autism spectrum disorders. *Autism Spectrum Disorders*. 2012;6(2):578-588.
 308. Levinson LJ, Reid G. The effects of exercise intensity on the stereotypic behaviors of individuals with autism. *Adapt Phys Activity Quart*. 1993;10(3):255-268.
 309. Rosenthal-Malek A, Mitchell S. Brief report: the effects of exercise on the self-stimulatory behaviors and positive responding of adolescents with autism. *J Autism Dev Disord*. 1997;27(2):193-202.
 310. Pan C-Y. Effects of water exercise swimming program on aquatic skills and social behaviors in children with autism spectrum disorders. *Autism*. 2010;14(1):9-28.
 311. Yilmaz I, Yanardağ M, Birkan B, Bumin G. Effects of swimming training on physical fitness and water orientation in autism. *Pediatr Int*. 2004;46(5):624-626.
 312. Movahedi A, Bahrami F, Marandi SM, Abedi A. Improvement in social dysfunction of children with autism spectrum disorder following long term Kata techniques training. *Res Autism Spectrum Disorders*. 2013;7(9):1054-1061.
 313. Todd T, Reid G, Butler-Kisber L. Cycling for students with ASD: self-regulation promotes sustained physical activity. *Adapt Phys Activity Quart*. 2010;27(3):226-241.
 314. Koenig KP, Buckley-Reen A, Garg S. Efficacy of the get ready to learn yoga program among children with autism spectrum disorders: a pretest-posttest control group design. *Am J Occup Ther*. 2012;66(5):538-546.
 315. Rosenblatt LE, Gorantla S, Torres JA, et al. Relaxation response-based yoga improves functioning in young children with autism: a pilot study. *J Alternative Complement Med*. 2011;17(11):1029-1035.
 316. Toscano CV, Barros L, Lima AB, Nunes T, Carvalho HM, Gaspar JM. Neuroinflammation in autism spectrum disorders: exercise as a "pharmacological" tool. *Neurosci Biobehav Rev*. 2021;129:63-74.
 317. Ferreira JP, Ghiarone T, Junior CRC, et al. Effects of physical exercise on the stereotyped behavior of children with autism spectrum disorders. *Medicina (Mex)*. 2019;55(10):685.
 318. Olin SS, McFadden BA, Golem DL, et al. The effects of exercise dose on stereotypical behavior in children with autism. *Med Sci Sports Exercise*. 2017;49(5):983-990.
 319. Colasanto M, Madigan S, Korczak DJ. Depression and inflammation among children and adolescents: a meta-analysis. *J Affect Disord*. 2020;277:940-948.
 320. Liu JJ, Wei YB, Strawbridge R, et al. Peripheral cytokine levels and response to antidepressant treatment in depression: a systematic review and meta-analysis. *Mol Psychiatry*. 2020;25(2):339-350.
 321. Reschke-Hernández AE. History of music therapy treatment interventions for children with autism. *J Music Ther*. 2011;48(2):169-207.
 322. Brownell MD. Musically adapted social stories to modify behaviors in students with autism: four case studies. *J Music Ther*. 2002;39(2):117-144.
 323. Fees BS, Kaff M, Holmberg T, Teagarden J, Delreal D. Children's responses to a social story song in three inclusive preschool classrooms: a pilot study. *Music Ther Perspect*. 2014;32(1):71-77.

324. Kern P, Wolery M, Aldridge D. Use of songs to promote independence in morning greeting routines for young children with autism. *J Autism Dev Disord*. 2007;37:1264-1271.
325. Kern P, Aldridge D. Using embedded music therapy interventions to support outdoor play of young children with autism in an inclusive community-based child care program. *J Music Ther*. 2006;43(4):270-294.
326. Kaplan RS, Steele AL. An analysis of music therapy program goals and outcomes for clients with diagnoses on the autism spectrum. *J Music Ther*. 2005;42(1):2-19.
327. Kim J, Wigram T, Gold C. The effects of improvisational music therapy on joint attention behaviors in autistic children: a randomized controlled study. *J Autism Dev Disord*. 2008;38:1758-1766.
328. LaGasse AB. Effects of a music therapy group intervention on enhancing social skills in children with autism. *J Music Ther*. 2014;51(3):250-275.
329. Ulfarsdottir LO, Erwin PG. The influence of music on social cognitive skills. *Arts Psychother*. 1999;26(2):81-84.
330. Lim HA. Effect of "developmental speech and language training through music" on speech production in children with autism spectrum disorders. *J Music Ther*. 2010;47(1):2-26.
331. Lim HA, Draper E. The effects of music therapy incorporated with applied behavior analysis verbal behavior approach for children with autism spectrum disorders. *J Music Ther*. 2011;48(4):532-550.
332. Vaiouli P, Grimmer K, Ruich LJ. "Bill is now singing": joint engagement and the emergence of social communication of three young children with autism. *Autism*. 2015;19(1):73-83.
333. Paul A, Sharda M, Menon S, et al. The effect of sung speech on socio-communicative responsiveness in children with autism spectrum disorders. *Front Human Neurosci*. 2015;9:555.
334. Gee BM, Thompson K, St John H. Efficacy of a sound-based intervention with a child with an autism spectrum disorder and auditory sensory over-responsivity. *Occup Ther Int*. 2014;21(1):12-20.
335. Hillier A, Greher G, Poto N, Dougherty M. Positive outcomes following participation in a music intervention for adolescents and young adults on the autism spectrum. *Psychol Music*. 2012;40(2):201-215.
336. Finnigan E, Starr E. Increasing social responsiveness in a child with autism: a comparison of music and non-music interventions. *Autism*. 2010;14(4):321-348.
337. Thompson G, McFerran K, Gold C. Family-centred music therapy to promote social engagement in young children with severe autism spectrum disorder: a randomized controlled study. *Child Care Health Dev*. 2014;40(6):840-852.
338. Schwartzberg ET, Silverman MJ. Effects of music-based social stories on comprehension and generalization of social skills in children with autism spectrum disorders: a randomized effectiveness study. *Arts Psychother*. 2013;40(3):331-337.
339. Corbett BA, Shickman K, Ferrer E. Brief report: the effects of Tomatis sound therapy on language in children with autism. *J Autism Dev Disord*. 2008;38:562-566.
340. Rapp JT, Vollmer TR. Stereotype I: a review of behavioral assessment and treatment. *Res Dev Disabil*. 2005;26(6):527-547.
341. Vivanti G, Zhong HN. Naturalistic developmental behavioral interventions for children with autism. *Clinical Guide to Early Interventions for Children With Autism*. 2020:93-130.
342. Snyder TD, De Brey C, Dillow SA. Digest of Education Statistics 2014, NCES 2016-006. *National Center for Education Statistics*. 2016.
343. Murphy MA, Ruble LA. A comparative study of rurality and urbanicity on access to and satisfaction with services for children with autism spectrum disorders. *Rural Special Educ Quart*. 2012;31(3):3-11.
344. Burrell TL. Parents' involvement in ASD treatment: what is their role? *Cognit Behav Pract*. 2012;19(3):423-432.
345. Downer JT, Pianta RC. Academic and cognitive functioning in first grade: associations with earlier home and child care predictors and with concurrent home and classroom experiences. *School Psychol Rev*. 2006;35(1):11-30.
346. Webster-Stratton C, Herman KC. The impact of parent behavior-management training on child depressive symptoms. *J Counsel Psychol*. 2008;55(4):473.
347. Menting AT, de Castro BO, Matthys W. Effectiveness of the incredible years parent training to modify disruptive and prosocial child behavior: a meta-analytic review. *Clin Psychol Rev*. 2013;33(8):901-913.
348. Neitzel C, Stright A. Relations between mothers' scaffolding and children's academic self-regulation: establishing a foundation of self-regulatory competence. *J Fam Psychol*. 2003;17(1):147-159.
349. Cline KD, Edwards CP. The instructional and emotional quality of parent-child book reading and early head start children's learning outcomes. *Early Educ Dev*. 2013;24(8):1214-1231.
350. Ingersoll B, Wainer A. Initial efficacy of Project ImPACT: a parent-mediated social communication intervention for young children with ASD. *J Autism Dev Disord*. 2013;43:2943-2952.
351. Matson ML, Mahan S, Matson JL. Parent training: a review of methods for children with autism spectrum disorders. *Res Autism Spectrum Disorders*. 2009;3(4):868-875.
352. McConachie H, Diggle T. Parent implemented early intervention for young children with autism spectrum disorder: a systematic review. *J Eval Clin Pract*. 2007;13(1):120-129.
353. Wong C, Odom SL, Hume KA, et al. Evidence-based practices for children, youth, and young adults with autism spectrum disorder: a comprehensive review. *J Autism Dev Disord*. 2015;45:1951-1966.
354. Kim EM, Sheridan SM. Foundational aspects of family-school connections: definitions, conceptual frameworks, and research needs. *Foundational Aspects of Family-School Partnership Research*. 2015:1-14.
355. Sheridan SM, Bovaird JA, Glover TA, Andrew Garbacz S, Witte A, Kwon K. A randomized trial examining the effects of conjoint behavioral consultation and the mediating role of the parent-teacher relationship. *School Psychol Rev*. 2012;41(1):23-46.
356. Rispoli MJ, Franco JH, van der Meer L, Lang R, Camargo SPH. The use of speech generating devices in communication interventions for individuals with developmental disabilities: a review of the literature. *Dev Neurorehab*. 2010;13(4):276-293.
357. Mirenda P. Toward functional augmentative and alternative communication for students with autism: manual signs, graphic symbols, and voice output communication aids. *Lang Speech Hear Serv Sch*. 2003;34(3):203-216.
358. Lorah ER, Parnell A, Whitby PS, Hantula D. A systematic review of tablet computers and portable media players as

- speech generating devices for individuals with autism spectrum disorder. *J Autism Dev Disord*. 2015;45:3792-3804.
359. Lorah ER, Tincani M, Dodge J, Gilroy S, Hickey A, Hantula D. Evaluating picture exchange and the iPad™ as a speech generating device to teach communication to young children with autism. *J Dev Phys Disab*. 2013;25:637-649.
 360. Schlosser R. Roles of speech output in augmentative and alternative communication: narrative review. *Augment Altern Commun*. 2003;19(1):5-27.
 361. Ganz JB, Earles-Vollrath TL, Heath AK, Parker RI, Rispoli MJ, Duran JB. A meta-analysis of single case research studies on aided augmentative and alternative communication systems with individuals with autism spectrum disorders. *J Autism Dev Disord*. 2012;42:60-74.
 362. Schlosser RW, Koul RK. Speech output technologies in interventions for individuals with autism spectrum disorders: a scoping review. *Augment Altern Commun*. 2015;31(4):285-309.
 363. Mineo BA, Ziegler W, Gill S, Salkin D. Engagement with electronic screen media among students with autism spectrum disorders. *J Autism Dev Disord*. 2009;39:172-187.
 364. Schmidt M, Laffey JM, Schmidt CT, Wang X, Stichter J. Developing methods for understanding social behavior in a 3D virtual learning environment. *Comput Hum Behav*. 2012;28(2):405-413.
 365. Bailenson J, Patel K, Nielsen A, Bajscy R, Jung S-H, Kurillo G. The effect of interactivity on learning physical actions in virtual reality. *Media Psychol*. 2008;11(3):354-376.
 366. Blascovich J, Loomis J, Beall AC, Swinthe KR, Hoyt CL, Bailenson JN. Immersive virtual environment technology as a methodological tool for social psychology. *Psychol Inquiry*. 2002;13(2):103-124.
 367. Wallace S, Coleman M, Bailey A. An investigation of basic facial expression recognition in autism spectrum disorders. *Cogn Emotion*. 2008;22(7):1353-1380.
 368. Kandalaft MR, Didehbani N, Krawczyk DC, Allen TT, Chapman SB. Virtual reality social cognition training for young adults with high-functioning autism. *J Autism Dev Disord*. 2013;43:34-44.
 369. Pennisi P, Tonacci A, Tartarisco G, et al. Autism and social robotics: a systematic review. *Autism Res*. 2016;9(2):165-183.
 370. Diehl JJ, Crowell CR, Villano M, Wier K, Tang K, Riek LD. Clinical applications of robots in autism spectrum disorder diagnosis and treatment. *Comprehensive Guide to Autism*. 2014:411-422.
 371. Diehl JJ, Schmitt LM, Villano M, Crowell CR. The clinical use of robots for individuals with autism spectrum disorders: a critical review. *Res Autism Spectrum Disord*. 2012;6(1):249-262.
 372. Ricks DJ, Colton MB. *Trends and Considerations in Robot-Assisted Autism Therapy*. IEEE; 2010:4354-4359.
 373. Scassellati B, Admoni H, Matarić M. Robots for use in autism research. *Annu Rev Biomed Eng*. 2012;14:275-294.
 374. Feil-Seifer D, Matarić MJ. *Toward Socially Assistive Robotics for Augmenting Interventions for Children With Autism Spectrum Disorders*. Springer; 2009:201-210.
 375. Scassellati B. *How Social Robots Will Help Us to Diagnose, Treat, and Understand Autism*. Springer; 2007:552-563.
 376. Cabibihan J-J, Javed H, Ang M, Aljunied SM. Why robots? A survey on the roles and benefits of social robots in the therapy of children with autism. *Int J Social Robot*. 2013;5:593-618.
 377. Kumazaki H, Muramatsu T, Yoshikawa Y, et al. Role-play-based guidance for job interviews using an android robot for individuals with autism spectrum disorders. *Front Psychiatry*. 2019;10:239.
 378. Anzalone SM, Tilmont E, Boucenna S, et al. How children with autism spectrum disorder behave and explore the 4-dimensional (spatial 3D+ time) environment during a joint attention induction task with a robot. *Res Autism Spectrum Disord*. 2014;8(7):814-826.
 379. Robins B, Dautenhahn K, Boekhorst RT, Billard A. Robotic assistants in therapy and education of children with autism: can a small humanoid robot help encourage social interaction skills? *Universal Access Inform Soc*. 2005;4:105-120.
 380. Kozima H, Nakagawa C, Kawai N, Kosugi D, Yano Y. *A Humanoid in Company With Children*. IEEE; 2004:470-477.
 381. Kozima H, Nakagawa C, Yasuda Y. *Interactive Robots for Communication-Care: A Case-Study in Autism Therapy*. IEEE; 2005:341-346.
 382. Maniram J, Karim SB, Oosthuizen F, Wiafe E. Pharmacological management of core symptoms and comorbidities of autism spectrum disorder in children and adolescents: a systematic review. *Neuropsychiatr Disease Treat*. 2022;18:1629-1644.
 383. Yu Y, Chaulagain A, Pedersen SA, et al. Pharmacotherapy of restricted/repetitive behavior in autism spectrum disorder: a systematic review and meta-analysis. *BMC Psychiatry*. 2020;20(1):1-11.
 384. Lim JJ, Anagnostou E. Biomedical interventions for autism spectrum disorder. *Neurodevelopmental Pediatrics: Genetic and Environmental Influences*. Springer; 2023:327-335.
 385. Deb S, Roy M, Limbu B, Bertelli M. Anti-anxiety medications and novel treatments for autism. *Handbook of Autism and Pervasive Developmental Disorder: Assessment, Diagnosis, and Treatment*. Springer; 2022:1157-1172.
 386. Hurwitz R, Blackmore R, Hazell P, Williams K, Woolfenden S. Tricyclic antidepressants for autism spectrum disorders (ASD) in children and adolescents. *Cochrane Database System Rev*. 2012:CD008372.
 387. Mathew S, Bichenapally S, Khachatryan V, et al. Role of serotonergic antidepressants in the development of autism spectrum disorders: a systematic review. *Cureus*. 2022;14(8):e28505.
 388. Häge A, Banaschewski T, Buitelaar JK, et al. Glutamatergic medication in the treatment of obsessive compulsive disorder (OCD) and autism spectrum disorder (ASD)—study protocol for a randomised controlled trial. *Trials*. 2016;17(1):1-16.
 389. Jobski K, Höfer J, Hoffmann F, Bachmann C. Use of psychotropic drugs in patients with autism spectrum disorders: a systematic review. *Acta Psychiatr Scand*. 2017;135(1):8-28.
 390. Rodrigues R, Lai MC, Beswick A, et al. Practitioner review: pharmacological treatment of attention-deficit/hyperactivity disorder symptoms in children and youth with autism spectrum disorder: a systematic review and meta-analysis. *J Child Psychol Psychiatr*. 2021;62(6):680-700.
 391. Farmer CA, Aman MG. Aripiprazole for the treatment of irritability associated with autism. *Expert Opin Pharmacother*. 2011;12(4):635-640.
 392. Hesapcioglu ST, Ceylan MF, Kasak M, Sen CP. Olanzapine, risperidone, and aripiprazole use in children and adolescents with autism spectrum disorders. *Autism Spectrum Disorders*. 2020;72:101520.

393. Reiersen AM, Handen B. Commentary on 'Selective serotonin reuptake inhibitors (SSRIs) for autism spectrum disorders (ASD)'. *Evidence-Based Child Health Cochrane Rev J*. 2011;6(4):1082-1085.
394. Williams K, Wheeler DM, Silove N, Hazell P. Cochrane review: selective serotonin reuptake inhibitors (SSRIs) for autism spectrum disorders (ASD). *Evidence-Based Child Health Cochrane Rev J*. 2011;6(4):1044-1078.
395. Reddihough DS, Marraffa C, Mouti A, et al. Effect of fluoxetine on obsessive-compulsive behaviors in children and adolescents with autism spectrum disorders: a randomized clinical trial. *JAMA*. 2019;322(16):1561-1569.
396. Green J, Garg S. Annual research review: the state of autism intervention science: progress, target psychological and biological mechanisms and future prospects. *J Child Psychol Psychiatry*. 2018;59(4):424-443.
397. Murari K, Abushaibah A, Rho JM, Turner RW, Cheng N. A clinically relevant selective ERK-pathway inhibitor reverses core deficits in a mouse model of autism. *EBioMedicine*. 2023;91:104565.
398. Pacheva I, Ivanov I. Targeted biomedical treatment for autism spectrum disorders. *Curr Pharm Des*. 2019;25(41):4430-4453.
399. Majhi S, Kumar S, Singh L. A review on autism spectrum disorder: pathogenesis, biomarkers, pharmacological and non-pharmacological interventions. *CNS Neurol Disord Drug Targets*. 2023;22(5):659-677.
400. Liu Y, Yang Z, Du Y, Shi S, Cheng Y. Antioxidant interventions in autism spectrum disorders: a meta-analysis. *Prog Neuropsychopharmacol Biol Psychiatry*. 2022;113:110476.
401. Bent S, Lawton B, Warren T, et al. Identification of urinary metabolites that correlate with clinical improvements in children with autism treated with sulforaphane from broccoli. *Mol Autism*. 2018;9:1-12.
402. Hendouei F, Sanjari Moghaddam H, Mohammadi MR, Taslimi N, Rezaei F, Akhondzadeh S. Resveratrol as adjunctive therapy in treatment of irritability in children with autism: a double-blind and placebo-controlled randomized trial. *J Clin Pharm Ther*. 2020;45(2):324-334.
403. Mousavinejad E, Ghaffari MA, Riahi F, Hajmohammadi M, Tiznobeyk Z, Mousavinejad M. Coenzyme Q10 supplementation reduces oxidative stress and decreases antioxidant enzyme activity in children with autism spectrum disorders. *Psychiatry Res*. 2018;265:62-69.
404. Nikoo M, Radnia H, Farokhnia M, Mohammadi M-R, Akhondzadeh S. N-acetylcysteine as an adjunctive therapy to risperidone for treatment of irritability in autism: a randomized, double-blind, placebo-controlled clinical trial of efficacy and safety. *Clin Neuropharmacol*. 2015;38(1):11-17.
405. Bent S, Hendren RL, Zandi T, et al. Internet-based, randomized, controlled trial of omega-3 fatty acids for hyperactivity in autism. *J Am Acad Child Adolesc Psychiatry*. 2014;53(6):658-666.
406. Yui K, Koshiba M, Nakamura S, Kobayashi Y. Effects of large doses of arachidonic acid added to docosahexaenoic acid on social impairment in individuals with autism spectrum disorders: a double-blind, placebo-controlled, randomized trial. *J Clin Psychopharmacol*. 2012;32(2):200-206.
407. Hendren RL, James SJ, Widjaja F, Lawton B, Rosenblatt A, Bent S. Randomized, placebo-controlled trial of methyl B12 for children with autism. *J Child Adolesc Psychopharmacol*. 2016;26(9):774-783.
408. Lynch R, Diggins EL, Connors SL, et al. Sulforaphane from broccoli reduces symptoms of autism: a follow-up case series from a randomized double-blind study. *Global Adv Health Med*. 2017;6:2164957x17735826.
409. Al-Ayadhi LY, Elamin NE. Camel milk as a potential therapy as an antioxidant in autism spectrum disorder (ASD). *Evid-Based Complement Altern Med*. 2013;2013:602834.
410. Sporn MB, Liby KT. NRF2 and cancer: the good, the bad and the importance of context. *Nat Rev Cancer*. 2012;12(8):564-571.
411. Yang J, Fu X, Liao X, Li Y. Nrf2 activators as dietary phytochemicals against oxidative stress, inflammation, and mitochondrial dysfunction in autism spectrum disorders: a systematic review. *Front Psychiatry*. 2020;11:561998.
412. Liu X, Lin J, Zhang H, et al. Oxidative stress in autism spectrum disorder—current progress of mechanisms and biomarkers. *Front Psychiatry*. 2022;13:813304.
413. Frye RE, Sequeira J, Quadros E, James S, Rossignol D. Cerebral folate receptor autoantibodies in autism spectrum disorder. *Mol Psychiatry*. 2013;18(3):369-381.
414. James SJ, Melnyk S, Fuchs G, et al. Efficacy of methylcobalamin and folinic acid treatment on glutathione redox status in children with autism. *Am J Clin Nutr*. 2009;89(1):425-430.
415. Frye RE, Melnyk S, Fuchs G, et al. Effectiveness of methylcobalamin and folinic acid treatment on adaptive behavior in children with autistic disorder is related to glutathione redox status. *Autism Res Treat*. 2013;2013:609705.
416. Naviaux RK, Curtis B, Li K, et al. Low-dose suramin in autism spectrum disorder: a small, phase I/II, randomized clinical trial. *Ann Clin Transl Neurol*. 2017;4(7):491-505.
417. Pangrazzi L, Balasco L, Bozzi Y. Natural antioxidants: a novel therapeutic approach to autism spectrum disorders? *Antioxidants*. 2020;9(12):1186.
418. Al-Amin MM, Rahman MM, Khan FR, Zaman F, Reza HM. Astaxanthin improves behavioral disorder and oxidative stress in prenatal valproic acid-induced mice model of autism. *Behav Brain Res*. 2015;286:112-121.
419. Ajami M, Pazoki-Toroudi H, Amani H, et al. Therapeutic role of sirtuins in neurodegenerative disease and their modulation by polyphenols. *Neurosci Biobehav Rev*. 2017;73:39-47.
420. Bertolino B, Crupi R, Impellizzeri D, et al. Beneficial effects of co-ultramicrosized palmitoylethanolamide/luteolin in a mouse model of autism and in a case report of autism. *CNS Neurosci Ther*. 2017;23(1):87-98.
421. Bozzatello P, Brignolo E, De Grandi E, Bellino S. Supplementation with omega-3 fatty acids in psychiatric disorders: a review of literature data. *J Clin Med*. 2016;5(8):67.
422. Niederhofer H. First preliminary results of an observation of Ginkgo Biloba treating patients with autistic disorder. *Phytother Res*. 2009;23(11):1645-1646.
423. Singh R, Kisku A, Kungummaraj H, et al. Autism spectrum disorders: a recent update on targeting inflammatory pathways with natural anti-inflammatory agents. *Biomedicine*. 2023;11(1):115.
424. Liao X, Li Y. Nuclear factor kappa B in autism spectrum disorder: a systematic review. *Pharmacol Res*. 2020;159:104918.
425. Xin P, Xu X, Deng C, et al. The role of JAK/STAT signaling pathway and its inhibitors in diseases. *Int Immunopharmacol*. 2020;80:106210.

426. Parker-Athill E, Luo D, Bailey A, et al. Flavonoids, a prenatal prophylaxis via targeting JAK2/STAT3 signaling to oppose IL-6/MIA associated autism. *J Neuroimmunol.* 2009;217(1–2):20–27.
427. Zawadzka A, Cieřlik M, Adamczyk A. The role of maternal immune activation in the pathogenesis of autism: a review of the evidence, proposed mechanisms and implications for treatment. *Int J Mol Sci.* 2021;22(21):11516.
428. Choi GB, Yim YS, Wong H, et al. The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring. *Science.* 2016;351(6276):933–939.
429. Marchezan J, Winkler dos Santos EGA, Deckmann I, Dos Santos Riesgo R. Immunological dysfunction in autism spectrum disorder: a potential target for therapy. *NeuroImmunoModulation.* 2019;25(5–6):300–319.
430. Arteaga-Henrıquez G, Gisbert L, Ramos-Quiroga JA. Immunoregulatory and/or anti-inflammatory agents for the management of core and associated symptoms in individuals with autism spectrum disorder: a narrative review of randomized, placebo-controlled trials. *CNS Drugs.* 2023;37(3):215–229.
431. Sıafıs S, Çıray O, Wu H, et al. Pharmacological and dietary-supplement treatments for autism spectrum disorder: a systematic review and network meta-analysis. *Mol Autism.* 2022;13(1):1–17.
432. Hafizi S, Tabatabaei D, Lai M-C. Review of clinical studies targeting inflammatory pathways for individuals with autism. *Front Psychiatr.* 2019;10:849.
433. Liu J, Gao Z, Liu C, et al. Alteration of gut microbiota: new strategy for treating autism spectrum disorder. *Front Cell Dev Biol.* 2022;10:792490.
434. Rao AV, Bested AC, Beaulne TM, et al. A randomized, double-blind, placebo-controlled pilot study of a probiotic in emotional symptoms of chronic fatigue syndrome. *Gut Pathogens.* 2009;1(1):1–6.
435. Silk D, Davis A, Vulevic J, Tzortzis G, Gibson G. Clinical trial: the effects of a trans-galactooligosaccharide prebiotic on faecal microbiota and symptoms in irritable bowel syndrome. *Aliment Pharmacol Ther.* 2009;29(5):508–518.
436. Liu G, Yuan L. Analysis on the etiology, pathogenesis and syndrome differentiation of children with autism in traditional Chinese medicine. *Liaoning J Trad Chin Med.* 2007;9:1226–1227.
437. Jiang X, Cai Z, Zhang Z, Li A, Cheng Y, Lyu Y. Combined treatment of children with autism with modified yinhuo decoction and therapeutic interventions. *Chin J Trad Chin Med Pharm.* 2016;31(10):4322–4324.
438. Zhou N, Li Y, Jiang X, Lu Y. Clinical observation of supplemented Lizhong decoction in treating children autism. *J New Chin Med.* 2015;47(6):200–202.
439. Wu H, Wu Z. Trinity” traditional chinese medicine treatment of autism. *Chin Med Herald.* 2006;11:116–117.
440. Kang D-W, Adams JB, Gregory AC, et al. Microbiota transfer therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. *Microbiome.* 2017;5(1):1–16.
441. Zhang S, Chen Q, Kelly CR, et al. Donor screening for fecal microbiota transplantation in China: evaluation of 8483 candidates. *Gastroenterology.* 2022;162(3):966–968.e3.
442. Wang J, Cao Y, Hou W, et al. Fecal microbiota transplantation improves VPA-induced ASD mice by modulating the serotonergic and glutamatergic synapse signaling pathways. *Transl Psychiatry.* 2023;13(1):17.
443. Kang D-W, Adams JB, Coleman DM, et al. Long-term benefit of microbiota transfer therapy on autism symptoms and gut microbiota. *Sci Rep.* 2019;9(1):5821.
444. Saurman V, Margolis KG, Luna RA. Autism spectrum disorder as a brain–gut–microbiome axis disorder. *Dig Dis Sci.* 2020;65(3):818–828.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Zhuang H, Liang Z, Ma G, et al. Autism spectrum disorder: pathogenesis, biomarker, and intervention therapy. *MedComm.* 2024;5:e497. <https://doi.org/10.1002/mco2.497>