Gut microbiota alterations in autism spectrum disorder: Implications for microbiota-gut-brain axis mechanisms and therapeutic strategies (Review)

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Abstract. Autism spectrum disorder (ASD) is a complex neurodevelopmental condition often accompanied by chronic gastrointestinal (GI) disturbances. Recent evidence suggests that these GI issues may be biologically linked to ASD through the microbiota-gut-brain axis (MGBA). The present review aimed to highlight how alterations in the gut microbiota contribute to ASD symptoms and to explore potential microbiome-based therapeutic strategies. Recent research findings regarding gut microbial composition in individuals with ASD were reviewed, focusing on changes in beneficial and harmful microbial taxa, associated inflammatory and metabolic alterations, and their implications for neural signaling within the MGBA. Data on emerging therapeutic interventions, such as probiotics, prebiotics, and fecal microbiota transplantation, were also evaluated. Studies reveal notable microbial dysbiosis in ASD, including reduced levels of *Bifidobacterium* and *Prevotella* and an increased abundance of *Clostridium* species. These alterations are linked to intestinal inflammation, impaired gut barrier integrity, and disturbances in neurotransmitter production. Furthermore, the highly restricted dietary patterns and food selectivity commonly observed in individuals with ASD are likely to reduce microbial diversity, particularly affecting fiber-dependent taxa, such as *Prevotella*. Concurrently, the severity of specific ASD traits, particularly repetitive and rigid behaviors, has been closely correlated with GI discomfort and distinct microbial imbalances. Microbial metabolites, such as short-chain fatty acids, appear to influence brain function, mood, and social behavior. Initial therapeutic trials indicate that microbiota-targeted interventions may improve both GI symptoms and behavioral outcomes. Current evidence supports a significant association between alterations in the gut microbiota and ASD pathophysiology via the bidirectional MGBA. While further studies are required to establish causality and refine therapeutic approaches, emerging microbiota-based treatments highlight the importance of adopting a holistic model that integrates neurological, immunological, and microbial aspects in understanding and managing ASD. However, the strength of mechanistic evidence varies across immune, neural, and endocrine pathways, with immune-related mechanisms supported by the most consistent ASD-specific data.

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Contents

1. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by persistent deficits in social communication and social interaction, restricted and repetitive patterns of behavior, interests, or activities (1). The etiology of ASD is multifaceted, including both genetic and environmental factors (2). Children with ASD often encounter difficulties in pragmatic communication skills, particularly in sustaining discussions, and may exhibit repeated behaviors that disrupt their everyday functioning (3). Early signs commonly appear during the first 2 years of life, although diagnoses often occur later (4). Core diagnostic features of ASD include restricted and repetitive behaviors (RRBs) and highly restricted, fixated interests (5). Early identification and evidence-based intervention are essential to improve long-term functional outcomes (6).

Data from 2016 demonstrate an ASD prevalence rate of 18.5 per 1,000 children aged 8 years, equivalent to 1 in 54 children. Boys have been found to be 4.3-fold more likely to be affected than girls. The results indicate a geographic variation in prevalence, with the lowest rates among Hispanic children (15.4), while rates were similar among non-Hispanic White children (18.5), non-Hispanic Black children (18.3), and Asian/Pacific Islander children (17.9). Among children with available intelligence data, 33% have an intellectual disability (IQ ≤70), with this rate being higher among girls (39%) than boys (32%) and also higher among Black (47%) and Hispanic (36%) children than White children (27%). The mean age of first ASD diagnosis was 51 months across all groups, where lower IQ was discovered in Black children later than White children (48 vs. 42 months) (7).

Recent scientific evidence indicates that gastrointestinal (GI) issues are among the most common comorbidities in children with ASD. A systematic review published in 2022 reported wide-ranging estimates of GI symptoms in children with ASD, with prevalence ranging widely across studies, reaching up to 91% in some reports. That systematic review also noted that GI symptoms were frequently associated with greater ASD severity, although findings varied across studies (8). The association between the gut microbiota and ASD is bidirectional. The specific behavioral profile of ASD, particularly extreme food selectivity and restricted diets of often processed, low-fiber foods, may function as a key contributor to gut dysbiosis. Core ASD symptoms, such as RRBs, are exacerbated by underlying GI discomfort and altered microbial signaling, making it essential to analyze microbiota changes in conjunction with these specific behavioral traits. Digestive issues are common in children with ASD and may be associated with alterations in the gut microbiota. Several studies have shown increases in the relative abundance of specific taxa, such as *Pseudomonas* spp., and shifts within the phylum Firmicutes, with decreases in other taxa, including *Bacillus subtilis* (9-11). However, findings across ASD microbiome studies are inconsistent, with variability in the reported microbial signatures and diversity indices. This heterogeneity reflects differences in participant age, dietary patterns, antibiotic exposure, geographic background, sample size, and microbiome profiling methodologies (e.g., 16S rRNA gene sequencing vs. shotgun metagenomics) (12,13). All of which affect taxonomic resolution and interpretation of results. Failure to control for restricted dietary patterns and food selectivity in ASD cohorts may confound microbiome comparisons with neurotypical controls, thereby contributing further to discrepancies across studies (14).

Fecal microbiota transplantation (FMT), including modified protocols, such as microbiota transfer therapy (MTT), as well as probiotic interventions, has demonstrated improvements in GI symptoms, with inconsistent effects on behavioral outcomes. Further research is required on the complex association between the gut and the brain in ASD (15). The association between ASD and the gut microbiota has emerged as a major research area in recent years. Once considered to be merely a part of the digestive system, research has shown that the GI tract possesses an intrinsic enteric nervous system that communicates bidirectionally with the central nervous system through the gut-brain axis. This communication facilitates bidirectional information flow and impacts mood, cognition, and overall brain function (16).

The primary interventions used to modulate the gut microbiota are FMT, probiotics, and prebiotics. These therapies have been investigated primarily for their effects on GI symptoms, with some studies also exploring potential associations with behavioral outcomes; however, the available evidence remains preliminary, heterogeneous, and inconclusive (17-20). Modulation of gut microbiota composition may influence gut-brain signaling pathways, although the clinical relevance of these effects remains uncertain. The present review discusses the efficacy of probiotics, prebiotics, and dietary interventions in treating ASD based on the current understanding of gut bacterial imbalances associated with the condition (21-23). An overview of the bidirectional communication between the gut microbiota and the central nervous system is illustrated in Fig. 1.

2. Methodology of the literature review

The present study was written as a narrative review in an aim to present recent evidence on the gut microbiome and the microbiota-gut-brain axis (MGBA) in ASD. The review was not prospectively registered as the aim was to provide a descriptive and integrative synthesis instead of a systematic or quantitative meta-analysis.

A structured literature search was conducted using major scientific databases: PubMed, Scopus, Web of Science, and Google Scholar. The search continued until May, 2025. The search strategy included combinations of key words, such as 'Autism Spectrum Disorder', 'gut microbiota', 'microbiome', 'gut-brain axis', 'microbiota-gut-brain axis', 'short-chain fatty acids', 'probiotics', 'prebiotics', 'fecal microbiota transplantation', and 'neurodevelopment'.

The screening process was conducted in two stages using predefined eligibility criteria. Titles and abstracts were initially screened for relevance, followed by full-text evaluation. Any discrepancies in study selection were resolved through

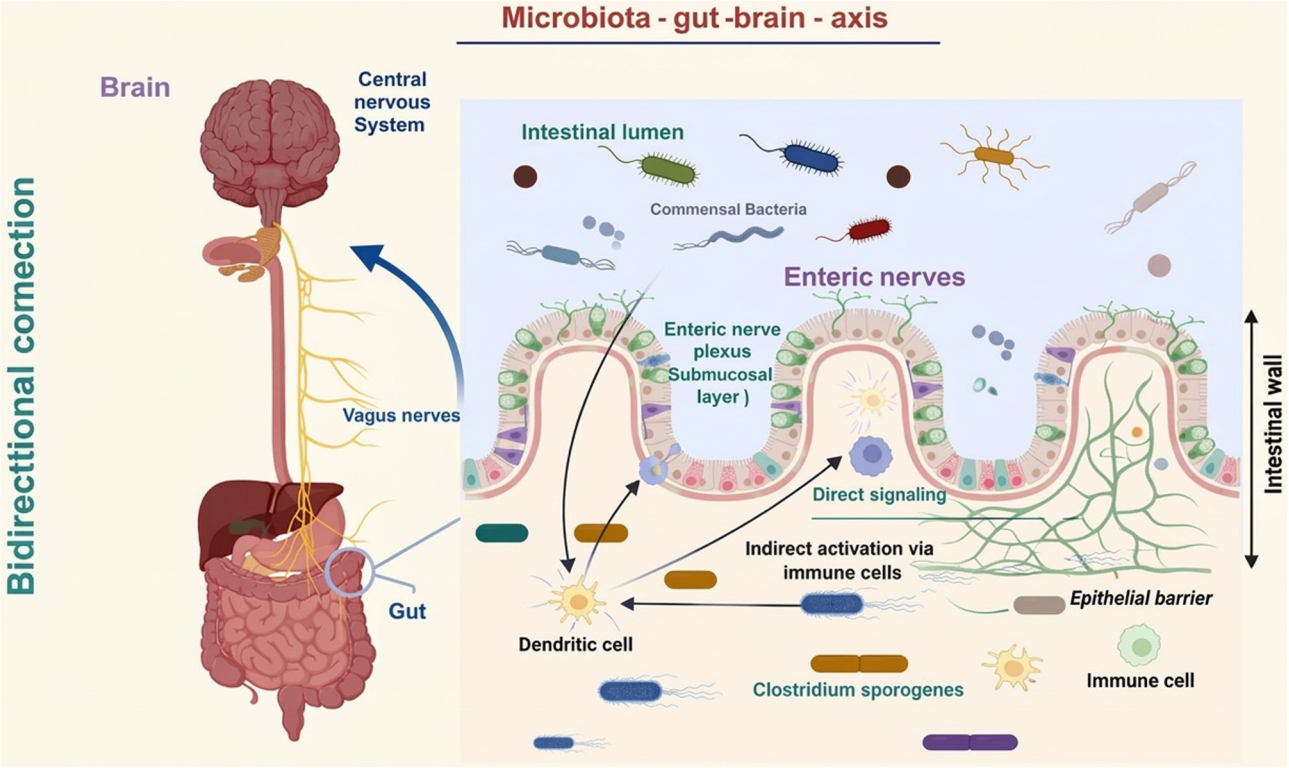

Figure 1. Schematic overview of the bidirectional communication between the central nervous system and the gastrointestinal tract via the microbiota-gut-brain axis (MGBA). Commensal gut microorganisms interact with intestinal epithelial cells, immune cells and the enteric nervous system. Signals are transmitted through neural pathways, particularly the vagus nerve, as well as immune and metabolic routes, enabling reciprocal gut-brain communication. Enteric nerve fibers are depicted within the intestinal wall (lamina propria and submucosal layers) and do not extend into the intestinal lumen. Neural activation is illustrated as an indirect process mediated through epithelial signaling, immune cell activation, and microbial metabolites rather than direct bacterial-neuronal contact.

discussion and consensus. A total of 180 records were identified. Following the removal of duplicates and title/abstract screening, 80 full-text articles were assessed for eligibility, and 50 studies were ultimately included in the final narrative synthesis.

The inclusion criteria consisted of peer-reviewed articles published in the English language, encompassing human observational studies, clinical trials, randomized controlled trials, animal studies, and pertinent *in vitro* investigations that explored associations between gut microbiota and ASD-related outcomes. Conference abstracts lacking full texts were excluded, and non-peer-reviewed sources were used to mitigate methodological bias and enhance the clarity of the results, case reports with insufficient methodological detail, and studies that did not examine neurodevelopmental or ASD-related outcomes.

A formal meta-analysis or standardized risk-of-bias assessment was not conducted based on significant differences in study designs, populations, interventions, and outcome measures. The evidence was instead organized by these studies (*in vitro* studies, animal models, observational human studies, and clinical trials) and synthesized narratively to provide a comprehensive overview. A formal risk-of-bias scoring system (e.g., Cochrane or Newcastle-Ottawa Scale) was not applied due to the design of the review. The evidence was critically appraised based on study design hierarchy, sample size, and methodological transparency. Consequently, randomized controlled trials and replicated human cohort studies were assigned to form the conclusions of the review. Conversely, small-scale pilot trials, open-label studies, and preclinical investigations were interpreted with appropriate caution and discussed separately from human clinical data. This structured approach ensures interpretative rigor while maintaining the integrative nature of the narrative synthesis. While the present review follows a narrative design, efforts were made to enhance transparency and reproducibility through search strategy, eligibility criteria, and study selection procedures.

3. Autism spectrum disorder

ASD is defined by persistent difficulties in social communication and restricted, repetitive patterns of behavior, with considerable heterogeneity in clinical presentation, including variability in cognitive function, language abilities, comorbid conditions, and GI symptom burden (24,25). These features are observed across diverse populations worldwide (26). This disorder may be associated with cognitive impairments and motor difficulties in some individuals, although the presentation varies widely across the spectrum (27).

According to the most recent surveillance data from the Centers for Disease Control and Prevention (CDC)'s Autism and Developmental Disabilities Monitoring (ADDM) Network, the identified prevalence of ASD among 8-year-old children in the USA increased from 1 in 44 (2018 surveillance) to 1 in 36 (2020 surveillance) and most recently, to ~1 in 31 children (3.2%) based on 2022 surveillance data (28). This

upward trend may reflect improvements in diagnostic awareness, screening practices, and service access rather than a true increase in biological incidence. In a regional study conducted in Riyadh, Saudi Arabia, recent estimates revealed a prevalence of ~2.5% (1 in 40 children), with the condition being 3-fold more common among males than females (29).

Although early brain development and neuronal restructuring contribute to the underlying neurobiological mechanisms of ASD, behavioral evaluation remains essential for diagnosis, as there are currently no definitive biological biomarkers. Historically, the classification of ASD has included a variety of developmental disorders, such as Asperger's syndrome, which are now subsumed under ASD in DSM-5. Furthermore, mental health disorders, particularly attention deficit hyperactivity disorder, and genetic disorders such as fragile X syndrome can co-exist with ASD (26).

GI symptoms in individuals with ASD. GI complications are common in individuals with ASD and may exacerbate challenges associated with the condition. Nausea, vomiting, diarrhea, chronic constipation, food allergies, and gastroesophageal reflux disease are common GI issues (30). Among children with ASD, constipation is one of the most frequently reported GI complaints and has been associated in some studies with increased behavioral dysregulation and repetitive behaviors (8,30). Children with ASD often lack the expressive communication skills to describe GI pain, which may manifest outwardly as an increase in rigid, repetitive behaviors or irritability. Therefore, alterations in specific bacterial taxa, including increased abundance of certain *Clostridium* species, particularly *Clostridium perfringens*, identified in case-control analyses, have been reported in some children with ASD; however, direct causal links between these microbial findings and behavioral manifestations remain unconfirmed (31). Metabolomic analyses have reported alterations in dicarboxylate and energy-related metabolic pathways in some individuals with ASD (32).

Some individuals with ASD may exhibit GI functional disturbances that could affect carbohydrate fermentation patterns; however, consistent evidence of intrinsic sugar malabsorption in ASD remains limited (32). Increased intestinal wall permeability has been reported in some children with ASD; however, findings are inconsistent across studies, possibly due to differences in permeability assessment methods (e.g., lactulose-mannitol testing vs. biomarker-based assays), small sample sizes, and variability in participant selection criteria. Such increased permeability, when present, may allow bacteria to cross the intestinal barrier and may contribute to systemic inflammation, as reported in some cohorts (33). This condition has been hypothesized to involve alterations in the production of barrier-strengthening proteins and changes in tight junction structure. Bacterial substances, such as lipopolysaccharides (LPS), may disrupt barrier integrity and contribute to inflammatory reactions that affect brain function (33-35).

Elevated circulating levels of LPS can activate innate immune pathways by Toll-like receptor 4 (TLR4), leading to the increased production of pro-inflammatory cytokines, such as interleukin (IL)-6, tumor necrosis factor-α (TNF-α), and IL-1β. These cytokines may affect brain function by altering blood-brain barrier permeability, promoting microglial activation and modulating neurotransmitter systems. Microglial activation, in particular, has been implicated in synaptic remodeling and neuroinflammatory processes relevant to ASD. Additionally, systemic inflammation may interact with the hypothalamic-pituitary-adrenal (HPA) axis and vagal signaling pathways, thereby contributing to changes in stress responsiveness and behavioral regulation (34-36). Studies have reported the decreased relative abundance of *Bifidobacterium* and increased levels of certain *Clostridium* spp. in some ASD cohorts; however, findings remain heterogeneous and population-specific (37,38).

These bacteria may produce microbial metabolites with neuroactive potential, including short-chain fatty acids (SCFAs), such as butyrate, propionate, and acetate, as well as aromatic compounds, such as p-cresol and tryptophan-derived metabolites. SCFAs may affect neurological pathways through immune modulation and epigenetic regulation and interact with vagal afferent signaling. Propionate has been shown in preclinical models to induce neuroinflammatory responses and repetitive behaviors, whereas butyrate has been associated with anti-inflammatory and barrier-supportive effects. Additionally, the microbial modulation of tryptophan metabolism may affect serotonin availability; however, gut bacteria can produce or regulate γ-aminobutyric acid (GABA), thereby potentially affecting excitatory-inhibitory balance within neural circuits. These pathways collectively illustrate how microbial metabolites may interact with immune, neural, and endocrine components of the MGBA (39,40). Although scientific evidence suggests associations between microbiota alterations, GI symptoms, and behavioral features in ASD, causal associations remain unclear. Studies have found a link between the severity of autistic behaviors and the repetition of intestinal symptoms, highlighting the need for further research into gut health in individuals with ASD (41,42).

Association between GI symptoms and ASD severity. Studies have indicated that digestive disorder prevalence estimates range from ~20% to >70% across studies on children with ASD, which reflects substantial heterogeneity that may stem from differences in age groups studied, based on parent-reported symptoms vs. clinical diagnosis, variability in GI symptom definitions, and differences in study design (43,44). Several studies have examined associations between GI symptoms and ASD. For example, the study by Adams *et al* (45) reported that 70% of children with ASD experienced GI problems, with a link between the severity of autistic symptoms and GI problems in children. In a similar context, Chaidez *et al* (46) reported an association between the health of the digestive system and the behavioral manifestations of ASD, as children with ASD and digestive symptoms exhibited more extreme behaviors compared to their healthy peers. Microbiota-based investigations have also identified differences in gut microbial composition between children with ASD and neurotypical controls, although these studies were not primarily designed as epidemiological prevalence analyses (47).

A large-scale population-based study found that children with ASD were far more likely to experience digestive issues, such as constipation, stomach pain, or reflux, compared to their neurotypical peers. There may be a complex association between gut health and everyday well-being, as these GI issues were frequently associated with increased anxiety,

physical discomfort, and social interaction difficulties (46). Other assessments using standardized Rome IV-based criteria have also reported variability in the association between GI symptoms and ASD severity, highlighting inconsistencies in measurement approaches (48).

The need for further research into how gut health may, or may not, influence the living experiences of children with ASD was highlighted by a study conducted by Jiang *et al* (49), which focused on four groups of toddlers ages 17 to 37 months. The first group included children with ASD who did not have issues with the digestive system, while the second group included children with ASD who suffered from GI issues. The third and fourth groups included children with atypical development, without and with GI issues, respectively. The results of that study demonstrated that the prevalence of digestive disorders was higher in children with ASD compared to children without ASD. However, these GI issues were not significantly associated with ASD symptom severity (49). In another study conducted by Chandler *et al* (50), the symptoms of the digestive system that were reported by parents were searched for in children with ASD compared to healthy children. That study included 132 children with ASD, 81 children with special educational needs but no ASD, and 82 children who developed naturally. The study did not find any connection between the symptoms of the digestive system and the level of intelligence or the severity of the symptoms of ASD (50).

Findings regarding the association between GI symptoms and ASD severity remain inconsistent, likely due to differences in study design, age ranges, definitions of GI symptoms, and behavioral assessment tools. Additional sources of variability include small cohort sizes, cross-sectional study designs, and differences in behavioral assessment scales (e.g., ADOS, CARS, parent-report tools), which may yield divergent estimates of ASD severity. Moreover, selective eating behaviors and restricted diets common in ASD may independently influence GI symptoms and microbial composition, thereby complicating attempts to disentangle direct associations between GI burden and ASD severity.

4. The gut microbiota

The 'gut microbiota' includes a diverse array of microorganisms, including bacteria, archaea, viruses, and eukaryotic microorganisms that inhabit the GI tract. These microorganisms have co-evolved with the host, forming a complex symbiotic association that influences multiple aspects of host physiology. The human GI tract harbors trillions of microorganisms. The mammalian gut microbiota is shaped by long-term host-microbe co-evolution, with diet, host genetics, age, and antibiotic exposure acting as major determinants of microbial composition and adaptive functional capacity (51).

Current estimates suggest that the total number of microbial cells in the human body is broadly comparable to the number of human cells and that the collective microbial genome contains substantially more genes than the human genome (52).

In humans, the gut microbiota consists of trillions of bacteria living symbiotically with the host, contributing substantially to intestinal biomass. This intricate ecosystem comprises not only bacteria but also viruses, fungi, protozoa, and archaea (53).

The term 'microbiota' collectively describes these microorganisms, while the 'microbiome' refers to their complete set of genetic material, including genes and gene products. Four major bacterial phyla, *Bacteroidetes*, *Firmicutes*, *Proteobacteria,* and *Actinobacteria*, predominate in a healthy adult gut microbiota, accounting for the majority of the bacterial community. Additionally, minor taxa such as Verrucomicrobiota and *Fusobacteria* are present at lower relative abundance in healthy adults (54).

Functions of the gut microbiota in health. The gut microbiota is a fundamental component of human health and contributes to host physiology through a variety of biological functions. The gut microbiota helps to break down proteins, fats, and complex carbohydrates that are otherwise difficult for the host to digest. The intestinal bacteria convert dietary fiber into SCFAs, such as butyrate, propionate, and acetate, which play a role in providing energy and promoting colon health (55).

The gut microbiota may affect immune development by promoting immune cell differentiation and regulating inflammatory responses in mucosal immunity, producing antimicrobial peptides, and regulating inflammatory responses. This effect helps in maintaining the balance of the immune environment and preventing inappropriate or excessive immune responses to commensal antigens and non-pathogenic stimuli (56,57). Moreover, the intestinal microbes play a defensive role by competing with pathogens for nutrients and places suitable for adhesion and by producing antimicrobial compounds that inhibit pathogen growth (58).

The gut microbes produce essential vitamins, particularly vitamins B and K, which play a crucial role in numerous physical processes; B vitamins are necessary for the health of the nervous system and energy production, while vitamin K contributes to the blood clotting process (59-61). Apart from these functions, intestinal microbes participate in multiple metabolic pathways, including the metabolism of bile acids, xenobiotics, and undigested dietary fiber. These activities may influence host metabolism and energy homeostasis and have been associated with host energy balance and adiposity (62,63). In addition, the gut microbiota contribute to the gut-brain axis, a bidirectional communication network linking the GI tract and the central nervous system.

Microbial metabolites, including neurotransmitter precursors and SCFAs, may influence brain function and behavior, and alterations in gut microbiota composition have been shown to be associated with several neurological and psychological conditions (64,65).

Functions of the gut microbiota in ASD. The gut microbiota has been increasingly investigated in relation to ASD, primarily through observational and preclinical evidence suggesting associations with physiological and neurobiological processes. The MGBA has been described as a bidirectional communication network linking the GI tract and the brain, through which gut microbes may modulate neural function and behavior (66). Gut microbes play a role in regulating intestinal permeability, which has been investigated in the context of ASD. Disruptions in microbial balance have been associated

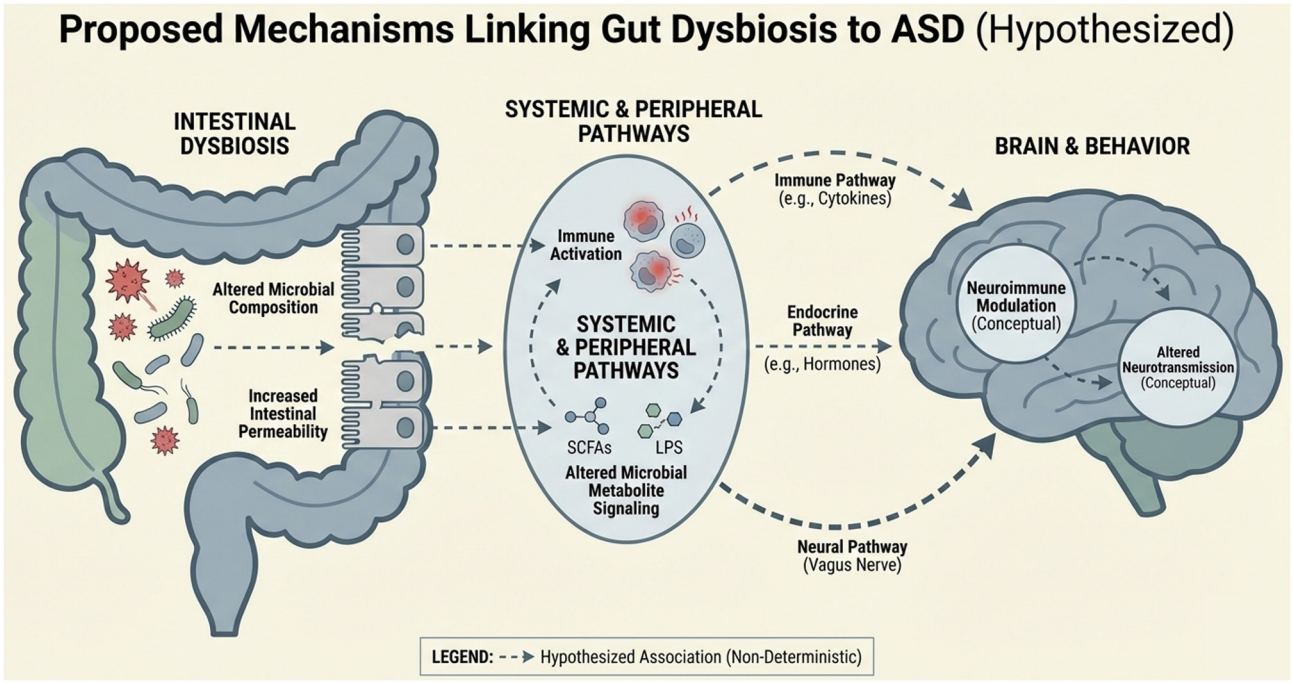

Figure 2. Mechanistic illustration of proposed pathways through which gut dysbiosis may influence neurobiological processes relevant to ASD. Alterations in gut microbial composition may contribute to increased intestinal permeability, immune activation, and changes in microbial metabolites such as SCFAs and LPS. These signals may reach the brain through systemic circulation or neural pathways, including the vagus nerve, potentially contributing to neuroimmune modulation and altered neurotransmission. The pathways shown are hypothesis-generating and are not indicative of established causality. ASD, autism spectrum disorder; SCFAs, short-chain fatty acids; LPS, lipopolysaccharides.

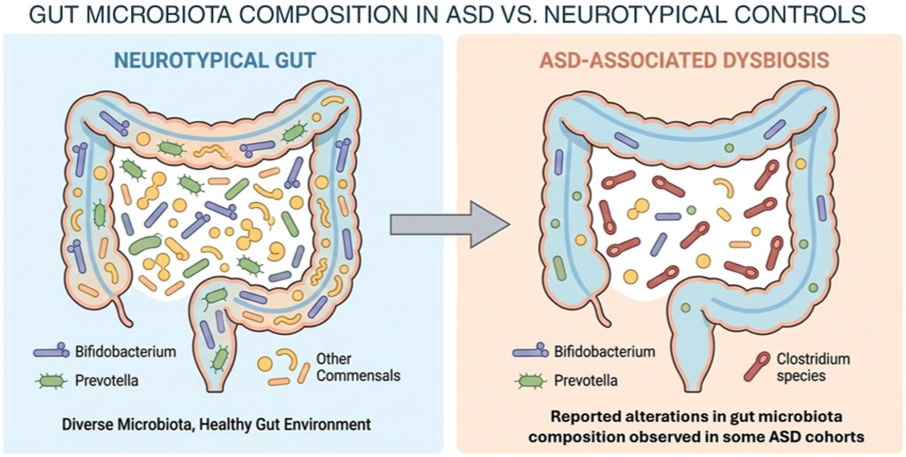

Figure 3. Conceptual comparison of gut microbiota composition in neurotypical individuals and individuals with ASD, as reported in some cohorts. Neurotypical gut microbiota is generally characterized by higher microbial diversity and increased abundance of beneficial taxa such as *Bifidobacterium* and *Prevotella*. By contrast, ASD-associated dysbiosis has been described in some studies by reduced microbial diversity and increased abundance of specific taxa, including *Clostridium* species. These patterns are variable and not consistently observed across all populations. ASD, autism spectrum disorder.

with increased intestinal permeability, a phenomenon often referred to as 'leaky gut', although its clinical significance in ASD remains under debate. Microbial components or byproducts may translocate into the bloodstream, potentially influencing systemic immune signaling and neurobiological processes. The proposed mechanisms linking gut dysbiosis to neurobiological processes relevant to ASD, including immune activation, changed microbial metabolite signaling, and vagal pathways, are summarized in Fig. 2. Increased intestinal permeability has been reported in a subset of individuals with ASD, although its association with symptom severity remains unclear and inconsistent across studies, potentially due to heterogeneity in study populations, differing definitions of 'leaky gut', and the lack of standardized biomarkers across cohorts (67).

The gut microbiota may modulate behavior and brain development via neuroendocrine, neuroimmune, and autonomic nervous system pathways. Alterations in gut microbiota composition have been shown to be associated with several conditions, including psychiatric and inflammatory disorders,

and are being explored in the context of ASD (15). Additionally, gut microbes produce metabolites, such as SCFAs, butyrate, propionate, and acetate, by breaking down dietary fiber. SCFAs may influence neural signaling through immune, endocrine, or vagal pathways, rather than through the direct penetration of the blood-brain barrier. Several studies and meta-analyses have reported alterations in the gut microbiota composition in subsets of individuals with ASD compared with neurotypical controls (68-70); however, the specific taxa and direction of change vary considerably across cohorts (Fig. 3). Behavioral properties intrinsic to ASD, particularly restricted dietary intake and sensory-driven food aversions, may form microbial profiles and influence observed metabolite patterns, thereby interacting with the proposed MGBA mechanisms. Changes in SCFA levels have been observed in individuals with ASD, which may suggest that these metabolites are involved in pathways associated with behavioral features observed in ASD (68).

At the molecular level, microbial metabolites may affect neuronal circuit development and synaptic function through multiple converging mechanisms. SCFAs, particularly butyrate, function as histone deacetylase (HDAC) inhibitors, thereby modulating gene expression involved in synaptic plasticity and neuronal differentiation. Altered SCFA profiles may affect microglial maturation and activity, synaptic pruning processes that are critical during early neurodevelopment. Excess propionate exposure in preclinical models has been associated with altered glutamatergic and GABAergic signaling, disruption of excitatory-inhibitory balance, and behavioral phenotypes resembling ASD. Furthermore, immune mediators induced by gut dysbiosis, such as IL-6 and TNF-α, may affect synaptic transmission by modifying neurotransmitter receptor expression and influencing long-term potentiation and synaptic remodeling. Through these interconnected immune, metabolic, and epigenetic pathways, gut microbiota alterations may indirectly shape neuronal connectivity and circuit-level function relevant to ASD (68).

Gut microbes produce a range of metabolites relevant to neural signaling, including bile acids, glutamate, dopamine, norepinephrine, serotonin, SCFAs, GABA, and histamine, which interact with the central nervous system (71,72). Alterations in gut microbiota composition affect neural circuits involving the amygdala, a functional brain area connected to complex information processing, emotional regulation, decision-making, and social behaviors. This connection is based primarily on preclinical models and associative human studies (73,74). However, the therapeutic relevance of targeting the gut microbiota for modulating ASD-related amygdala dysfunction remains uncertain (75). Changes in gut microbiota have been linked to genetic, epigenetic, and environmental influences during early development, which affect the development of the amygdala and white matter connections with other brain regions (76).

The strength of the evidence linking gut microbiota-related processes to ASD varies across different biological pathways. Immune-related mechanisms, which include altered cytokine profiles and low-grade inflammation, are supported by a substantial body of ASD-specific research involving both human and animal studies. Neural mechanisms, such as vagal signaling and neurotransmitter modulation, are mainly supported by preclinical models and associative data from human populations. Conversely, endocrine and metabolic pathways, including stress hormone modulation and the involvement of the HPA axis, are largely inferred from the broader literature on the gut-brain axis, with limited ASD-specific validation.

Factors influencing gut microbiota composition in autistic patients. While gut microbiota development follows a dynamic trajectory across the lifespan, several factors may form microbiota composition in individuals with ASD. In individuals with ASD, several factors, including environmental exposures, diet, medication use, and genetic predispositions, affect gut microbiota composition. These factors may contribute to inter-individual variability in microbiota profiles observed across ASD cohorts.

At the molecular level, these factors may affect gut microbiota composition by altering microbial metabolic niches, host immune signaling, and epithelial barrier regulation. Changes in substrate availability, stress-related neuroendocrine signaling, and immune-mediated selective pressures can reform microbial community structure by favoring taxa with specific metabolic capacities. Moreover, host-derived factors such as antimicrobial peptides, mucin secretion, and immunoglobulin A coating may selectively modulate bacterial colonization and persistence, thereby contributing to long-term shifts in microbial equilibrium (77). Postnatal colonization is affected by mode of delivery, feeding practices, and early environmental exposures. After birth, several factors, such as antibiotic use, exposure to infections, breastfeeding vs. formula feeding, diet, stress, and genetics, influence the composition of the gut microbiota. These factors collectively influence the diversity of the gut microbiota and its long-term function (78).

These factors may contribute to inter-individual variability in microbiota profiles observed across ASD cohorts, as well as to symptom heterogeneity, particularly given differences in diet selectivity, medication exposure, and environmental influences that vary widely between study populations. These factors include environmental influences, diet, medications, and genetic predispositions. The gut microbiota composition in ASD is shaped by a complex interplay of genetic, environmental, and early-life factors, including diet, antibiotic exposure, and mode of delivery (Fig. 4), as described below:

Dietary patterns. Nutrition is a crucial factor, as children with ASD often follow restricted eating habits, resulting in limited dietary diversity and deficiencies in certain nutrients. These dietary patterns may influence the composition of the gut microbiota, promoting the growth of some microbial communities while inhibiting others.

A diet rich in processed foods and low in dietary fiber has been shown to be associated with shifts in gut microbiota composition, including the reduced abundance of beneficial taxa and the increased prevalence of potentially pathogenic species (63,79). For example, the excessive intake of simple carbohydrates may contribute to gut dysbiosis by favoring the expansion of opportunistic microbial populations and promoting overall microbial imbalance (63).

Resistant starch, oligosaccharides, and dietary fiber are types of complex carbohydrates that can positively modify the composition of beneficial microorganisms in the gut, such as (*Bifidobacterium*

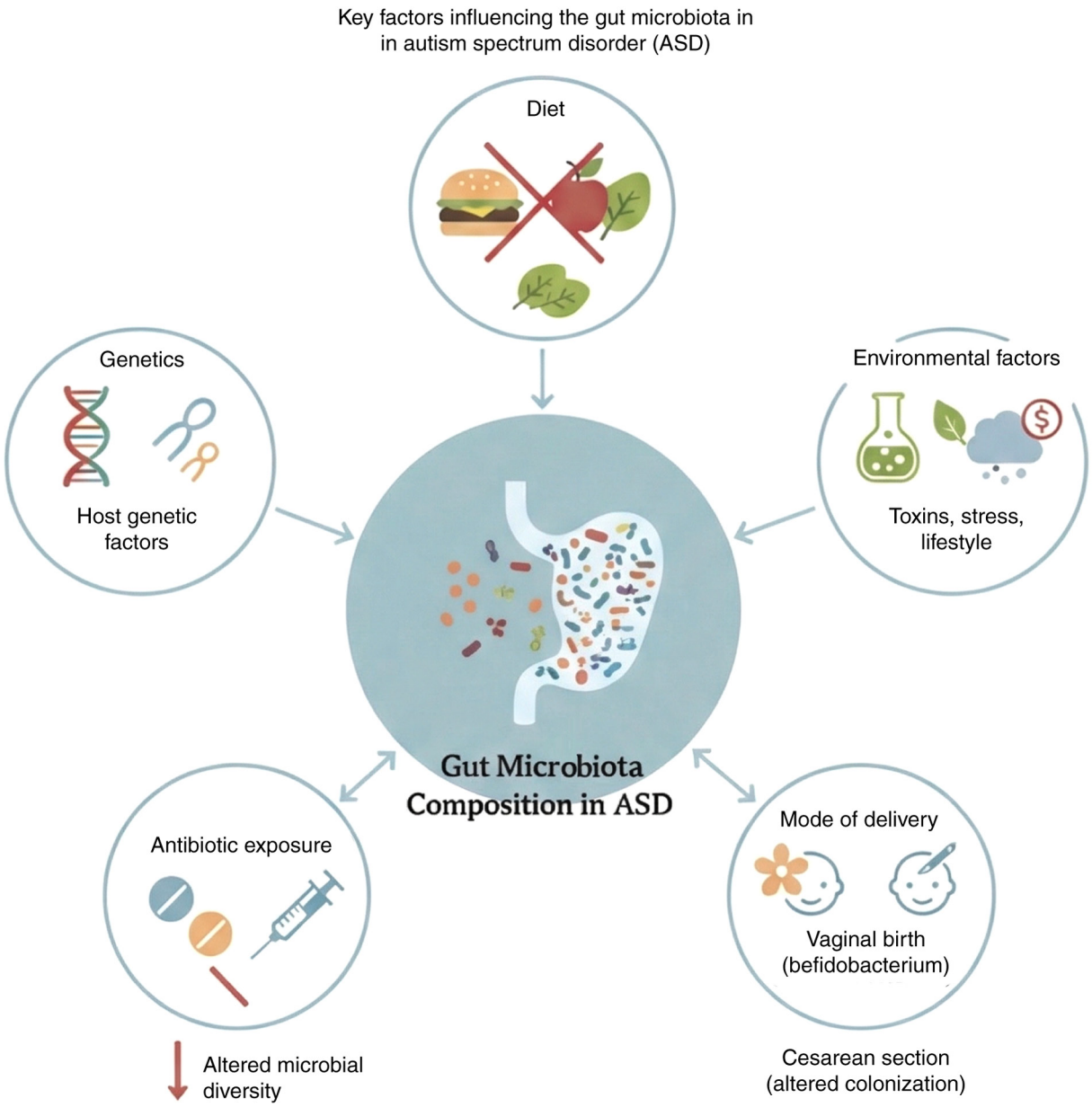

Figure 4. Conceptual diagram illustrating key factors proposed to influence gut microbiota composition in individuals with ASD. These factors include dietary patterns, antibiotic exposure, mode of delivery (vaginal vs. cesarean), host genetic background, and environmental influences such as stress and lifestyle. The combined effects of these factors may contribute to inter-individual variability in gut microbiota profiles observed across ASD populations. ASD, autism spectrum disorder.

spp., *Lactobacillus* spp., *Akkermansia muciniphila*, and other fiber-associated taxa, including *Faecalibacterium, Roseburia, Bacteroides, Prevotella, Ruminococcus,* and specific *Clostridium* spp.). Beyond compositional changes, dietary fiber fermentation produces SCFAs that function as signaling molecules. SCFAs activate G-protein-coupled receptors, such as GPR41 and GPR43, and may inhibit HDACs, thereby affecting epithelial gene expression, inflammatory signaling, and mucosal immune responses. Reduced fiber intake may therefore decrease butyrate availability, impair tight junction protein expression (e.g., occludin and claudins), and increase intestinal permeability, potentially reinforcing inflammatory and microbial instability pathways (55,80).

The restricted diets typically observed in ASD, often termed 'food selectivity' or 'picky eating', are characterized by a strong preference for starches, snacks, and highly processed foods, accompanied by a strict aversion to fruits, vegetables, and whole grains. This behavioral rigidity directly limits the intake of dietary fiber. Consequently, fiber-degrading bacteria, such as *Prevotella* and *Bifidobacterium*, are frequently found at reduced levels in ASD cohorts (79). This restricted diet-induced depletion of beneficial taxa results in the lower production of SCFAs, such as butyrate, which is essential for gut barrier integrity (55). Thus, the unique microbial signature often attributed to ASD may be, at least in part, a secondary consequence of the restrictive dietary behaviors inherent to the disorder, although direct causal associations remain to be established, creating a self-perpetuating cycle of dysbiosis.

Mode of delivery. The method of delivery affects the composition of the gut microbiota of newborns. During the

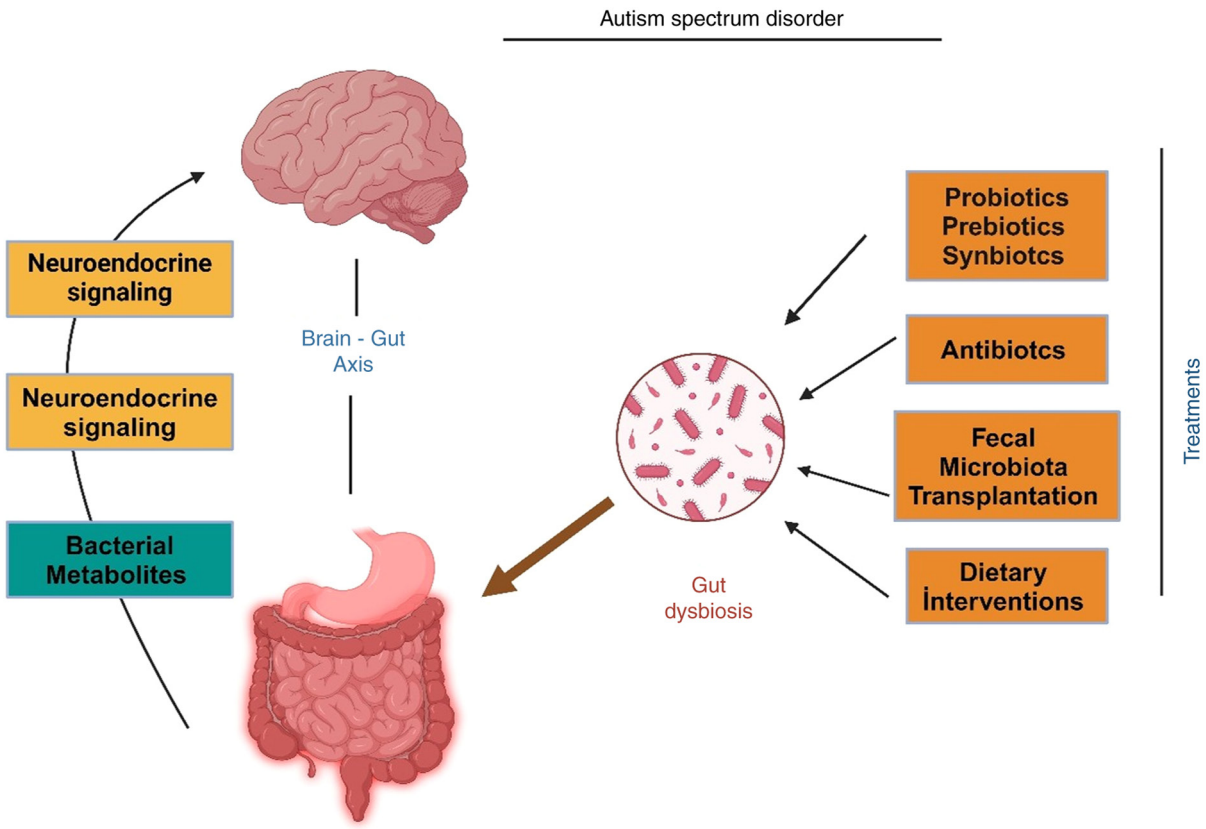

Figure 5. Conceptual overview of the microbiota-gut-brain axis in autism spectrum disorders. Gut dysbiosis is hypothesized to influence neurodevelopment through microbial metabolites, neuroendocrine signaling, immune modulation, and neural pathways. Therapeutic approaches targeting the gut microbiota are shown illustratively to indicate potential points of intervention within this axis, without implying established clinical efficacy.

birth process, the baby is colonized with microorganisms. During vaginal birth, the baby receives the vaginal microbiome of the mother, while during cesarean delivery, the baby receives bacteria from the skin of the mother (81).

It has been estimated in specific cohorts that maternal fecal microbiota may contribute substantially to the early gut microbiota of vaginally delivered infants, with lower similarity observed following cesarean delivery (82). Furthermore, data demonstrated that compared to infants born via cesarean delivery, who exhibited an enrichment of certain Gram-negative taxa associated with early-life dysbiosis, vaginally delivered babies had higher levels of beneficial microbes, such as *Lactobacillus*, *Bacteroides,* and *Bifidobacterium*, health-associated microbes commonly enriched in early-life gut microbiota. Early microbial colonization during this critical developmental window may affect immune imprinting processes, including regulatory T-cell differentiation and mucosal immune tolerance. Delayed colonization by obligate anaerobes may alter microbial metabolite exposure during neonatal immune maturation, potentially affecting epithelial barrier development and long-term microbial stability (81,83,84).

Microbial changes in the gut of newborns born via cesarean section are associated with a range of health issues, including an increased risk of infection, and have been explored as contributors to neurodevelopmental outcomes, although evidence linking delivery mode directly to ASD remains inconclusive (83-85). Similarly, Korpela (84) and Inchingolo *et al* (85) assessed the impact of delivery modality on the gut microbiota. They discovered that infants born via cesarean section were more prone to respiratory illnesses during their first year of life and had more infections, such as *Enterococcus* and *Klebsiella*.

Antibiotic use. The gut microbiota is significantly affected by antibiotic use, particularly during early development. This can lead to a decrease in microbial diversity and has been explored as a potential factor associated with neurodevelopmental outcomes, although evidence specifically linking antibiotic exposure to ASD remains limited and inconclusive, and causal associations remain unestablished. Antibiotics can eliminate both beneficial and harmful bacteria, which can lead to gut dysbiosis, a disrupted microbial balance (86).

Studies have demonstrated that the long-term, excessive use of antibiotics reduces the number of beneficial bacteria in the gut, such as *Bifidobacterium* and *Lactobacillus*, while increasing the growth of harmful bacteria, such as certain species of *Clostridium* and Enterobacteriaceae (87,88). This change in microbial balance may contribute to an altered intestinal permeability, immune activation, and shifts in microbial metabolite-mediated neurotransmitter pathways, which have been proposed in experimental and observational studies as potential mechanisms influencing GI and neurobehavioral features observed in ASD (89,90).

The antibiotic-induced depletion of anaerobic taxa may reduce SCFA production and alter bile acid metabolism, thereby reshaping microbial ecological niches (70). The

concurrent expansion of Gram-negative bacteria may increase luminal LPS levels, activating TLR4-mediated signaling and downstream pro-inflammatory cytokine pathways. In addition, the disruption of microbial tryptophan metabolism may affect serotonin precursor availability, further modifying host-microbe interactions (89,90).

5. Microbiota-gut-brain axis

An increasing body of research describes the MGBA as a bidirectional communication system linking GI function with neural and neurodevelopmental processes, including those investigated in the context of AS, as conceptually illustrated in Fig. 5 (91). While numerous MGBA mechanisms, such as neuroendocrine signaling via the hypothalamic-pituitary-adrenal (HPA) axis, immune system modulation, vagus nerve stimulation, and the production of microbial metabolites (e.g., SCFAs), have been described in neurological and psychiatric disorders, evidence supporting their role in ASD remains heterogeneous and varies by pathway (92,93).

Research indicates that GI symptoms are prevalent in children with ASD and have been reported to co-occur with behavioral and emotional features, such as anxiety, sleep disturbances, and irritability, although the directionality of these associations is unclear (94,95). Alterations in gut microbiota composition have been proposed as one of several factors that may contribute to these associations, as neurological, endocrine, and immunological pathways all influence neurological function. Gut-derived signals may affect brain function through mechanisms involving microbial metabolites, vagal signaling, and immune-mediated pathways, which have been explored in relation to ASD-related features (14).

Studies suggest that MGBA signaling may differ between children with ASD and neurotypical controls, although findings are heterogeneous (96,97). Individuals with ASD experience digestive symptoms, such as constipation and abdominal pain, which have been reported to co-occur with behavioral features including rigidity and sensory hypersensitivity. An imbalance in the gut microbiota is often observed, with alterations in gut microbiota composition reported in some ASD cohorts, including changes in the relative abundance of taxa within the *Bacteroidetes* phylum and certain *Clostridium* species, which have been hypothesized to relate to immune and neuroinflammatory pathways explored in ASD research, although evidence remains inconclusive (34,77).

Immune-mediated gut-brain signaling exhibits the most consistent association with ASD-related features, whereas neural and endocrine pathways are supported mainly by indirect or preclinical evidence. Specific ASD behaviors, particularly repetitive behaviors, have been linked to microbial metabolic output (98,99). For instance, altered levels of certain SCFAs (such as elevated propionate relative to butyrate) have been shown in animal models to induce repetitive behaviors in preclinical models and neuroinflammation (34).

At a mechanistic level, immune activation represents one of the most biologically plausible links between gut dysbiosis and ASD pathogenesis. Increased intestinal permeability may facilitate the systemic translocation of microbial-derived molecules such as LPS, which can activate TLR4 signaling and promote the release of pro-inflammatory cytokines, including IL-6, TNF-α, and IL-1β. These circulating mediators may alter blood-brain barrier permeability and promote microglial activation. Activated microglia regulate synaptic pruning and remodeling during neurodevelopment; dysregulated microglial signaling has been implicated in atypical synaptic density and excitatory-inhibitory imbalance reported in ASD. Persistent low-grade inflammation may therefore influence circuit maturation within cortical and limbic regions involved in social cognition and behavioral flexibility (34,56).

Neural communication through the vagus nerve represents another key MGBA pathway. Microbial metabolites and inflammatory mediators may stimulate vagal afferents projecting to the nucleus tractus solitarius, which integrates autonomic input and relays signals to higher-order regions, including the amygdala and hypothalamus. An altered vagal tone has been associated with anxiety, stress reactivity, and emotional dysregulation, features reported in ASD populations. Although direct causal evidence is limited, this neural route presents a framework linking peripheral microbial alterations with central behavioral modulation (100,101).

Endocrine mechanisms further integrate microbiota-brain signaling. Immune activation and microbial metabolites may affect the function of the HPA axis, altering cortisol secretion and stress responsiveness. Dysregulated HPA axis activity has been described in subsets of individuals with ASD and may contribute to heightened anxiety, irritability, and sensory hypersensitivity. Additionally, gut microbes modulate tryptophan metabolism, potentially shifting its availability between serotonin synthesis and the kynurenine pathway, which affects neuroinflammatory tone and neurotransmitter balance (68,102). Through the convergence of immune, neural, and endocrine pathways, changes in the gut microbiota may contribute to neurodevelopmental processes relevant to ASD, although definitive causality remains to be established. The detailed molecular and cellular mechanistic evidence described above is derived from preclinical animal models and *in vitro* investigations (16,35,39). While these models provide biological insight into immune activation, microglial signaling, neurotransmitter modulation, and HPA axis interactions, the direct validation of these mechanisms in well-characterized human ASD cohorts is limited. Therefore, extrapolation from experimental systems to clinical ASD populations needs to be undertaken, and translational research is required to confirm these mechanistic pathways in humans. Thus, the dietary preferences of ASD individuals not only shape the microbiome, but also alter the MGBA signaling in a manner that can exacerbate the very repetitive behaviors that limit their diet.

6. Gut microbiota-targeted therapeutic interventions in ASD

Currently, there is no approved pharmacological treatment targeting the core symptoms of ASD, although behavioral, educational, and supportive interventions are widely used. Despite evidence suggesting associations between gut microbial alterations and ASD, researchers focus on techniques for treating the illness by manipulating the gut microbial community as a potential experimental or adjunctive therapeutic strategy. In parallel, computational approaches, such as virtual toxicity screening and molecular docking, are

combined with microbiological and translational research pipelines to accelerate the prioritization of promising therapeutic candidates before experimental validation (103). These microbiota-targeted strategies may include oral prebiotics, probiotics, nutritional supplements, FMT, and modified protocols, such as MTT (104).

It is critical to understand the role of gut microbiota in the pathophysiology of ASD. These emerging approaches, behavioral, speech, and social therapies, along with nutritional and medical treatments, can alleviate symptoms (105-107). The association of gut microbes with ASD has been demonstrated in numerous studies, as these microbes play a critical role in regulating brain development, function, and behavior (68). Clinical studies and animal models have demonstrated an interaction between ASD behavior and gut microbiota. Animal studies have shown that the transplantation of microbiota from individuals with neurodevelopmental conditions can induce autism-like behaviors in germ-free mice, although translation to human ASD remains uncertain (108,109). Research has shown that symptoms, such as bloating, stomach pain, diarrhea, and constipation, are common in individuals with ASD and have been reported to be associated with ASD symptom severity in some studies (108-111). The strength of evidence supporting microbiota-targeted interventions in ASD varies substantially across study designs. While randomized controlled trials remain limited and often report modest or subgroup-specific effects, much of the available evidence is derived from open-label studies, small pilot trials, animal models, and *in vitro* experiments. Therefore, current findings need to be interpreted with caution.

To facilitate comparison across different microbiota-targeted interventions, Table I summarizes clinical and preclinical studies investigating probiotics, prebiotics, synbiotics, and FMT in ASD. Before examining specific microbiota-targeted interventions, establishing these strategies is essential, considering the robust mechanistic evidence underpinning microbiota-gut-brain interactions in ASD. The most compelling evidence suggests that ASD involves immune-related pathways, particularly dysregulation of inflammatory responses and impairment of gut barrier integrity, thereby highlighting these processes as potential therapeutic targets (112,113). Neural mechanisms, including neurotransmitter signaling and vagal modulation, are supported by animal models and correlational studies conducted in human populations (16,71,74,101). Endocrine and metabolic pathways, including the regulation of stress hormones, are inferred from research concerning the wider MGBA. These distinctions are essential for the accurate interpretation of therapeutic outcomes and underscore the experimental character of current microbiome-based interventions in ASD.

Research has indicated that the maternal consumption of a high-fat diet during pregnancy leads to changes in the gut microbiota of newborns, which may increase the risk of developing ASD. Breastfeeding for ≥6 months reduces the risk of developing this disorder; formula feeding introduces the bacteria *Clostridium* difficile into the gut of newborns. Furthermore, even short-term antibiotic use may cause long-term changes in the gut microbiome (82).

The study by Xiao *et al* (114) revealed that children who took antibiotics in the first 3 years of life experienced changes in the composition of their gut microbiota. Immunodeficiencies, Crohn's disease, obesity, inflammatory bowel disease, and behavioral abnormalities, particularly in children with ASD, are among the disorders in which gut bacterial imbalances are frequently present (114). Human and animal studies have shown that ASD is associated with alterations in metabolites and gut microbiota composition (68).

Prenatal treatment with the antiepileptic drug valproic acid in mice has been shown to cause phenotypic changes in the *Firmicutes* and *Bacteroidetes* phyla, as well as behaviors resembling ASD (115). Another study demonstrated that compared to their neurotypical siblings and healthy controls, children with ASD frequently have different gut microbiota compositions that are marked by increased microflora and decreased microbial diversity, which may aggravate ASD symptoms (116). Children with ASD may have more severe ear infections and use antibiotics more frequently, which could cause *Desulfovibrio* bacteria to overgrow and produce virulence components linked to ASD, a mechanism extensively highlighted by Finegold's research (117). Patients with GI issues and ASD have also been found to have elevated *Sutterella* levels, which are related to mucosal metabolism. However, other studies, such as those by Gondalia *et al* (118) and Son *et al* (119), revealed no appreciable variations in the composition of gut microbiota between autistic individuals and their sibling controls (118,119).

Children with ASD have been reported to exhibit an increased abundance of specific *Clostridium* species, particularly *Clostridium histolyticum* and *Clostridium perfringens*. As suggested by Ellen Bolte in 1998, this overgrowth is associated with alterations in the gut microbiota induced by antibiotics (68,107,120). Neurobehavioral and GI symptoms in regressive ASD cases temporarily improved with vancomycin treatment; however, the issues returned once the antibiotic was terminated, probably as a result of *Clostridium* spores (121). Children with ASD and PDD-NOS are more likely to have elevated levels of potentially harmful *Clostridium* metabolites, such as phenols and p-cresol (122). *Clostridium* bacteria are resistant to glyphosate. While good bacteria, environmental variables such as the pesticide glyphosate may also contribute to ASD through the proliferation of these bacteria (68,107).

Malabsorption and digestive dysfunction are common issues among autistic individuals. Duodenal microbiota diversity and disaccharidase activity differ between patients and healthy controls. A small decrease in bacterial diversity has been observed in children with ASD; however, oral microbiome investigations have not revealed any substantial differences in bacterial diversity in ASD (106). *Prevotella*, which is crucial for vitamin production and glucose metabolism, was shown to be at lower levels in patients (123).

Due to their propensity to release toxins, such as ammonia and yeasts, *Candida albicans* bacteria are more common in toddlers diagnosed with ASD (68). However, studies with large sample sizes are required to fully elucidate the role that fungi play in ASD. The concept of the MGBA has been proposed based on extensive research, where the body can regulate the gut microbiota through neural, immune, and endocrine pathways, assisting in the maintenance of the environmental balance and response to changes (114). Gut microbes and their metabolites can influence the body through the MGBA. A previous study

Table I. Comparison of studies on gut microbiome interventions for autism spectrum disorder.

Intervention	Type of study	Study subjects	Outcomes	Limitations	(Refs.)
Prebiotics, probiotics, synbiotics	Animal study	BTBR mice	Improved social behaviors, reduced repetitive behaviors	Limited translation to humans	(109)
Probiotics	Animal study	BTBR mice	Improved social behavior in some groups, changes in gut microbiota	Animal model	(110)
Probiotics	Open-label trial	Children with ASD (n=30)	Improved GI symptoms and parent-reported behavioral scores	Small sample size, no control group, subjective outcomes	(127)
FMT/probiotics	Meta-analysis of RCTs	Children with ASD (=336 across studies)	Suggestive associations between microbiota interventions and behavioral outcomes	Included animal studies. Limited low-quality of human studies; more research needed	(104)
Probiotic + colostrum	Double-blind RCT (pilot, crossover)	Children with ASD and GI symptoms (n=8)	Improved GI symptoms in some participants	Very small sample size	(21)
Probiotics	Case study	Boy with ASD and severe cognitive disability (n=1)	Improved core ASD symptoms	Single case study, limited generalizability	(128)
Probiotics	Double-blind RCT	Preschoolers with ASD (n=85)	No significant effect on overall ASD severity; subgroup-specific behavioral effects	Heterogeneity; subgroup analysis	(111)
Prebiotics, probiotics, synbiotics	*In vitro* gut microbiome model	Fecal samples from ASD children	Positive modulation of gut microbiota	*In vitro* models may not reflect *in vivo* physiology	(129)
Prebiotics	Double-blind RCT	Children with ASD (n=33)	Improved GI symptoms and parental quality of life; no effect on core ASD symptoms	Small sample size	(106)
Fecal microbiota transplantation (FMT)	Open-label clinical trial	Children with ASD (n=18)	Improved GI and ASD symptoms, changes in gut microbiota	Small sample size, no control group	(38)

ASD, autism spectrum disorder; FMT, fecal microbiota transplantation; RCT, randomized controlled trial; GI, gastrointestinal.

demonstrated that alterations in MGBA signaling have been proposed as a potential contributing mechanism in ASD (68). Research has indicated that gut microbes influence both the intestinal and central nervous systems via immune, endocrine, neurological, and metabolic pathways (122). The symbiotic association between gut microbes and the host is crucial for maintaining survival and health. These microbes provide essential nutrients, aid in the metabolism of toxins, drugs, and food ingredients by the body, and form a barrier that shields the body from infections (124).

The gut microbiota produces a variety of beneficial metabolites, such as butyrate and lactic acid, that have anti-inflammatory, antitumor, and antimicrobial properties. These compounds are necessary to protect the body from harmful bacteria, regulate immunological responses, and preserve overall health. Butyrate is an SCFA that is vital for colon cells to use as an energy source. It also aids in reducing inflammation and strengthening the intestinal barrier. However, the primary producers of lactic acid, which maintains the balance of the gut microbiome and reduces the risk of infection and inflammation, are bacteria such as *Lactobacillus*. Thus, these metabolites highlight the vital role the microbiome plays in human health by promoting intestinal and systemic immune functions. This knowledge paves the way for potential microbiome-based therapeutic applications to enhance beneficial microbial communities and related metabolites in the body (124). In addition, the gut microbiota significantly

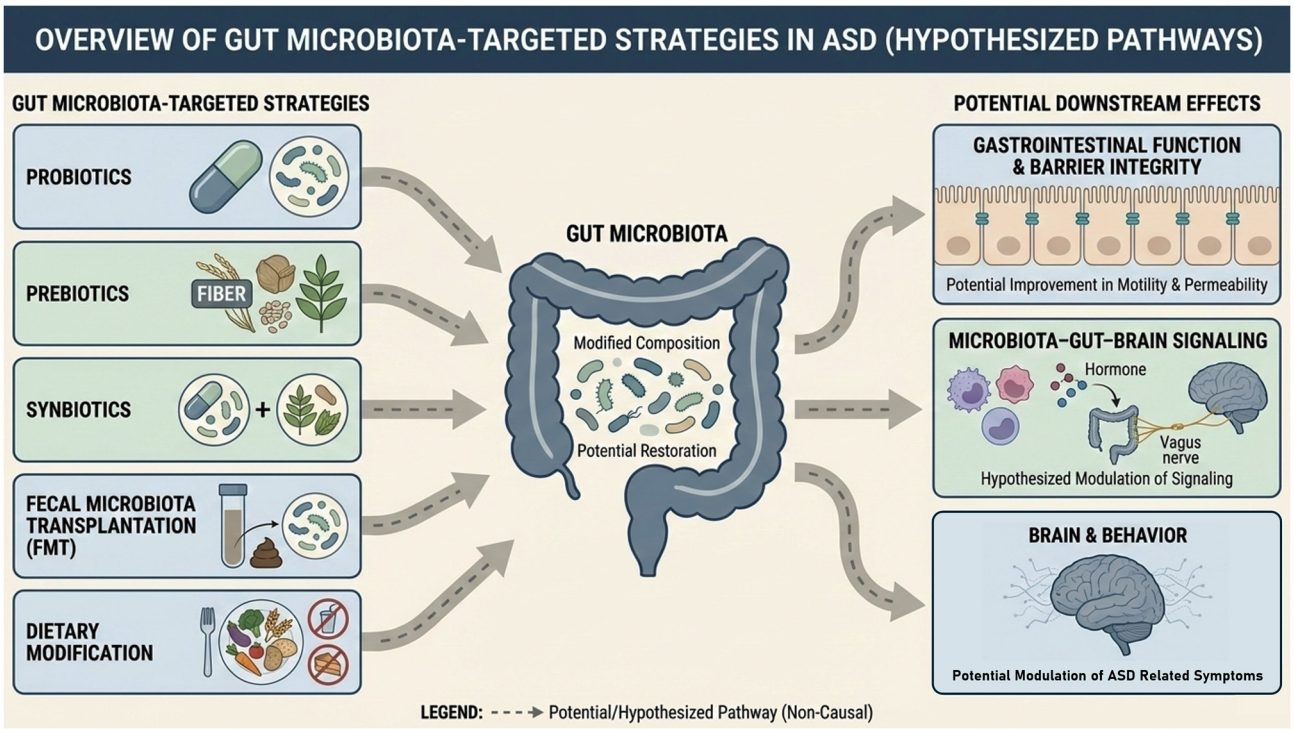

Figure 6. Overview of gut microbiota-targeted interventions explored in ASD. Approaches such as probiotics, prebiotics, synbiotics, fecal microbiota transplantation and dietary modification are hypothesized to modulate gut microbial composition and microbiota-gut-brain signaling. These interventions may influence gastrointestinal symptoms, gut barrier integrity and neuroimmune pathways, although reported effects on core ASD symptoms remain variable across studies. ASD, autism spectrum disorder.

influences the development of the adaptive immune system of the host. Increasing evidence suggests that microbial metabolites play a critical role in the development and function of the central nervous system. Animal studies have also shown that the absence of a gut microbiota early in life can cause alterations in the genetic pathways of the amygdala, a region associated with emotions, fear, and social behaviors (105,110,125).

The MGBA is a complex two-way communication system that involves many physiological pathways, such as immunological responses, metabolic processes, neuronal signaling, gut barrier permeability, and neuroendocrine mechanisms (89). A 'leaky gut', which is brought on by an imbalance in gut microbes that support the integrity of the gut barrier, is common in individuals with ASD (68). This increased permeability may allow microbial components to enter the bloodstream, triggering systemic inflammation and immunological responses. The gut microbiota plays a role in immune dysregulation in people with ASD, as demonstrated by increased microglia activity and elevated inflammatory markers (116). Furthermore, metabolites such as SCFAs, which are produced by gut microbes, have an impact on the metabolism and immune response of the body. Alterations in SCFA profiles have been reported in association with behavioral features of ASD (122). Additionally, gut microbes influence the levels of neurotransmitters such as GABA and serotonin, which in turn help control behavior and mood. They also may influence the HPA axis, which links gut function and the response of the body to stress. These pathways may therefore be targets for therapeutic interventions in ASD and demonstrate the complex connections between gut microbes and neurological processes (68).

Some interventions aim to modify the gut microbiome, such as FMT, probiotics, prebiotics, and certain antibiotics; these have been explored experimentally, although their effects are transient and raise concerns regarding long-term microbiome disruption in conditions, such as ASD via their effects on the MGBA. FMT involves introducing beneficial bacteria from a healthy donor, which helps restore microbial balance and improve microbial diversity in individuals with bacterial imbalances, potentially improving digestive and behavioral symptoms (48). Both probiotics (live beneficial bacteria) and prebiotics (non-digestible fibers that stimulate the growth of beneficial bacteria) contribute to supporting gut health by rebalancing the gut microbiome. These approaches have been shown to enhance microbial diversity within the gut, positively impacting immune, neurological, and endocrine responses and potentially improving symptoms of ASD. The use of synergistic probiotics, which combine probiotics and prebiotics, may further improve the promotion of metabolic balance and microbial diversity in the gut (124,126).

At the molecular level, several mechanisms have been proposed to explain the beneficial effects of microbiota-targeted interventions in ASD. Probiotic strains, such as *Lactobacillus* and *Bifidobacterium,* may enhance epithelial barrier integrity by upregulating tight junction proteins, including occludin and claudins, thereby reducing intestinal permeability and limiting systemic translocation of pro-inflammatory microbial components. This barrier-stabilizing effect may attenuate circulating cytokine levels (e.g., IL-6, TNF-α), potentially reducing microglial activation and downstream neuroinflammatory signaling within cortical and limbic regions (68,89).

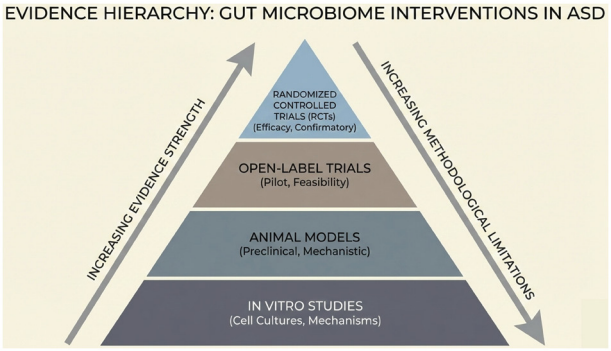

Figure 7. Evidence hierarchy summarizing the current landscape of gut microbiome intervention studies in ASD. The pyramid illustrates increasing levels of evidence from *in vitro* studies and animal models to open-label clinical trials and randomized controlled trials. While higher levels of evidence provide greater rigor and generalizability, they are also associated with methodological challenges, including heterogeneity, limited sample sizes, and variability in intervention protocols. ASD, autism spectrum disorder.

Prebiotics and dietary fibers may exert therapeutic effects primarily through selective fermentation and the increased production of SCFAs, particularly butyrate. Butyrate serves not only as an energy source for colonocytes but also functions as an HDAC inhibitor, thereby modulating gene expression related to synaptic plasticity, immune regulation, and oxidative stress responses. The restoration of balanced SCFA profiles may contribute to improved gut barrier function, reduced inflammatory tone, and the stabilization of excitatory-inhibitory neurotransmitter balance (124,126).

FMT and MTT may exert broader ecosystem-level effects by restoring microbial diversity, reintroducing fiber-fermenting taxa, and normalizing metabolite production. This ecological restructuring may reduce the overrepresentation of toxin-producing bacteria (e.g., certain *Clostridium* species) and lower levels of potentially harmful metabolites such as p-cresol. Improved microbial diversity may also influence tryptophan metabolism and serotonin availability, thereby modulating central neurotransmission and stress-related endocrine signaling. Although direct mechanistic confirmation in ASD populations remains limited, these converging immune, metabolic, and neurochemical pathways provide a plausible biological basis for reported improvements in GI and behavioral symptoms (51,68).

The study by Liber and Więch (20) reported on FMT in children with ASD (20). They reported that FMT was associated with improvements in GI symptoms and selected behavioral measures by modifying microbial composition, restoring SCFA-producing taxa, and potentially normalizing systemic inflammatory and neurotransmitter-related biomarkers (20). Notably, interventions such as MTT and FMT have demonstrated potential in breaking the cycle between GI discomfort and behavioral symptoms (22,38). Long-term follow-ups of MTT in ASD children have reported not only a sustained improvement in GI health and increased microbial diversity (such as increased *Bifidobacterium* and *Prevotella*), but also reductions in certain behavioral measures, including improvements in repetitive behaviors in some participants and acceptance of new foods (15). Based on the proposed role of the MGBA in ASD, several microbiota-targeted interventions have been explored as experimental therapeutic strategies (Fig. 6). Santocchi *et al* (111) conducted a randomized controlled trial of probiotic supplementation in preschoolers with ASD. While there were no significant differences in the overall ASD severity score, the authors noted in their subgroup analysis that children without GI issues had improved in core ASD symptoms (111). Shaaban *et al* (127) conducted a prospective, open-label trial on probiotics in Egyptian children with ASD and found that probiotic treatment improved both behavioral and GI symptoms.

In their study, Nettleton *et al* (109) examined the effects of consuming probiotics, synbiotics, and prebiotics in a mouse model of ASD. They discovered that probiotic and synbiotic treatments improved sociability and repetitive behaviors (109). In another study using a mouse model of ASD, Pochakom *et al* (110) evaluated the effects of selective probiotic administration and discovered improvements in the gut flora. Reported improvements vary considerably across studies, likely reflecting differences in probiotic strain specificity, dosage, treatment duration, baseline GI status of participants, outcome measurement tools, and study design (open-label vs. randomized controlled trials). These methodological inconsistencies complicate direct comparisons across studies and contribute to heterogeneous findings (68). In addition, differences in baseline dietary rigidity and food selectivity among participants may influence both initial microbiota composition and responsiveness to microbiota-targeted therapies, representing an underexplored variable in current clinical trials. Taken together, findings from microbiota-targeted interventions in ASD remain heterogeneous, with variability likely reflecting differences in study design, intervention type, duration, and outcome measures. The strength of evidence supporting gut microbiome interventions in ASD varies substantially across study designs, as summarized in Fig. 7.

7. Conclusion and future perspectives

ASD can be understood in a broader context, considering the complex and changing interactions between the gut and the brain. The present review focused on the modulatory effects of the gut microbiome on gut-brain communication, illustrating how microbial changes can affect physiological processes pertinent to behavior and cognition, extending beyond the confines of digestive health. Alterations in gut microbiota composition have been documented in specific populations with ASD and have been investigated concerning GI symptoms, anxiety, and behavioral characteristics, focusing on gut health as a significant domain for further research in ASD. Microbial metabolites, such as neurotransmitters and SCFAs, function as crucial signaling molecules within this axis; conversely, disruptions in microbiota composition have been suggested to compromise gut barrier integrity and immune signaling, potentially affecting gut-brain communication pathways.

Early clinical and preclinical studies have explored treatments that target the microbiota, including probiotics, prebiotics, and FMT. Although some studies suggest improvements in GI symptoms, behavioral outcomes remain inconsistent, likely due to differences in intervention protocols, participant stratification, duration of follow-up, and the use of non-standardized behavioral assessment tools.

These limitations highlight the need for rigorously designed, adequately powered, long-term randomized controlled trials.

Future investigations would benefit from longitudinal study designs that integrate detailed behavioral profiling, including restricted dietary patterns and repetitive behaviors, with comprehensive microbiome analyses. Such an approach would allow for the clarification of the temporal dynamics between gut microbial alterations and ASD symptom trajectories and help determine whether observed microbiota differences reflect primary pathophysiological mechanisms or secondary consequences of ASD-related behavioral traits. It is imperative that future microbiome research in ASD meticulously accounts for the confounding effects of restricted diets. The unique dietary selectivity and repetitive behaviors of ASD patients are not just clinical symptoms, but also important modulators of gut microbiome composition. Distinguishing whether microbial dysbiosis is a primary etiology of ASD or a secondary byproduct of rigid eating habits remains a critical frontier.

Current evidence suggests that immune-related microbiota-gut-brain mechanisms are the most strongly supported targets for microbiome-based interventions in ASD. By contrast, neural and endocrine pathways are mostly supported by preclinical or indirect evidence and need more validation through well-designed clinical studies. These viewpoints encourage a more integrated understanding of ASD as a condition influenced by the interactions between gut function, immune system signaling, and neurodevelopmental processes, rather than just a brain-centered disorder.

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Authors' contributions

All authors (SSA, MOM, RAA, OAA, HMK, AMS, RA, OAA, NA, MMW, and RMM) contributed to the conceptualization of the study, data collection, data analysis, data review, writing the first draft of the manuscript, preparation of graphical work and drawing figures. MMW and OA critically revised the manuscript. All authors have read and agreed to the published version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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