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THE GUT-BRAIN AXIS IN AUTISM SPECTRUM DISORDER: MICROBIOTA-TARGETED THERAPIES AND NEUROBIOLOGICAL INSIGHTS

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ABSTRACT

Introduction: Autism spectrum disorders (ASD) are complex neurodevelopmental disorders with an etiology that includes genetic, environmental, immunological, and neurobiological factors. Increasing evidence suggests that the gut-brain axis (GBA), a bidirectional communication system linking the gastrointestinal tract with the central nervous system, may play a key role in their pathogenesis.
Research objectives: This review aimed to provide an overview of current insights into the relationship between gut microbiota and brain function in the context of ASD, with a specific focus on neuroimmunological, neuroendocrine, and metabolic pathways.
Methods: The methodology involved an analysis of scientific literature focusing on studies published in the last 15 years, sourced from PubMed, Google Scholar, and Web of Science databases. The review included experimental, clinical, and review studies related to gut microbiota, dysbiosis, immune response, hypothalamic-pituitary-adrenal (HPA) axis activation, stress, and microbiota-targeted interventions.
Key findings: Children with ASD exhibit characteristic alterations in microbiota composition, increased intestinal permeability, and chronic inflammation. Dysbiosis disrupts the metabolism of neurotransmitters (GABA, serotonin, dopamine), affecting brain function. A relationship has been identified between the microbiota and activation of the HPA axis as well as the stress response. Factors such as cesarean section delivery, antibiotic therapy, and feeding methods modify the microbiota in early life. Probiotic, prebiotic, and microbiota transplantation therapies may improve ASD symptoms.
Conclusions: Modulation of the gut microbiota through probiotics, prebiotics, and Fecal Microbiota Transplantation (FMT) may support ASD treatment. However, further clinical research and ethical guidelines are necessary to ensure their safe application in children.

KEYWORDS

Autism, Gut Microbiota, Gut-Brain Axis, Short-Chain Fatty Acids, Dysbiosis, Microbiota-Targeted Interventions

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1. Introduction

Autism spectrum disorders (ASD) are a heterogeneous group of neurodevelopmental conditions marked by challenges in social interaction, limited range of interests, and repetitive behavioral patterns. Their etiology is multifactorial and includes both genetic and environmental factors, such as early infections, diet, and gut microbiota [1].

In recent years, increasing attention has been directed toward the role of the **gut-brain axis**, a complex communication system linking the gastrointestinal tract with the central nervous system. A growing body of evidence suggests that disturbances in gut microbiota may influence the development and manifestation of ASD symptoms, both behavioral and somatic [2,3]. Children with autism frequently experience gastrointestinal disturbances, including constipation, diarrhea, and abdominal pain, the severity of which correlates with neurological symptoms [4].

This paper seeks to provide an overview of the current understanding of how the gut-brain axis contributes to the pathophysiology of ASD, to examine the molecular mechanisms involved in this interaction, and to assess potential therapeutic approaches centered on gut microbiota modulation. A comprehensive approach to this issue reveals new opportunities for supportive therapy in ASD and contributes to a better understanding of its neurobiological foundations.

2. Methodology

A literature analysis covering mainly the years 2010-2025 was conducted using the PubMed, Web of Science, and Google Scholar databases. Experimental, clinical, and review studies related to microbiota, neuroimmunology, and microbiota-based therapies in ASD were included. The data were compared and synthesized while maintaining quality and currency criteria.

3. The gut-brain axis: definition and mechanisms of action

The gut-brain axis (GBA) constitutes a complex and dynamic signaling network that links the gastrointestinal tract with the central nervous system (CNS), incorporating the autonomic nervous system (ANS), endocrine signaling, and immune pathways. A pivotal component of this axis is the intestinal microbiota - a diverse population of microorganisms inhabiting the gut, which impacts brain function through the release of bioactive metabolites, modulation of immune responses, and interaction with neurotransmitter systems [5].

This bidirectional system functions in two directions: neural signals from the brain influence digestive processes (e.g., motility and secretion), while microbial activity in the gut transmits feedback signals that affect brain function and behavior [6]. Communication within the GBA occurs mainly via the following mechanisms:

- **vagal signaling:** The vagus nerve serves as a principal communication route, responsible for transmitting about 90% of afferent signals from the gut to the brain. It regulates both enteric and central nervous system activity and responds to microbial metabolites and gut status. Alterations in vagal tone have been implicated in behavioral and neuropsychiatric disorders, including ASD [7, 8, 12]. As a bidirectional conduit, it governs motor, secretory, and immune responses in the gastrointestinal tract [9].
- **cytokine and immune mediator involvement:** The immune system plays a central role in GBA signaling by releasing cytokines in response to dysbiosis or intestinal barrier dysfunction. Pro-inflammatory mediators such as IL-6 and TNF-α can compromise the blood-brain barrier (BBB), initiate neuroinflammatory cascades, and interfere with neural signaling, which is associated with behavioral symptoms seen in ASD [10, 11].
- **microbial-derived neurotransmitters and precursors:** Gut bacteria produce or influence levels of neurotransmitters like GABA, serotonin, and dopamine. For instance, species from the genera *Lactobacillus* and *Bifidobacterium* synthesize GABA, which has anxiolytic properties and may modulate social behaviors linked to ASD [12, 13]. Around 90% of serotonin in the body is produced in the gut, regulated by microbial activity such as *Bifidobacterium dentium*, which enhances expression of the TPH1 enzyme critical for serotonin synthesis [14]. Dopaminergic and other aminergic compounds are also produced in the gut and influence higher cognitive and motivational functions [15].
- **systemic dissemination of microbial metabolites:** Short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate are the result of bacterial fermentation of indigestible fibers. These metabolites modulate immune function, impact the permeability of intestinal and BBB, and stimulate G-protein-coupled receptors on vagal afferents. SCFAs also affect gene expression and neurotransmission within the CNS, while supporting neuroendocrine and immune balance [16, 17].
- **CNS-active compound synthesis:** Gut microbiota synthesize neuroactive molecules that influence CNS operations. For example, *Lactobacillus* and *Bifidobacterium* strains produce GABA, a neurotransmitter associated with reduced anxiety [12]. Furthermore, serotonin production, largely occurring in the gut, is shaped by microbial modulation [18]. When the structural integrity of the intestinal or BBB is disrupted, commonly referred to as”leaky gut" or”leaky brain", this may lead to widespread immune activation and inflammation within the nervous system [19]. Immune system disturbances driven by microbial imbalance can contribute to neuroinflammatory states relevant to psychiatric conditions like ASD [20].

In summary, the GBA acts as a regulatory interface integrating hormonal, immune, and neural cues that influence neurodevelopment and behavior. Disruption of this axis is increasingly linked to the etiology of psychiatric conditions, notably autism.

4. Gut dysbiosis and its role in the pathogenesis of ASD

There is a growing body of evidence indicating that the gut microbiota plays a key role in the development and function of the nervous system, influencing both embryological development and later cognitive and behavioral functions. The term „dysbiosis” refers to an imbalance in the composition and function of the gut microbiome, which may lead to the development of various pathologies, including autism spectrum disorder [21].

Studies have shown that children with ASD often have a different gut microbial composition compared to their neurotypical counterparts. Observations include, among others, increased abundance of *Clostridium* species and reduces levels of *Bifidobacterium* and *Prevotella* [22, 23]. This alteration may lead to disturbances in the production of short-chain fatty acids (SCFAs), which play a crucial role in regulating immune responses, intestinal barrier function, and neural signaling [16].

SCFAs, such as butyric acid, are primarily produced by gut bacteria and exhibit neuroprotective and anti-inflammatory properties [24]. A reduction in their levels may increase intestinal barrier permeability, known as „leaky gut", allowing bacterial endotoxins such as lipopolysaccharides (LPS) to enter systemic circulation. LPS can activate the immune system and induce neuroinflammatory responses in the brain, as demonstrated in individuals with ASD [25].

Elevated LPS levels and microglial activation in the brain may lead to dysfunction in neurotransmitter systems, particularly GABA and glutamate, which has been documented in animal models of autism [26]. Additionally, dysbiosis affects tryptophan metabolism and may disrupt serotonin production in the gut, further influencing social and emotional behaviors [27].

An imbalanced microbiota may also modulate immune functions, contributing to the chronic low-grade inflammation observed in many children with ASD [28]. There is evidence of immune dysregulation and elevated levels of pro-inflammatory cytokines such as IL-6, IL-1β, and TNF-α in patients with ASD, which may be associated with gut microbiota and intestinal barrier integrity [29].

In conclusion, alterations in gut microbiota composition contribute substantially to the development of ASD by disrupting neurological, immune, and metabolic pathways. Restoring a healthy gut microbiota may represent a future therapeutic target for alleviating ASD symptoms.

5. Immunological mechanisms linked to the gut-brain axis in ASD

Immune dysfunction is one of the significant hallmarks observed in children with ASD, with the gut-brain axis playing a central role in this context. Interactions between the gut microbiota, the intestinal barrier, and the immune system influence both the development and functioning of the central nervous system. The scientific literature increasingly emphasizes the importance of immunological signaling pathways in the pathogenesis of ASD [30].

Children with ASD exhibit increased intestinal permeability, which may allow translocation of bacterial components such as lipopolysaccharides (LPS) into systemic circulation. The presence of LPS activates Toll-like receptors (TLR4) on immune cells, initiating a signaling cascade that leads to the production of pro-inflammatory cytokines, including interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-alpha (TNF-α) [31, 10].

Studies have demonstrated that children with ASD have elevated levels of pro-inflammatory cytokines in both peripheral blood and cerebrospinal fluid. Microglial and astrocyte activation in the central nervous system, as a response to inflammatory signals, has been documented in post-mortem studies and positron emission tomography (PET) imaging of individuals with autism [32, 33]. Such neuroinflammatory activity can affect neurogenesis, synaptic plasticity, and brain development processes that are critical for the development of cognitive and social functions.

The gut immune system, particularly gut-associated lymphoid tissue (GALT), also plays a role in modulating inflammatory responses. Dysbiosis may lead to reduced activity of regulatory T cells (Tregs), which possess anti-inflammatory properties. Their deficiency may promote autoimmunity and chronic inflammation [29, 34]. Patients with ASD have also shown increased activity of Th17 lymphocytes and elevated levels of interleukin-17 (IL-17), which can cross the BBB and potentially influence fetal brain development [35].

In the context of the GBA, mast cell activation is also noteworthy. These cells, located in the intestinal mucosa, are capable of releasing histamine, tryptase, and a variety of cytokines. Excessive activation of mast cells has been observed in children with ASD and may contribute to the development of neurobehavioral symptoms [36].

In summary, immune disturbances play a fundamental role in the pathogenesis of ASD, with gut microbiota composition and intestinal barrier integrity being key to maintaining immunological balance. Therapeutic interventions aimed at restoring eubiosis and modulating immune responses may represent a promising treatment strategy for ASD.

6. ASD metabolic disturbances of gut microbiota in children with ASD

The gut microbiota plays a crucial role in maintaining the body's metabolic homeostasis, and its composition and functions directly affect the operation of the GBA. In children with ASD, significant alterations have been observed in both the species composition and metabolic activity of the gut microbiome, which may contribute to the development of neurobehavioral disorders [37, 38].

One of the main mechanisms through which the microbiota influences the nervous system is the production of SCFAs, such as butyric, acetic, and propionic acids. SCFAs are produced through the fermentation of dietary fibers by gut bacteria and play a key role in regulating intestinal barrier function, immune responses, and neurotransmitter metabolism [16]. Studies have shown altered SCFA levels in children with ASD, particularly elevated propionic acid, which can cross the BBB and affect neuronal function [39].

Although propionic acid is physiologically present in the gut, its excessive levels may have neurotoxic effects, impacting mitochondrial function, altering GABA and glutamate levels, and increasing oxidative stress in neural cells [26]. In animal models, administration of propionate induced stereotypical behaviors and social deficits resembling autism symptoms [40].

Moreover, metabolic disturbances in the microbiota may lead to increased production of ammonia, phenols, indoles, and other toxic metabolites that can negatively affect the nervous system [41]. In children with ASD, increased activity of *Clostridium* species has been observed, which produce potentially neurotoxic metabolites and enzymes involved in catecholamine metabolism [42].

Another relevant aspect is the production of neuroactive metabolites from tryptophan. Under conditions of eubiosis, tryptophan is converted into serotonin and melatonin, neurotransmitters responsible for regulating sleep and mood. In children with ASD, disturbances in tryptophan metabolism have been reported, including increased conversion along the kynurenine pathway, leading to the formation of neurotoxic metabolites such as quinolinic acid [43].

In the context of ASD, the microbiota's role in bile acid and lipid metabolism is also significant. Abnormalities in this area may impair the absorption of fat-soluble vitamins (A, D, E, K), which are critical for nervous system development [44].

In summary, gut dysbiosis results in significant metabolic disturbances that affect brain function through the production and modulation of bioactive compounds. Understanding these processes may provide the foundation for the development of novel therapeutic strategies for ASD, including dietary interventions, probiotic supplementation, or FMT.

7. Therapeutic interventions targeting the gut-brain axis in ASD

An increasing number of studies suggest that interventions targeting the gut microbiota may offer therapeutic benefits for managing autism symptoms by modulating the GBA. Among the most extensively studied therapeutic strategies are: probiotics, prebiotics, elimination diets, FMT, as well as pharmacological interventions aimed at altering bacterial metabolism.

- **Probiotics and prebiotics**

Probiotics, defined as live microorganisms that confer health benefits to the host, have been studied in the context of ASD primarily for their potential to reduce gastrointestinal symptoms and improve behavioral outcomes. A meta-analysis by Qiao et al. [45] found that probiotics may alleviate autism-related symptoms, including hyperactivity and stereotypic behaviors, while also reducing digestive issues. The most commonly used strains include *Lactobacillus rhamnosus*, *Bifidobacterium longum*, and *Lactobacillus plantarum* [46].

Prebiotics, which are indigestible food components that promote the growth of beneficial bacteria, also demonstrate modulatory effects on the GBA. A study by Grimaldi et al. [47] showed that supplementation with galactooligosaccharides (GOS) in children with ASD improved cognitive functioning and reduced anxiety symptoms.

- **Elimination diet**

The use of a gluten-free and casein-free (GFCF) diet is one of the most popular, though controversial, interventions in ASD. Some reports suggest that certain children may benefit from the elimination of these proteins from the diet, potentially due to their impact on the gut microbiota and intestinal barrier function [48]. However, randomized clinical trials have yielded conflicting results, and therefore, the GFCF diet is not currently recommended as a routine intervention without prior confirmation of food sensitivity [49].

- **Fecal microbiota transplantation (FMT)**

Fecal microbiota transplantation (FMT) is an innovative method that involves transplanting gut microbiota from a healthy donor into the gastrointestinal tract of a patient. A study by Krigsman et al. [50] demonstrated that FMT in children with ASD resulted in a sustained reduction of autistic symptoms and

improvement in gastrointestinal function for at least two years following the intervention. These effects were associated with an increased microbial diversity and the normalization of SCFAs levels [51].

- **Pharmacological modulators of the microbiota**

Certain medications, such as antibiotics (e.g., rifaximin) and microbial enzyme inhibitors, can influence the composition and function of the gut microbiota. Although the use of antibiotics carries potential risks due to side effects, their short-term application in cases of significant dysbiosis in patients with ASD has been the subject of clinical investigations [52].

- **Psychobiotics and postbiotics**

A new emerging field involves psychobiotics and probiotic strains that, through their influence on the GBA, may exert anxiolytic and antidepressant effects. In preclinical studies on mice, administration of *Bifidobacterium infantis* demonstrated antidepressant-like effects by modulating tryptophan metabolism and reducing inflammatory cytokines [53].

Postbiotics - metabolic products of microorganisms, are also gaining attention as potential interventions in ASD, particularly short-chain fatty acids (SCFAs) and tryptophan-derived metabolites.

8. The role of genetics and epigenetics in the microbiota-brain interaction in ASD

Recent advances in GBA research have shown that genetic and epigenetic components may modulate the influence of the microbiota on brain development and autistic behaviors [54,55].

Family, twin, and association studies have shown that ASD is characterized by high heritability, estimated at 50–90% [56]. Hundreds of genetic variants associated with ASD have been identified, involving genes related to synapse development, neuroplasticity, and immune regulation [57]. Among these are SHANK3, CHD8, NRXN1, CNTNAP2, and MECP2 - genes whose expression may be modified by environmental factors, including microbiota-derived products [58,59].

It has been demonstrated that certain genetic variants influence gut microbiota composition, potentially leading to dysbiosis and secondary immune and metabolic disturbances. For example, mutations in the MET gene, which is involved in immune response and intestinal barrier function, have been linked to reduced microbial diversity in children with ASD [60]. Other studies have shown that genes related to inflammatory responses, such as IL6 and TLR2, may determine the host's reaction to microbial composition, thereby modulating the course of ASD [61].

Epigenetics refers to inheritable but reversible changes in gene activity that occur without altering the underlying DNA sequence. Key epigenetic processes encompass DNA methylation, histone alterations, and microRNA-mediated regulation. These mechanisms are susceptible to modulation by environmental influences, such as the structure of the gut microbiota and the metabolites it produces [62].

One of the key mechanisms involves the action of SCFAs, particularly butyrate, which functions as a histone deacetylase (HDAC) inhibitor. HDAC inhibition leads to enhanced expression of genes related to neuroplasticity and inflammatory regulation [63]. In individuals with ASD, altered methylation of gene promoters involved in neurogenesis and synaptogenesis has been observed, which may be secondary to gut dysbiosis and SCFAs deficiency [64].

The influence of the microbiota on the epigenome is particularly significant during critical periods of brain development, namely, the prenatal and early childhood stages. Studies using animal models have shown that germ-free models, which lack microbiota, exhibit altered gene expression in the prefrontal cortex and hippocampus, correlating with behavioral abnormalities resembling ASD [65]. Reintroduction of microbiota in young animals can partially reverse these changes, suggesting the plasticity of the brain's epigenome [66].

Furthermore, certain gut bacteria, such as *Bacteroides fragilis* and *Lactobacillus rhamnosus*, have been linked to modulation of gene expression in the brain via their influence on the HPA axis and cytokine levels [10, 67]. It has been demonstrated that their presence can affect histone acetylation and DNA methylation in brain regions responsible for emotions and social functions [68].

MicroRNAs (miRNAs) are small molecules that regulate the stability and translation of mRNA. In patients with ASD, altered levels of various miRNAs have been detected in plasma, brain tissue, and the gastrointestinal tract [69]. Studies suggest that the microbiota can regulate miRNA expression through the production of metabolites or via direct interactions with intestinal epithelial cells [70]. For instance, Lactobacillus has been shown to affect the expression of miR-155, a regulator of immune response and neurogenesis [71].

Changes in miRNA expression may, in turn, influence genes involved in synapse development (e.g., SHANK, NEUROLIGIN) and neuronal cytoskeleton organization, thereby modulating neuronal plasticity and activity, processes that are critically important in the pathogenesis of ASD [72].

An increasing body of evidence indicates that genetic susceptibility to ASD may be amplified by environmental stimuli, such as maternal diet, prenatal infections, and neonatal microbiota composition. For example, activation of the IL-17 pathway in pregnant mice led to the development of autism-like phenotypes in offspring, a phenomenon linked to microbiota disruption and altered gene expression in the brain [35].

Additionally, studies on microbiota inheritance have shown that children inherit not only genes, but also microbial patterns from their mothers, which can influence their neurobehavioral development through epigenetic mechanisms [73]. Within this framework, factors such as dietary patterns, antibiotic use, and the method of birth (cesarean versus vaginal delivery) are crucial in shaping the neonatal gut microbiota composition.

9. Gut-brain axis, the HPA axis, and stress in patients with ASD

In recent years, increasing attention has been devoted to the role of the GBA in the pathophysiology of ASD, particularly regarding its interaction with the hypothalamic-pituitary-adrenal (HPA) axis, which plays a central role in regulating the body's response to stress.

The HPA axis serves as a central neuroendocrine system that governs the body's homeostatic reactions to both physical and emotional stress. Activation of this axis leads to the release of corticotropin-releasing hormone (CRH) from the hypothalamus, which stimulates the pituitary gland to secrete adrenocorticotropic hormone (ACTH), ultimately prompting the adrenal glands to produce cortisol, the main stress hormone. Cortisol then exerts negative feedback on the hypothalamus and pituitary to regulate the stress response [74]. In children with ASD, HPA axis dysfunction has been observed, including altered diurnal cortisol rhythms, elevated or reduced salivary cortisol levels, and abnormal stress reactivity [75, 76].

The gut microbiota influences HPA axis regulation through immunological, metabolic, and neuroendocrine mechanisms. Animal studies have shown that germ-free mice exhibit a hyperactive HPA axis, with significantly elevated corticosterone levels after stress exposure compared to conventionally raised mice. Interestingly, colonization of the gastrointestinal tract with *Lactobacillus farciminis* or *Bifidobacterium infantis* normalized the stress response in these animals [53, 66], suggesting that the microbiota functions as a stress buffer by modulating glucocorticoid receptor expression in the brain and pro-inflammatory cytokine levels.

Psychological stress also affects the integrity of the intestinal barrier, leading to increased permeability ("leaky gut") and the translocation of bacterial components such as lipopolysaccharides (LPS) into systemic circulation. LPS activates the immune response and stimulates the production of pro-inflammatory cytokines (e.g., IL-6, TNF-α), which can cross the BBB and modulate hypothalamic neuron activity [77]. This mechanism may result in heightened HPA axis activation and chronic inflammation, both of which are observed in individuals with ASD [11].

HPA axis dysfunction in ASD may be primary (due to genetic predisposition and neuroanatomical changes in the CNS) or secondary, stemming from chronic environmental stress and sensory processing issues. Children with ASD often show increased sensitivity to sensory stimuli (e.g., sound, light, touch), which may lead to chronic HPA axis activation and excess cortisol production [78]. Clinical studies have also reported abnormal diurnal cortisol rhythms, in some children with ASD, cortisol levels are too low in the morning and elevated in the evening, correlating with sleep disturbances and mood disorders [79].

Communication between the microbiota and the HPA axis occurs through multiple mechanisms, including the vagus nerve, which transmits sensory signals from the gut to the brain. Gut bacteria also produce neuroactive metabolites such as SCFAs, tryptophan, and its derivatives (e.g., indole, serotonin, melatonin), all of which influence synaptic plasticity and neuroendocrine functions [62]. SCFAs exhibit anti-inflammatory effects and may attenuate HPA axis activation by modulating GPR41/GPR43 receptors and stimulating the production of gut peptides like GLP-1 and PYY [17].

In the context of ASD, GBA and HPA axis dysregulation are strongly interconnected. Gut dysbiosis may lead to HPA overactivation and chronic stress, which in turn affect brain structure and function. Neuroimaging studies have shown that children with ASD exhibit reduced hippocampal volume and structural alterations in the amygdala - brain regions responsible for emotional regulation and stress response [80]. Moreover, prenatal stress and inflammation during pregnancy can disrupt neonatal microbiota colonization, resulting in long-term alterations in HPA axis activity and increased susceptibility to ASD [18].

From a therapeutic perspective, modulation of the gut microbiota (e.g., via probiotics, prebiotics, elimination diets, or fecal microbiota transplantation) may influence HPA axis function and reduce autism

symptoms. Clinical studies have shown that probiotics containing *Lactobacillus rhamnosus* or *Bifidobacterium infantis* can alleviate stress-related symptoms and improve social behaviors in children with ASD [46, 81]. These interventions may work by normalizing cortisol levels, reducing inflammatory markers, and improving intestinal barrier integrity.

In conclusion, the interplay between the HPA axis and gut microbiota constitutes a complex regulatory network that significantly contributes to the development of ASD. Dysregulation of this system contributes to chronic stress, neuroinflammation, and the exacerbation of autistic symptoms. Understanding the interdependence between these systems may aid in the development of effective, individualized therapies for individuals with ASD.

10. The impact of cesarean delivery and infant feeding method (breastfeeding vs. formula feeding) on the gut microbiota in children with ASD

The gut microbiota plays a critical role in the development of the immune and nervous systems, with its formation beginning during the perinatal period. Numerous studies indicate that both the mode of delivery and the type of infant feeding can significantly influence the composition of the gut microbiome - an influence that may be especially important in the context of autism spectrum disorder (ASD).

Cesarean section delivery is associated with substantial differences in microbial colonization compared to vaginal birth. Infants born vaginally are colonized primarily by maternal vaginal microbiota, such as *Lactobacillus* and *Prevotella*, whereas those born via cesarean section are colonized by skin-associated (*Staphylococcus* spp.) and environmental bacteria [82]. This altered microbial profile may lead to delayed immune system maturation and neuroimmune dysregulation.

In the context of ASD, it has been shown that children delivered by cesarean section have a slightly increased risk of developing the disorder. Meta-analyses point to a significant but modest association between operative delivery and the likelihood of ASD development [83]. A potential mediating mechanism may be the impaired development of the gut microbiota, which affects the GBA, as well as immune and endocrine system functions.

Another crucial factor influencing microbiota development is feeding method during infancy. Breastfeeding not only provides essential nutrients but also delivers human milk oligosaccharides (HMOs), which promote the growth of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus* [84]. Breast milk also contains antibodies, enzymes, and growth factors that support immune system development and protect the intestinal barrier. In contrast, formula feeding is associated with less diverse microbiota, greater abundance of opportunistic bacteria, and slower microbial maturation [85].

Studies suggest that children with ASD were breastfed less frequently and for shorter durations compared to neurotypical peers. In a study by Al-Farsi et al. [86], breastfeeding for a duration shorter than six months has been strongly linked to a heightened risk of developing autism. Thus, breastfeeding may exert a protective effect by supporting eubiosis, reducing inflammation, and promoting serotonergic neurotransmitter development.

Importantly, the effects of birth and feeding mode may be cumulative. Infants delivered via cesarean section and formula-fed exhibit the most disrupted microbiota composition, characterized by reduced *Bifidobacterium* and increased presence of potentially pathogenic bacteria [87]. Such microbiota profiles are frequently observed in children with ASD, suggesting a potential pathophysiological link between perinatal factors and neurodevelopmental disorders.

An additional consideration is that infant microbiota composition may have lasting effects on future health. Microbial colonization during the first months of life determines the stability of the microbiome in subsequent years and may influence neurobiological function, immune competence, and stress response [88].

To conclude, the method of birth and the approach to infant nutrition are key determinants in the development of a child's gut microbiome. Cesarean delivery and formula feeding may lead to unfavorable microbiome alterations, potentially contributing to the development of ASD through effects on the immune system and the GBA. In the context of early autism prevention, it is worth considering strategies that support probiotic colonization and promote breastfeeding, especially in at-risk populations.

11. The impact of early childhood antibiotic therapy on the gut microbiota and the risk of ASD

Early-life antibiotic therapy may disrupt the natural colonization process of the neonatal and infant gut, leading to long-lasting alterations in microbiome composition. These changes can affect the development of the GBA, as well as neuroimmune and neuroendocrine mechanisms relevant to ASD. During the prenatal period and the first two years of life, the microbiota develops rapidly, and antibiotic exposure during this window may result in reduced species diversity, a decrease in *Bifidobacterium* populations, and an increase in

opportunistic strains [89, 90]. Even a single course of antibiotics can shift the microbiome toward a mature profile lacking beneficial species [91, 92].

Population-based studies from Taiwan and Sweden, involving large child cohorts (over 900,000 individuals), have found a small but statistically significant increase in ASD and ADHD risk following antibiotic exposure during the first two years of postnatal development (adjusted HR ~1.06–1.46) [89]. However, sibling-controlled analyses, which account for genetic and environmental confounders, have shown that this association disappears or becomes non-significant, suggesting that the observed correlation may not reflect a causal relationship [89, 93]. A 2021 meta-analysis reported a slightly elevated overall risk of ASD (OR 1.13; 95% CI 1.07–1.21), but no significant association in sibling comparison studies (OR 1.04; 95% CI 0.97–1.11) [93].

Mechanistic findings, largely based on animal models, support the idea that early-life antibiotic exposure leads to dysbiosis, characterized by reduced levels of *Bifidobacterium*, *Lactobacillus*, and other strains involved in immune regulation and neurotransmitter balance. This dysbiosis results in increased oxidative stress, HPA axis dysregulation, intestinal and BBB permeability, and altered expression of immune-regulatory molecules [93, 94, 95]. In such models, early antibiotic-treated rats exhibit heightened anxiety, poorer cognitive performance, and social behavior deficits [92, 95].

From a clinical standpoint, while epidemiological data suggest a possible subtle link between infant antibiotic therapy and ASD, high-quality studies, especially sibling-controlled designs, do not confirm a causal effect. Variations in findings likely reflect differences in methodology, classification, healthcare access, and socioeconomic factors [93].

The class of antibiotic may also matter, broad-spectrum antibiotics, particularly macrolides and sulfonamides, have more frequently been associated with increased ASD risk in Danish and Canadian cohorts [95, 96]. However, once again, sibling analyses point to environmental factors as the primary determinants of this association.

Given this uncertainty, mechanistic insights into the vulnerability of the infant microbiome to antibiotic disruption remain important, especially in the context of early immune development, HPA axis regulation, and neural signaling. Dysbiosis can lead to gut permeability and elevated LPS levels, which cross the BBB, promote neuroinflammation, and affect neurogenesis and synaptic function, key factors in ASD pathophysiology [89, 97].

Moreover, the recovery of the microbiota following antibiotic treatment may be prolonged. In children, microbiome diversity can remain diminished for years, potentially increasing susceptibility to other neurodevelopmental risk factors [92, 95].

Given the inconclusive evidence, clinical guidelines remain cautious: antibiotic therapy is an important medical tool, and effective infection prevention should rely on best practices in antibiotic selection, dosage, and duration for infants. At the same time, supporting immunity through diet, breastfeeding, and potentially, microbiota supplementation with probiotics or synbiotics may prove beneficial [89, 98]. There remains a strong need for further systematic studies, particularly those involving sibling controls, antibiotic class-specific analyses, and long-term follow-ups, to more precisely assess the relationship between early antibiotic exposure and ASD risk.

- **Ethical and social aspects of microbiota-based therapies in children with ASD**

Interest in microbiota-based therapies for the treatment of ASD is growing rapidly, particularly in light of the increasing number of studies demonstrating a link between gut microbiota composition and nervous system functioning. Among these therapies are probiotics, prebiotics, FMT, and diets designed to modulate the microbiome.

While the potential benefits of these interventions are promising, important ethical and societal questions arise, especially regarding their use in children, who represent a particularly vulnerable population.

- **Consent and autonomy in therapeutic decision-making**

Children with ASD are not fully capable of making independent therapeutic decisions, which means that the entire responsibility for treatment choices lies with their legal guardians. This raises critical questions regarding the adequate communication of information to parents and their ability to assess the risks and benefits of interventions that are often still experimental. There is a lack of consistent standards governing how information about FMT or probiotics is presented, which may lead to misinterpretation of research findings and the generation of unrealistic expectations [99].

Parents may be particularly vulnerable to social pressure, medical marketing, or desperation, especially when their child exhibits severe or treatment-resistant symptoms. This can increase the risk of impulsive or poorly informed decisions [100].

Some methods, such as FMT, raise additional ethical concerns related to the transfer of biological material from donor to recipient. Although screening regulations for donor pathogens exist, the long-term impact of the transplanted microbiota on a child's health remains poorly understood. The introduction of a foreign microbial ecosystem may carry unforeseen consequences, calling into question the ethical justification of using such methods in children without long-term safety data [101].

- **Equity and access to treatment**

Another crucial consideration is equitable access to microbiota-based therapies. Many of these interventions, especially FMT performed in clinical settings, remain costly and inaccessible to families with lower socioeconomic status. These disparities in access risk exacerbating existing health and social inequalities, particularly for children with ASD, who already face barriers in accessing specialist diagnostics and therapies [102].

Moreover, such interventions are often not reimbursed by public healthcare systems, as their efficacy and safety are still under investigation. This creates a situation where parents must choose between conventional care and private, experimental treatments, frequently at significant financial, emotional, and logistical cost.

From a social justice perspective, there is a pressing need for policy reform to ensure that access to innovative therapies is not solely determined by a family's financial resources [103]. Ensuring fair, evidence-based integration of microbiota-targeted treatments into public health systems is essential to prevent deepening disparities in care for children with ASD.

- **Risk of stigmatization and cultural responsibility**

An important yet often overlooked social dimension of microbiota-based therapies is the issue of stigma associated with mental and neurodevelopmental disorders. These interventions may inadvertently reinforce the notion that ASD is a condition to be "fixed" through a simple microbiological solution. Such a perspective risks undermining the importance of neurodiversity and may encourage pathologizing attitudes toward autism, rather than promoting an understanding and acceptance of different ways of functioning [104].

Cultural factors also play a significant role. Some communities may view microbiota manipulation, particularly FMT, as an infringement on natural order, invoking skepticism or resistance. Conversely, others may expect a rapid cure, increasing pressure on both parents and children to pursue interventions without fully considering their implications [105].

It is therefore essential that both scientific and clinical communities ensure that communication regarding microbiota-based therapies is grounded in robust scientific evidence, while also respecting the complexity of ASD. ASD should not be reduced to a microbial imbalance, and messages about these treatments must incorporate a multidimensional view of health.

Crucially, parents must be informed not only of potential benefits, but also of the current limitations, including the lack of definitive long-term safety and efficacy data. Promoting informed, nuanced understanding is key to ensuring ethical and responsible use of these emerging therapeutic approaches.

- **Recommendations and future directions**

From an ethical standpoint, there is a clear need to develop comprehensive guidelines that define both indications and contraindications for the use of microbiota-based therapies in children with ASD. Future research should not only focus on efficacy and safety, but also on the social impact of these interventions, for example, how they influence family and societal perceptions of autism, the child's self-esteem, and the quality of life of caregivers.

It is also recommended to implement a mandatory informed consent process for every child participating in microbiota therapy studies, ensuring that parents or guardians make decisions based on complete, transparent information. Furthermore, all such research should be subjected to ethical review by independent bioethics committees.

There is a pressing need for educational campaigns targeting both caregivers and professionals, aimed at promoting informed and responsible decision-making. These efforts should highlight not only potential benefits but also the uncertainties and limitations associated with emerging microbiota interventions.

In conclusion, while microbiota-based therapies represent a promising avenue for the treatment of ASD symptoms, their implementation must be approached with care, critical reflection, and consideration of the broader ethical and social context. Only with appropriate legal and ethical regulations, centered on the child's well-being, equitable access, and transparent communication, can these therapies be safely and fairly integrated into clinical practice.

12. Discussion

A review of the available data indicates that the GBA plays a significant role in the pathogenesis of ASD. Key mechanisms underpinning the microbiota-brain interaction in ASD include gut dysbiosis, altered microbial metabolism, HPA axis hyperactivity, and chronic inflammation. Notably, specific bacterial species have been linked to the severity of autistic symptoms, including sensory disturbances, anxiety, and cognitive impairments.

Environmental factors such as cesarean delivery, early-life antibiotic use, and infant feeding methods have been shown to influence microbiota development during critical periods of brain maturation. Disruptions in microbiota composition may modulate the stress response via the HPA axis and alter neurotransmission (e.g., GABA, serotonin, SCFAs), thereby affecting behavior and neurological development.

The findings suggest that interventions such as probiotics, prebiotics, elimination diets, and FMT may alleviate ASD symptoms by restoring microbial balance and reducing inflammation. However, the implementation of such therapies, especially in children, raises important ethical considerations that require further in-depth analysis.

In summary, while microbiota-targeted approaches offer promising therapeutic potential, their application must be guided by rigorous scientific validation, ethical oversight, and informed decision-making that prioritizes the well-being of the child.

13. Conclusions

The collected data clearly indicate that the gut microbiota plays a key role in the pathophysiology of ASD, influencing the functioning of the GBA, immune responses, neurotransmission, and HPA axis activity. An imbalance in the gut microbiota, known as dysbiosis, may compromise the integrity of both the intestinal and BBB, trigger microglial activation, and promote persistent inflammation, factors that are mirrored in the neurobiological characteristics of autism. Key mechanisms include disrupted SCFAs production, altered neurotransmitter synthesis (e.g., GABA, serotonin), abnormal tryptophan metabolism, and the influence of microbiota on epigenetics and gene expression related to neuroplasticity.

Environmental factors, such as mode of delivery, infant feeding, and early-life antibiotic use, play a significant role in shaping the microbiome and may modulate ASD risk. Cesarean delivery and formula feeding are correlated with reduced microbial diversity and a dominance of opportunistic species, which may increase vulnerability to GBA dysregulation. Similarly, early antibiotic exposure may result in long-lasting microbiome alterations, although epidemiological data are inconclusive regarding a direct causal link to ASD. Instead, evidence points to indirect mechanisms, including immune modulation, oxidative stress, and neurotransmitter imbalance.

With a growing number of intervention studies, several promising therapeutic strategies aimed at modulating the microbiota have emerged. These include probiotic and prebiotic supplementation, elimination diets (e.g., GFCF), FMT, and newer approaches such as psychobiotics and postbiotics. Probiotics, particularly from the *Lactobacillus* and *Bifidobacterium* genera, show potential in reducing neurobehavioral symptoms, improving HPA axis function by lowering inflammatory markers, normalizing cortisol levels, and enhancing gut barrier integrity. While still experimental, FMT has shown encouraging results, indicating possible long-term improvement in both ASD symptoms and gastrointestinal function.

Equally important, however, are the ethical and social challenges related to the use of microbiota therapies in children. The need for parental decision-making, the lack of long-term safety data, economic barriers to access, and the potential for stigmatization of children with ASD pose serious concerns requiring systemic regulation. The introduction of clinical guidelines, educational initiatives, and the expansion of therapy access within public health systems is essential for their ethical and equitable implementation.

The therapeutic outlook for individuals with ASD should embrace an integrated approach, combining insights from microbiome science, neuroimmunology, genetics, and epigenetics with practical dietary and microbial interventions. Future research should prioritize the identification of dysbiosis biomarkers, the personalization of microbiota-targeted therapies, and the long-term evaluation of early-life interventions.

Ultimately, the goal is not only to alleviate symptoms but to enhance the quality of life for individuals with ASD and their families through individualized, effective, and safe therapeutic approaches rooted in multidisciplinary knowledge and compassionate care.

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