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A Potential Link between Gut Leakiness and Sex Differences in Autism Spectrum Disorder (ASD)

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ABSTRACT

Background: Autism is categorized as autism spectrum disorder (ASD), characterized by recurrent behaviors and difficulties with social communication that can affect different body systems and are linked to gut microbiota dysbiosis (GM). The importance of altered GM in autistic people, the ways in which these changes could result in leaky gut, and the possible connection between sex differences and this problem are all highlighted in this paper. Main body: Research indicates that increased intestinal barrier permeability may play a significant role in the pathological changes seen in autism; it makes it easier for gut-derived endotoxins to enter the brain, where they activate the TLR4-MyD88-NF-κB signaling cascade and create an inflammatory environment. There have been differences found in the amounts of various bacterial metabolites, such as beta cresol, short-chain fatty acids, lipopolysaccharides, and bacterial toxins, in the blood and urine of children with autism. Additionally, the importance of particular proteins like zonulin, lysozyme, and calprotectin as biomarkers that can detect the leaky gut in ASD at an early stage has been shown. Bacterial metabolite leakage in these patients may be explained by disruption of the gut blood-brain barrier. As a result, a number of microbiota manipulation techniques have been developed to balance out sex differences. Male ASD sufferers are four times as likely as female ASD sufferers. Additionally, in both human and animal models of this illness, such as the maternal immune activation (MIA) mice model, the composition of GM is dependent on sex. However, very few studies have taken sex's biological impact into account when assessing how GM affects symptoms of ASD. MIA increases pro-inflammatory cytokines and chemokines in the mother, such as interleukin, IL-6, and IL-17α, which interfere with the development of the fetal brain. According to recent studies, MIA causes GM dysbiosis. This is significant because the GM, which lives in the gastrointestinal (GI) tract, contributes to the development of the immunological, neurological, and metabolic systems through the microbiota-gut-brain axis, resulting in characteristics that resemble ASD in the MIA model.Research exploring the impact of GM on ASD etiology primarily focuses on men, ignoring the recipient's and donor's sex/gender, even though biological distinctions linked to sex, GM, and leaky gut have been discovered to be correlated with each other. In the MIA model, sex-mediated gut-immune interactions were found in the few research that take into account the biological impact of sex.The limited studies that consider the biological effect of sex revealed sex-mediated gut-immune interactions in the MIA model. Conclusion: Given that many people with autism have gastrointestinal problems, this review emphasizes the possible link between ASD and GM. The involvement of altered GM in autistic people, how these changes result in leaky gut, and the possible connection between leaky gut and sex differences are all covered. Furthermore, this study offers a number of promising therapeutic therapies, including as intestinal proteins, some probiotics, and chemicals derived from bacteria, as novel approaches to reestablish a healthy GM.

Keywords: *Autism, Gut Microbiota, Sex differences, Gut leakiness, Gut -Brain Axis, Inflammatory Markers*

Introduction

Autism spectrum disease (ASD) is a complex neurodevelopmental disorder (NDD) characterized by limited or repetitive behaviors, communication issues, and social interaction challenges [1]. 1% of children globally suffer with ASD [2], which has a significant impact on the social and ancestral economies [3]. Although gene mutations are the main cause of ASD development [4], a child's gender is a predictor similar to the frequency of familial history [5, 6], with males having a 4-fold higher risk than girls. Therefore, it is likely that early developmental sex-specific variables contribute to the genesis of ASD [6, 7].

About 46.8% of ASD patients have gastrointestinal (GI) symptoms, including bloating, diarrhea, constipation, nausea, vomiting, and stomach discomfort. When compared to their peers without GI symptoms, children with ASD who frequently display GI symptoms exhibit higher levels of hyperactivity, social disengagement, irritability, and stereotypical behaviors [8]. Researchers are becoming more interested in GM as a possible underlying cause of ASD because of the known link between GI problems and ASD. Recent research has shown that people with ASD have altered GM composition, a phenomenon known as "dysbiosis" [8]. ASD may arise as a result of altered bacterial metabolites and a weakened gut barrier brought on by dysbiosis in GM [8]. According to a research by Kang et al. [9] on 18 kids with ASD, Microbiota Transfer Therapy (MTT) reduced GI issues and symptoms related to autism [8]. When these kids were monitored two years later, it was discovered that the improvements in GI and core ASD symptoms continued even after the medication was discontinued [10]. Furthermore, 112 boys with normal development and 92 boys with ASD were studied by Wong et al. [11]. They found that, regardless of the presence of functional gastrointestinal disorders (FGID), there was a substantial difference in the GM composition between the two groups. This finding suggests that neuropsychiatric symptoms may be impacted by gut dysbiosis in people with ASD, regardless of their gastrointestinal condition [11]. Through the gut-brain axis, the GM can affect the brain's growth and function, which is why it is so important [12]. Nevertheless, no study examined the differences in GM between males and girls with ASD. Age has a major impact on the composition and functions of GM, particularly in the early stages of life, and is another crucial determinant in ASD-associated GM dysbiosis [8]. Despite the fact that ASD is a chronic condition, research on the differences in GM composition in middle and old age is rather uncommon.

Despite the fact that ASD is a chronic condition, there aren't many studies on how GM composition changes in middle and old age. ASD is known to be significantly influenced by gestational infection and the resulting maternal immune activation (MIA), which can be well mimicked in mouse studies [13]. MIA increases pro-inflammatory cytokines and chemokines in the mother, including interleukin IL-6 and IL-17α, which can interfere with the development of the fetal brain [14, 15]. Rodents are exposed to the viral mimic polyinosinic: polycytidylic acid (poly IC; 10) during pregnancy in order to establish the MIA model. Male offspring exposed to MIA are more likely than female offspring to display an ASD-like phenotype, just like in humans [13, 16].

According to recent studies, MIA causes the GM to become dysbiotic [17, 18]. This discovery is significant since the GM influence the immunological, metabolic, and neurological systems through the microbiota-gut-brain axis [17-22], which may result in characteristics that resemble autism spectrum disorder (ASD) in the MIA model. Despite the paucity of research on the biological impact of sex, it was discovered that sex plays a part in mediating the gut-immune interactions in animal models. Male mice given GM from human male ASD donors showed more pronounced behavioral changes [21], and sex-related differences in GM were linked to differences in social behavior [23, 24]. Male vulnerability to neurodevelopmental insults has been linked to the neuroimmune system, which has been shown to display sexual difference. For instance, during fetal development, male brain immune cells, particularly microglia, demonstrated increased susceptibility to

microbiota disruptions [25]. Despite this data, sex is often overlooked in preclinical studies examining how GM affects MIA/ASD symptoms since many of these studies primarily use male donors and receivers [25], and others do not reveal the sex of the animals used.

According to research, exposure to dietary or environmental heavy metals can upset the delicate balance of trace elements in the stomach. In people with ASD, these disruptions may aggravate gut disease by adversely affecting zinc (Zn) homeostasis. The oxidative stress, immunological dysregulation, and inflammation observed in the intestines of ASD patients may be related to the reduced absorption of zinc brought on by exposure to heavy metals [26]. In order to demonstrate the mechanistic relationship between intestinal permeability (leakiness) and sex differences in Autism Spectrum Disorder (ASD), this review was designed in a trial.

1. Sex differences

Gender and age have an impact in ASD diagnosis [27]. Males with ASD are typically diagnosed at an earlier age due to the more obvious symptoms, such as repetitive behaviors and speech impairments [28, 29]. On the other hand, females who exhibit less overt symptoms, such as anxiety and social camouflaging, are frequently diagnosed later in adolescence or adulthood, missing crucial opportunities for early intervention [29]. According to recent studies, the male-to-female diagnosis ratio is about 4:1 in childhood and drops to about 3:1 in adulthood, suggesting that female symptoms of ASD are becoming more widely recognized [5]. These results raise important questions about the effectiveness of treatment plans that were traditionally created for male managers as well as the suitability of contemporary research criteria of the disorder. Treatment responses also show differences across genders. Conventional medications like applied behavior analysis (ABA) and Cognitive Behavioral Therapy (CBT) work for 45% of women and 70% of men. Medications that emphasize social communication, adaptive behaviors, and emotional control appear to work better on women, underscoring the need for gender-specific treatment strategies. However, current ASD medications, which are often designed to be gender-neutral or male-centric, might not adequately address the unique needs of women [5]. ABA, for instance, tries to minimize specific behaviors and improve social expertise by strengthening methods; nevertheless, it has been shown to be less effective in treating females, who frequently display undetected symptoms like anxiety and social camouflaging. Similarly, CBT focuses on maladaptive behaviors and cognitive distortions, but it may not be able to address the particular emotional and social difficulties that female with ASD face.

2- Characterization of GM on gender and age groups bias

Although the impact of sex is often overlooked in preclinical studies that assess sex-specific risk variables, including GM, linked to ASD [22, 24]. The prevalence of several gut microorganisms known to have pathogenic implications, such as Bacteroidetes, Blautia, and Prevotellaceae, increased despite a general fall in diversity. Similar gut microbes have been linked to altered gut metabolomics and unbalanced neurotransmitters in ASD, according to prior study [30-34]. For example, propionic acid, a short-chain fatty acid (SCFA) that can pass through the blood-brain barrier, is produced by Bacteroidetes [24]. On the other hand, Blautia plays a role in the production of tryptophan, a precursor to serotonin [35]. Both the enteric and central nervous systems are influenced by serotonin and its modulators, and ASD has been shown to have impaired serotonergic transmission [36]. In particular, a subset of children with ASD exhibit elevated blood serotonin levels, which are significantly correlated with gastrointestinal problems, but persons with ASD typically have lower serotonin levels in the brain [37].

Consequently, an increase in Blautia may raise serotonin, which may have an impact on male recipient mice's ASD-like behaviors [24]. Furthermore, because high levels of Prevotellaceae cause inflammation, mucosal activation, and systemic T-cell activation [39], they are linked to the pathophysiology of a number of

illnesses, including ASD [38]. It has been suggested that differences in this microbe could function as a diagnostic marker for gut dysbiosis in ASD [24], despite conflicting results addressing the changes in Prevotellaceae abundance in ASD [24]. In conclusion, the higher frequency and intensity of ASD-like symptoms in people receiving MIA GM from males rather than females may be attributed to this sex-dependent expansion of pathogens. The similarities between the human status and the gut microbial composition of male MIA highlight the need to incorporate gender/sex considerations in both preclinical and clinical research regarding the gut microbiome's function in mediating ASD [24].

It is noteworthy that the microbiota of female donors in the MIA model also altered the recipients' gut bacterial composition, increasing the genera Lactobacillus and Rikenella, which may play a protective role [24]. The Ruminococcaceae family of Lactobacillus bacteria improves the integrity of the intestinal barrier, and probiotics made from Lactobacillus have been demonstrated to both reverse gut alterations associated with high-fat diets in mothers and lessen the detrimental effects of stress in rodents [24]. Furthermore, Rikenella is known to be an essential probiotic for reducing intestinal inflammation [41] and has been linked to resilience after early-life stress [40]. Consequently, these additional benefits offered by female-specific microbes probably protected recipient mice from the social behavior deficiencies linked to MIA (for example, females that exposed to female microbiota showed a higher social preference than those that exposed to male MIA microbiota). They may also help to explain the sex differences in the expression of ASD-like behavioral abnormalities in the MIA model. Female recipients of microbiota from both male and female donor's demonstrated overall social behavior, but did not favor social novelty as compared to males. This lack of preference for discovering new stimuli over familiar ones may indicate a deficit in social interaction and cognition among female candidates, or it may reveal inherent sex-dependent variations in behavior, as females have a lower tendency for novelty detection than males [24]. Nonetheless, females, regardless of the donor's gender or MIA status, displayed a general increase in basic activity, as seen by the overall distance walked in the broad field examination area. This increase could be attributed to increased hyperactivity or a stress reaction [42]. When it comes to ASD in humans, women may be better at hiding or disguising their social difficulties, but they nonetheless experience significant social anxiety and stress in new social settings [43]. For neurodevelopmental disorders like ASD, understanding sex differences at baseline is crucial for developing tailored interventions and support networks.

Pro-inflammatory cytokines such TNF-α, IL-6, and IL-1β are elevated in the MIA model of ASD [44]. While microbiota from female donors likewise increased IL-1β, only the transplant of MIA microbiota showed increased levels of IL-4 and IL-7. Interestingly, one unexpected effect of commensal bacteria was to increase the production of IL-1β to help repair the intestinal barrier [24], suggesting that the increase in this cytokine in females may have a protective purpose. Additionally, recipients of female microbiota, regardless of (host vaginal microbiota) Veh/MIA treatment, produced less (microphage inflammatory protein -2) MIP-2 and (tumor necrosis factor -α) TNF-α than recipients of male microbiota, suggesting a higher inflammatory response linked to male microbiota donation. In contrast to male gut microbial donation, this discovery is consistent with the GM profile and suggests a possible defensive immunological response from females [24].

It was also observed that sex-dependent impacts on the characteristics of brain immune cells, particularly microglia, were caused by variations in gut-immune interaction. Specifically, the male recipient mice's microglia morphology was altered by the MIA microbiota from male donors, which matched the neuroinflammation seen in ASD [45]. Therefore, it is possible that changes in GM associated with maternal immune activation (MIA) cause dysregulation of the gut-immune interactions, which in turn contributes to the reported sex differences in ASD-like behavioral deficits.

Notably, it was suggested that the observed disparities in GM between people with ASD and those without it are significantly influenced by age and gender. According to the alpha diversity analysis, the microbiota richness of guys with ASD differed significantly from that of their NT peers. In particular, species richness was lower in males with ASD than in NT males, which is consistent with a previous study that also found that those with ASD had less diversity and plenty [46].

However, the variation in microbial mass remained negligible when gender was not taken into account. This suggests that the evaluation of microbial diversity between ASD and NT groups may be impacted by gender bias [47]. On the other hand, this conclusion was not supported by the study conducted by Bhusri et al. [48]. According to Bhusri et al. [48], those with ASD have higher levels of Fusobacteriota, Fusobacteriaceae, and Fusobacterium. These increases were detected when compared between NT girls and females with ASD. The male groups did not, however, differ much from one another. Fusobacterium was found to be more common in males than in females when sex differences were taken into account [49]. The lack of significant differences between the ASD and NT male groups may be explained by this elevated baseline in healthy males. Ruminococcus gnavus was shown to be more prevalent in males with ASD. Conversely, a variety of bacteria, including species from the phyla Synergistota, Synergistaceae, and Clostridiales vadinBB60 group, were more prevalent in the NT group. Additionally, it was shown that the NT candidates had higher concentrations of Cloacibacillus, Oscillospiraceae UCG-002, Christensenellaceae R7 group, Eubacterium ruminantium group, Ruminococcus gauvreauii group, and Clostridia vadinBB60 group. ASD was also linked to Fusobacterium and Ruminococcus [50]. Some species of Fusobacterium and Ruminococcus gnavus have been linked to inflammation. However, the underlying mechanisms are complex and probably due to a variety of interrelated variables, such as toxin generation and interactions between bacterial surface chemicals and the immune system [48]. A previous study by Zheng et al. [50] discovered that people with ASD and constipation had lower levels of beneficial bacteria that produce butyrate, a short-chain fatty acid (SCFA) vital for gut health. Furthermore, it was shown that these people had significantly higher levels of Fusobacterium, which can cause intestinal inflammation. Additionally, Oscillospiraceae UCG-002, which was discovered to be more prevalent in NT males, was found to be inversely correlated with the incidence of depression. Thus, our findings indicate that GM may have a substantial impact on the emergence of ASD via influencing inflammation and mental health.

Gender has a significant impact on the composition and outcome of genetically modified organisms (GM), as demonstrated by the correlation between gender and bacterial taxa [48]. Remarkably, children with ASD were shown to have higher levels of Veillonella, a bacterium associated with poor oral hygiene in Thai children, which often led to periodontitis and dental caries [51]. GM has been linked to ASD [48], gastrointestinal problems, and neurodevelopmental disorders (including ASD), according to Ahrens et al. [52].

2.1. ASDs; sex biased reaction to environmental risk factors

It is important to stress that certain environmental substances, such atrazine and p-nitrotoluene, may have an impact on the behaviors of people with ASD. According to a study published by Gomez [53], male mice exposed to environmental contaminants in their early lives experience long-lasting changes in their GM as adults. The neuroendocrine system in the hypothalamus is disturbed by Bisphenol S (BPS). It is frequently present in a variety of food packaging materials, including sales receipts, cans, and baby bottles [54]. Fluorobenzoate degradation in the GM of the progeny mice was significantly altered when they were evaluated as adults. Therefore, exposure of mothers and infants to specific environmental toxicants, particularly endocrine toxins, may affect changes in GM in people with ASD and may be linked to the progression of the condition [48].

2.2 Intestinal Barrier Permeability (IBP)

It was found that, the structure and activity of proteins that are essential for maintaining the integrity of the intestinal barrier may be affected by specific genetic variables. Those with ASD may be more susceptible to intestinal permeability (IP) due to variations in genes related to tight junction proteins, mucus formation, and immunological response [26]. Changes in intestinal barrier integrity and inflammatory signaling have been observed in several ASD models, including Shank3–/– mice and prenatal zinc-deficient mice, where tight junction protein alterations have been reported. These changes suggest a possible link between zinc deficiency and malfunction of the gastrointestinal barrier in ASD. The importance of zinc shortage on gut health and more general physiological activities is highlighted by the dysregulation of tight junction proteins, such as ZO-1 and E-cadherin [26].

Because of the weakened barrier, microbially released compounds can enter the bloodstream, increasing pro-inflammatory cytokines and causing systemic inflammation. In consequence, systemic inflammation can reach and negatively impact the developing brain of people with ASD, where it increases the production of cytokines, activates microglia, and exacerbates neuroinflammation. ASD frequently results in aberrant immunological responses, which can worsen IP and affect the intestinal barrier's integrity.

Different elements of triggered immunity can impact the intestinal epithelia's tight junctions, impairing their ability to sustain barrier function [26]. Supporting research has shown that people with ASD have altered gut mucosa immunity [56, 57]. Variations in cytokine panels, immune cell types, and signaling pathways are indicative of dysregulated innate and adaptive immunity. The intestinal mucosa of people with ASDs had higher levels of pro-inflammatory cytokines such interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), indicating chronic intestinal inflammation [26].

Additionally, abnormalities in immune cell populations in the gastrointestinal mucosa of people with ASD included altered proportions of T helper cell subtypes and increased T cell activation [58]. These immunological abnormalities point to a complex relationship between gastrointestinal comorbidity and immune failure in ASDs. Furthermore, zinc shortage can cause gut pathology, but this does not rule out the potential that zinc deficiency-specific brain pathologies, such as anomalies in SH3 and multiple ankyrin repeat domains protein (SHANK) and the brain-derived neurotrophic (BDNF), also contribute to the ASD phenotype [59]. Actually, it's likely that a variety of cerebral and extra-cerebral diseases work together to cause ASD.

3. The role of leaky gut in the development of autism

Research has suggested that gram-negative bacteria create compounds called lipopolysaccharides (LPS), which can pass through the intestinal barrier (IB) and enter the brain, causing an inflammatory environment that affects brain function [60]. The gastric mucosa efficiently isolates a number of germs from the body by serving as a barrier between the body and the GI lumen. The gastrointestinal barrier, which is acknowledged as a key link between the brain and gut within the gut-brain axis, is the collective term for the protective properties of the GI mucosa [60]. In addition to gastrointestinal illnesses including inflammatory bowel disease and irritable bowel syndrome, dysbiosis has been linked to neurological and mental health issues like depression, stress, Parkinson's disease, Alzheimer's disease, and ASD.

Maintaining the IP's integral structure is essential for general health, even in cases of dysbiosis. When compared to healthy controls, a considerable percentage of abnormal IP values, as measured by the lactulose/mannitol ratio, have been found in both autistic patients and their relatives [61]. IP dysfunction has been found in both individuals with autism and even in their first-degree relatives.

Additionally, it was discovered that there was some damage to the tight junctions (TJs) in the intestines of the children with ASD; it should be noted that in some youthful autism cases, this damage might happen without the presence of gastrointestinal issues [62]. On the other hand, a recent study found no discernible difference in IP between children with autism and either their healthy siblings or unrelated controls [63]. Moreover, some close, healthy relatives of autistic individuals have been found to have elevated IP, suggesting that ASD is not the exclusive cause of loss intestinal integrity [64]. As a result, it is still unclear whether IP disruption causes autism or autism causes IP.

Increased IP, also known as "leaky gut," has been shown to exist in BTBR T+tf/J mice, an animal model for autism [60]. After receiving metformin, which helps to restore the integrity of the IP, these mice's behavioral problems significantly improved. These results suggest that autism may in fact be influenced by the "leaky gut" phenomena. Additionally, LPS from the gut may get through the damaged intestinal barrier and enter the brain, where they activate the pro-inflammatory microenvironment mediated by activation of the Toll-like receptor 4(TLR4), which seems to play a major role in the development of autism. Our claim is supported by the observed down-regulation of inflammatory cytokines linked to TLR4 signaling in the cerebral cortex after the IB is repaired. Few investigations have been done expressly to look into how aberrant intestinal permeability affects the neuropsychiatric symptoms of ASD [60].

According to a study by Li et al. [60], increased IB permeability may be a major factor in the pathological changes in autism. A gut-derived endotoxin enters the brain as a result, activating the TLR4-MyD88-NF-κB pro-inflammatory signaling cascade .Li et al. [60] reported a number of fascinating results that can be summarized as follows:

• After receiving metformin, marble burying, a measure of repetitive behaviors in BTBR mice, significantly decreased; this could be because of IP malfunction.

• IP malfunction led to a decreased inclination for social proximity in autistic mice; metformin therapy reversed this effect.

• Following metformin treatment, the expression of the upstream regulatory molecules TLR4, MyD88, and (Nuclear Factor kappa-light-chain-enhancer of activated B cells) NF-κB as well as the degree of macrophage infiltration and the pro-inflammatory cytokines monocyte chemoattractant protein-1 (MCP-1), IL-1β, and TNF-α reduced.

• Compared to mice treated with metformin, autistic animals treated with dextran sulfate sodium (DSS) as an indicator of gut leakiness had greater plasma LPS levels.

• As a result, Li et al. [60] postulated that improving autistic behavior in adult autistic mice may be achieved by using metformin to repair the IP.
Numerous studies have revealed a dynamic and reciprocal interaction between the gut and brain via the "gut-brain axis." Numerous intestinal metabolites, such as serotonin, short-chain fatty acids, indoles, and LPS, have been discovered in the blood and brain of autistic children [36, 65, and 66].

According to D'Eufemia et al. [62], 9 out of 21 (43%) autistic individuals had a changed IP, although none of the 40 controls had it [60]. This was one of the initial signs of autism's leaky gut. In 2010, Magistris et al. [67] discovered that abnormal IP, or a leaky gut, was present in over one-third (36.7%) of a cohort of autistic children. Furthermore, there is still more to learn about autism and leaky gut. Previous studies have shown that ASD patients have decreased intestinal TJ claudin [60]. Higher zonulin levels are associated with increased hyperactivity, decreased social functioning, and a higher CARS score. Autistic patients with GI issues and changed gut microbiota had an over-representation of the zonulin gene when compared to the other groups. Females exhibit much lower expression of zonula occludens-1 (ZO-1) in gut tissue than males, indicating that female sex hormones play a role in controlling the gut tight junction. In addition,

estrogen has been reported to reduce ZO-1 expression at both the mRNA and protein levels in the stomach. This may have contributed to autism's sex bias, in which girls are less likely than males to have autistic characteristics. In terms of gut permeability and its impact on gut microbiota, a phenomena associated with sex differences in mRNA expressions of the serine protease (PRSS) gene has been observed. PRSS is a trypsinogen-encoding gene that has been shown to produce "trypsin-like activity," a protein (PRSS) that is typically released from the intestines of IBS patients. Recent research has demonstrated that PRSS increases gut epithelial permeability and acts on submucosal neurons to generate gut hypersensitivity, a prevalent trait in the majority of autistic people. In Esnafoglu's study [68], serum zonulin, an indication of elevated IP, was considerably greater in autistic children compared to healthy controls, which was consistent with the results of Li et al. [60].

Studies have shown that people with ASD had higher amounts of serum endotoxins and gut metabolites, which suggests that a "leaky gut" may be a factor in this neurodevelopmental disorder [69]. One method that absorption of LPS from the gut lumen may affect autistic behaviors is through the activation of the brain's innate immune system by circulating pro-inflammatory cytokines [70]. LPS can activate TLR4 to cause systemic inflammation that affects the central nervous system via intracellular (TLR4/MyD88/NF-kB and TLR4/TRIF/IRF3 pathways) and extracellular (mediated by LPS-binding protein, the cluster of differentiation and myeloid differentiation factor 2 [MD-2] pathways) pathways [21]. The first MyD88-dependent pathway is started by the LPS/MD-2/TLR4 complex on the plasma membrane, while the second TLR4/TRIF transduction occurs in early endosomes after receptor endocytosis.

The MyD88-dependent pathway triggers the generation of pro-inflammatory cytokines; TLR4 recognizes LPS through the accessory protein MD2, and its intracellular TIR region binds to MyD88's carboxyl terminus. IRAK1, IRAK2, and IL-1 receptor-associated kinase-4 (IRAK4) are then recruited by the MyD88 terminal via homotypic contacts. The active IRAK4, IRAK1, and IRAK2 split from the MyD88/IRAK complex after being phosphorylated and bind to TNF receptor-associated factor 6 (TRAF6). TRAF6 then triggers the transforming growth factor B-activated kinase (TAK1) complex. TAK1 activates the downstream IκB kinase (IKK), which is made up of two kinases (IKKα and IKKβ), resulting in NF-κB activation. IκBα, an NF-κB inhibitor, is phosphorylated by IKK. Finally, free NF-κB enters the nucleus and causes inflammatory cytokines including TNF-α, IL-1, and IL-6 to be produced [71]. A growing body of research indicates that the pathogenesis of ASD may involve brain inflammation associated with increased inflammatory biomarkers such as TNF-α and IL-6 [60]. In the brain of fetal mice, activating TLR4 signaling in microglia through maternal LPS treatment dramatically raises the protein expression levels of TLR4, phospho-NFκB, p65, and pro-inflammatory cytokines, including TNFα; these offspring display ASD-like behavior with less social behavior, increased anxiety, and repetitive behaviors. Numerous studies have already demonstrated that neuroinflammation may be the main etiology of autism [72, 73].

4. Metal Imbalances in ASDs

It's noteworthy to note that a number of things, including illnesses, chemicals, drugs, and diet, might affect IP. For example, it has been suggested that dietary factors like gluten and casein exacerbate IP in certain people with ASD. Importantly, these studies, which primarily examined samples of hair, nails, and infant teeth, found that children with ASD had greater levels of lead (Pb), nickel (Ni), cadmium (Cd), and mercury (Hg) metals than neurotypical controls [74]. For example, children with ASD have persistently higher blood lead levels. The discovery that infants aged 0-3 years had the highest lead load [75] brought attention to the risk of early prenatal exposure (Table 1).

The cellular disorders linked to heavy metal exposure, such as oxidative stress, inflammation, and mitochondrial malfunction, are similar to those seen in ASD. By disrupting mitochondrial function, heavy metals exacerbate oxidative stress, a characteristic of ASD. For instance, by

inhibiting proteins and enzymes necessary for antioxidant defense, mercury worsens oxidative stress [76]. Children with ASD have mitochondrial damage, including increased production of hydrogen peroxide, decreased oxygen consumption, and overreplication and deletions of mitochondrial DNA [77]. These mitochondrial issues are consistent with ASD symptoms, suggesting a role in ASD development [78]. Furthermore, by increasing proinflammatory cytokines, heavy metals can cause autoimmunity and alter the expression of genes associated with oxidative stress and inflammation [26]. Most importantly, they compete with essential metals like zinc and calcium to disrupt cellular metabolism and signaling.
The link between various metal ions and ASD suggests that, rather than a single metal toxicity or deficiency, ASD may be linked to a specific metal panel. Recently, a model theory was proposed that states that changes made to one metal also affect other metals. Therefore, rather than a change in a single trace metal, ASD may be linked to a unique and potentially distinguishing metal profile. Hazardous metals may increase the risk of ASD through cellular disruption of zinc signaling. Conversely, a lack of zinc may increase the levels of dangerous metals and exacerbate their effects [74]. Certain metals, such as lead, can affect brain development directly or indirectly through mechanisms like gut-brain communication (Table 1).

Table (1): An review of studies looking at the connection between intestinal disorders, changes in the gut microbiota, and exposure to dangerous metals or zinc deficiency

Authors	Metal dysregulation	Gut pathologies	Microbiota changes
Eggers et al. [79]	The average levels of lead exposure were measured in the mother's entire blood during the second and third trimesters of pregnancy.	Changes in the microbiota of the gut Potential alterations in the gut microbiota linked to metals that could compromise the integrity of the gut barrier	Certain microbiome alterations were associated with prenatal lead exposure: three bacterial taxa were negatively connected with third-trimester lead exposure, and six bacterial taxa were negatively correlated with second-trimester lead exposure. .
Han et al. [80]	Individuals with elevated Cd levels who ingested contaminated food, water, or air	Inflammation, short-chain fatty acid (SCFA) metabolism, and metabolic syndrome are all declining. Changes in the microbiota of the gut	decreased butyrate-producing bacteria due to cadmium exposure
Zhai et al. [81]	Chinese children with ASD had hair samples that contained Hg, Pb, As, Cu, Zn, Fe, Ca, and Mg.	Constipation, bloating, diarrhea, and abdominal pain are all signs of GI dysfunction. Alterations in the gut microbiome. There is a positive correlation between GI issues and ASD severity. .	High levels of Pb or Hg are strongly correlated with the abundance of Parabacteroides and Oscillospira.

Shao and Zhu [82]	exposure to As, Cu, Pb, Zn, and Cd as a result of living near a mining and smelting site	Gut microbiota alterations	Following Pb exposure, Lachnospiraceae, Eubacterium eligens, Ruminococcaceae UGG-014, Erysipelotrichaceae UCG-003, Tyzzerella3, Bacteroides, Slackia, and Roseburia were all more common. Pb exposure: Prevotella 9, Bacteroides, Roseburia, and Proteus depletion
Chai et al. [83]	People whose diets are deficient in zinc (ZD)	Metabolic syndrome, inflammation, and potential barrier failure (sucharin, a pro-inflammatory metabolite, and taurocholic acid, a putative mediator triggering intestinal leakage) were all higher in the ZD group.	Probiotic bacteria *Kashiwanohense ifidobacterium* were decreased in the ZD group, but *Phocaeicola vulgatus*, *Alistipes putredinis*, *Bacteroides uniformis,* P*hocaeicola* sp000434735, and *Coprococcus eutactus* were significantly increased.

Research indicates sex differences in both profiles and clinical presentation of ASD .Studies suggest that males with ASD may have different metal exposure associations and processing capacities compared to females, who may have a protective effect and a higher liability threshold for diagnosis.

5. Therapeutic Interventions

Many children with ASD experience nutritional deficiencies due to inadequate intake, digestive issues, and compromised vitamin transport across the blood-brain barrier [84]. Research indicates that taking multivitamins throughout pregnancy may reduce the incidence of ASD, highlighting the importance of eating healthily during critical developmental phases [85]. Vitamins B1, B5, B6, and D mediate critical processes such as neurotransmitter production, synapse regulation, and metabolic activity. It has been demonstrated that taking supplements containing them can improve behavior, support brain function, and help manage ASD symptoms [86].

5.1. Microbiota Transplantation Treatments

ASD is a complicated collection of neurodevelopmental diseases linked to the dysbiosis of GM and its metabolites [8]. Thus, altering GM was one way to treat ASD [87]. the impact of probiotic Bifidobacterium and fecal microbiota transplantation on animal behavior and alterations in the global GM in the propionic acid (PPA) rat model of autism. Both treatments corrected the social impairment caused by PPA therapy [88], increased microbial diversity, promoted specific alterations in the microbiota, and were expected to modify the metabolic pathways of microorganisms. Growing research highlighting the importance of the GM brain axis and its connection to mental illnesses such as ASD has led to the development of many therapeutic strategies that focus on altering the GM. This included the use of probiotics, prebiotics, and fecal microbiota transplantation [87].

Bifidobacterium longum (*B. longum*) is one of the most widely used probiotic species since it is an important part of GM and has many beneficial effects on human well-being in general and mental health in particular [89-93]. In fact, *B. longum* lessened the severity of ASD in a number of human and animal investigations [8]. Fecal microbiota transplantation is another widely used strategy that has been demonstrated in numerous studies to improve ASD [10]. These two treatments were

used to a propionic acid rat model of autism in order to determine their effects on animal behavior and gut microbiota modulation. Streptococcus, Paraeggerthella, and Lachnospiraceae were found to be more common in the GM of rats given PPA, according to Abujamel et al. [8].

Clostridium is one of the most prevalent bacteria in the digestive system. Although this genus also includes certain pathogenic bacteria, it also contains a variety of helpful bacteria that perform essential functions in the gut [94]. For example, the bacterial clusters Clostridium IV and XIVa make up around 35% of gut microorganisms [95]. In Clostridium cluster IV, the two main prevalent species are Anaerofilum and Faecalibacterium [96-98]. The importance of butyrate in preserving overall health and mental health in particular is further demonstrated by the depletion of acyl-CoA thioesterase in a rat model of autism. Furthermore, neurodevelopmental problems, including ASD, have been linked to vitamin deficiencies and changes in lipid metabolism [8], and ubiquinone supplementation has been shown to alleviate ASD symptoms in children [99]. Another necessary amino acid that is involved in several physiological functions is histidine. Histamine, an organic nitrogen molecule important in neurotransmission and host immunity, can be produced via its metabolism [100]. Therefore, it is anticipated that PPA therapy will lead to a reduced excess of metabolic pathways that are either directly or indirectly associated with neurodevelopmental issues. On the other hand, it is expected that GM animals treated with BF will produce more vitamin B12, which will be associated with a contemporaneous decrease in the metabolism of peptidoglycan, beta lactam resistance genes, succinate, pyruvate, and nitrogen. Cobalamin, also referred to as vitamin B12, is a cofactor that contributes to the synthesis and methylation of antioxidants. Both biological activities have been demonstrated to be impaired in ASD. Additionally, B12 supplementation improved the clinical outcomes of the illness [101]. Furthermore, *Lpb. plantarum* may have neuroprotective qualities based on the synthesis of GABA, a neurotransmitter that regulates mood and anxiety that is often disturbed in people with ASD [102]. The association between *Lpb. plantarum* PS128 consumption and behavioral advantages in teenagers was further confirmed by a recent retrospective observational study involving 131 autistic individuals, particularly when concurrent GI symptoms were present [103]. A 12-year-old child with ASD showed improvements in GI symptoms and core symptoms after receiving a diversified multi-strain combination of 10 probiotics (#VSL3) for four weeks [104]. It is still uncertain whether multi-strain probiotics are more beneficial than single-strain formulations due to mixed results from multiple studies. Thus, results from clinical trials continue to be the primary criterion for guiding the selection of appropriate probiotic therapy [33]. For example, Tomova et al. [105] emphasize that the efficacy of probiotic *Lpb. plantarum* may also vary depending on the individual characteristics of the patient and the composition of their microbiota. More recently, He et al. conducted a comprehensive review and meta-analysis of seven probiotic intervention trials [106].

5.2. Anti- inflammatory Therapies:

Because of the importance of immunological dysregulation and intestinal inflammation, anti-inflammatory treatments are being explored in rodent models as well as human patients. Lowering gut inflammation may alleviate both gastrointestinal and behavioral symptoms in ASD [26].

5.3. Dietary Interventions: Diets free of gluten and casein, as well as the use of specific fibers to promote the growth of beneficial bacteria, have been studied in both human and rodent models [107,108]. Despite the mixed outcomes, these medicines show promise for a small subset of ASD patients, particularly those with severe gastrointestinal difficulties. Nonetheless, given the findings, metal homeostasis should be a main therapeutic target for ASD.

5.4. In addition, zinc supplementation has been linked to improved GM composition [109]. It inhibits harmful organisms while promoting the growth of beneficial microbes [110]. The immune system and overall digestive health rely on this balance in the GM. There have been almost no clinical trials in ASD. However, in a study of 79 autistic people, zinc supplementation showed potential. After zinc therapy, hyperactivity in

autistic children with GI pathology improved significantly, but not in autistic children without GI disease [26].

Conclusions

Understanding ASD remains a major challenge for the scientific community due to its complexity, which needs interdisciplinary study and therapy strategies. In this regard, studies on GM, inflammation, and IP in sex differences have provided critical information for understanding the convoluted pathophysiology of ASD. These findings have implications for microbiome-based therapeutics and biomarker-driven techniques in future treatment efforts, underlining the therapeutic potential of addressing gut barrier integrity and microbiota composition to alleviate both GI and behavioral symptoms in ASD.

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