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REVIEW ARTICLE

The relationship between autism spectrum disorder, gut-brain axis and gut microbiota

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Abstract

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder in which symptoms such as difficulty in social interaction, communication problems, limited interests, and limited behavioral patterns are observed. The prevalence of ASD has been increasing over the years, but its etiology has not been fully elucidated. Gastrointestinal (GI) symptoms are a common comorbidity in children with ASD, but the underlying mechanisms are unknown. Many studies have shown alterations in the composition of the gut microbiota and their metabolic products in patients with ASD. The gut microbiota influences brain development and behaviors through the neuroendocrine, neuroimmune, and autonomic nervous systems. In addition, abnormal gut microbiota is associated with several diseases, such as obesity, diabetes, autoimmune diseases, neurodegenerative diseases, and psychiatric diseases (ASD, depression, anxiety disorder, etc.). In this review, we aim to provide information about the bidirectional interactions between the central nervous system and the gastrointestinal tract (the gut-brain axis), the possible roles of the gut-brain axis and gut microbiota in the etiology of autism spectrum disorder, and current hypotheses.

Keywords: Autism spectrum disorder, Gut Microbiota, Gut-Brain axis

Introduction

Autism Spectrum Disorder (ASD) is a childhood neurodevelopmental disorder characterized by social and communicative deficits, repetitive and stereotyped behaviors, and limited interests [1]. The prevalence of this disorder which is gradually increasing today, was determined as 2.76% (1/36) according to 2020 data [2]. In the same study, it was also reported that ASD is 3.8 times more common in boys than in girls [2].

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The etiology of autism, characterized by a wide spectrum of clinical symptoms, is not yet fully known.

Studies have shown that children with ASD are frequently accompanied by gastrointestinal symptoms such as food intolerance, chronic constipation, nausea, vomiting, chronic diarrhea, gastroesophageal reflux, bloating, and indigestion, with rates ranging from 46% to 84 % [3]. It has also been reported that these symptoms may be related to the degree of deficits in social relationships, the severity of stereotypic behaviors, hyperactivity, and aggression in children with ASD [4]. The detection of these comorbid conditions and the determination of the relationship between certain neuropsychiatric disorders and the gastrointestinal system have brought attention to the investigation of the 'Gut-Brain Axis' in the etiology of autism.

The 'Gut-Brain axis' is a dynamic and bidirectional structure that includes many tissues and organs such as the brain, secretory glands, intestine, immune cells, and intestinal bacterial flora. It is suggested that the exchange of signals and information along this axis affects the chemical structure and behavior [5]. The vagal nerve, including sympathetic and parasympathetic branches of the autonomic nervous system; the bacterial cell wall, which activates the immune system and neuroendocrine pathways; tryptophan and short-chain fatty acids, metabolites of intestinal bacteria; neurotransmitters and neuropeptides are important communication tools of this axis [5].

In this review, we aim to provide information about the possible roles of the gut-brain axis and gut microbiota in the etiology of autism spectrum disorder and current hypotheses.

1. Gut Microbiota

The intestinal bacterial flora (gut microbiota), which contains millions of species and about 10^{14} microorganisms, was defined as microbiota in 2007 by the Human Microbiome Project (HMP) [6]. The number of these microorganisms colonizing various parts of the body, such as the skin, genitals, and intestines, is known to be about ten times greater than the number of cells in the human body. *Firmicutes* and *Bacteriodetes* bacterial families are predominant in this microbial structure, which shows different distributions in the gastrointestinal tract due to physiological and chemical properties. In addition to bacteria, this microbial diversity also includes eukaryotes, viruses, bacteriophages, and different families of fungi [6].

The gut microbiota, which begins to develop within a very short time after birth, is thought to affect human health directly or indirectly [7]. Studies have shown that the gut microbiota act as a barrier that prevents the proliferation of pathogenic organisms, contributes to the digestion of food, and degradation of toxic and waste substances, and plays an important role in the regulation of lipid and glucose metabolism, activation of the immune system and gene expression. Along with these functions, metabolites produced by the gut microbiota and released into the peripheral circulation have been reported to contribute to the development and function of the central nervous system [8].

The number and content of the gut microbiota, a symbiotic life with the human host, is affected by many factors such as age, genetics, dietary habits, geographical region, mode of birth, gestation period, antibiotic, pre-biotic or probiotic use [9]. The alteration or disruption of this system, which is normally in equilibrium, for any reason is defined as dysbiosis. This condition, which is closely related to immune dysregulation, is thought to lead to serious metabolic and inflammatory pathologies and predispose to many diseases [10].

2. The Gut-Brain Axis

Microbiology and psychiatry sciences, which have developed in different fields for many years, have started to be investigated together with the progress of metagenomic studies and the development of 16s ribosomal RNA sequence analysis methods. Research has shown that gut microbiota, whose species and number can be determined with developing methods, is effective in many physiological events such as digestion, growth, immune system, and body energy balance (homeostasis) [10]. The gastrointestinal tract contains the enteric nervous system, composed of primary afferent neurons and nerve endings. Changes occurring in the gastrointestinal tract are transmitted to the brain via the vagal nerve. The gastrointestinal tract also harbors different cell groups that release cytokines and neuroendocrine hormones that play an important role in the body's response to infection and inflammation. Heijtz and Clarke, investigating the relationship between gut

microbiota and disorders associated with the central nervous system and neuropsychiatric diseases, suggested a bidirectional interaction between the brain and the gut [11, 12]. Also, researchers have reported that this interaction is mediated through the neuroendocrine, autonomic, and enteric nervous systems and the immune system. This dynamic pathway, called the 'Gut-Brain axis', involves many tissues and organs such as the brain, secretory glands, gut, immune cells, and gut microbiota [13] (Figure 1).

The pathways and mechanisms thought to be involved in the Gut-Brain axis.

2.1.Vagal Nerve (N. Vagus): The 10th cranial nerve, N. Vagus, carries afferent and efferent nerve fibers related to motor, sensory and autonomic nervous systems [14]. It has important functions related to respiratory, circulatory, and digestive functions and forms a direct connection between the gut and the brain [14]. In the literature, it has been reported that c-FOS levels were increased in vagal sensory ganglia and some brain regions of animals infected with pathogenic *Citrobacter rodentium* and *Campylobacter jejuni* and that this bacterial alteration in the gastrointestinal tract may be associated with anxiety symptoms [15, 16].

2.2. Cell Wall Components and Immune Responses: The gut microbiota has a peptidoglycan cell wall structure that activates both humoral and cellular immunity. The cell wall of gram-negative bacteria in the microbiota contains peptidoglycan monomers, lipopolysaccharides (LPS), porins, and mannose-enriched glycans, while the cell wall of gram-positive bacteria contains peptidoglycan monomers and lipoteichoic acids [17]. These components in the cell wall structure can stimulate intestinal epithelial cells and trigger the production of molecules associated with neural signaling pathways. The humoral immune response is activated by the binding of pro-inflammatory microbial components known as pathogen-associated molecular patterns (PAMPs) to pattern recognition receptors (PRPs) such as Toll-like receptors (TLRs) and NOD-like receptors (NODs) on intestinal epithelial cell surfaces. This activation triggers the release of inflammatory cytokines and acts directly on the brain where the permeability of the blood-brain barrier permits and indirectly through the vagal nerve where it does not [7]. In addition, these microbial components support the development of the immune system by antigen presentation to immune cells such as lymphocytes and macrophages [18]. The lipopolysaccharide (LPS) structure in the cell wall

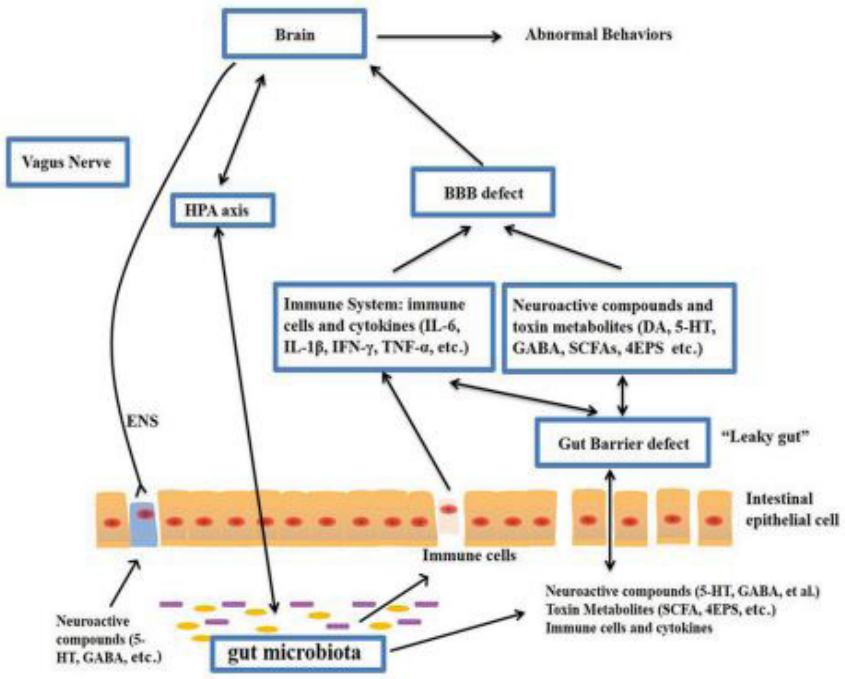

Figure 1: *The relationship between the gut microbiota and ASD[13]*

of gram-negative bacteria increases intestinal alkaline phosphatase production together with immunoglobulin A (Ig A), while the peptidoglycan layer enables the maturation of lymph follicles through NLR (NOD-like receptor). Maturing lymph follicles recognize intestinal bacterial flora via TLR (Toll-like receptor) and help cluster B lymphocytes [7, 18]. Further studies are needed to clarify the relationship between gut microbiota and the immune system.

2.3. Metabolites of the Gut Microbiota: Metabolites and nutritional components produced by microbial fermentation have important effects on brain function and immune response. One of the most important functions of the gut microbiota is the digestion of undigested nutrients through fermentation and the production of short-chain fatty acids (SCFA), which are an important source of energy for intestinal epithelial cells [7]. Short-chain fatty acids, including products such as acetate, propionate, butyrate, and lactate, support the immune system by preventing the accumulation of toxic substances as well as being an energy source for the body [19]. In addition, gut microbiota helps regulate the metabolism of tryptophan, an essential amino acid.

• **Short-chain Fatty Acids (SCFA):** Fatty acids have many functions, such as being a source of energy for cells, being present in the membrane structure, being involved in cellular functions, regulating gene expression and signaling pathways, and being involved in the synthesis of other lipid-structured mediators such as eicosanoids, which are chemical messengers [19]. The brain is an organ rich in fatty acids and fatty acid derivatives such as eicosanoids, lecithin, glycerophospholipids, sphingolipids, and prostaglandins. While fatty acids trigger inflammation by binding to specific immune cells such as T lymphocytes, B lymphocytes, and macrophages, some short-chain fatty acids produced by microbial fermentation have anti-inflammatory effects [20]. Most of the short-chain fatty acids such as acetate, butyrate, isobutyrate, hexonate, and propionate are produced by *Eubacterium, Roseburia, Faecalibacterium, Bifidobacterium, Lactobacillus* and *Enterobacter* species in the gut microbiota [21]. In the literature, it has been reported that fatty acids affect intestinal permeability, alter the lipoprotein profile, increase immune system functions, and acidify colonic pH [22]. It has also been reported that short-chain fatty acids, which are also involved in the enteroendocrine signaling pathway, bind to G protein-coupled receptors (GPR43, GPR41) receptors and initiate the release of neuropeptides such as peptide YY, glucagon-like peptide (GLP-1) and help regulate body homeostasis [22].

• **Tryptophan:** Tryptophan, an essential amino acid, is the precursor of substances such as serotonin, melatonin, and niacin. Tryptophan follows one of three different metabolic pathways: incorporation into tissue proteins, oxidation (kynurenine), and hydroxylation (serotonin pathway). While 3-10% of tryptophan participates in the hydroxylation pathway that forms chemical messengers such as serotonin and melatonin, 90% or more of tryptophan participates in the oxidation pathway that breaks the indole ring in its structure and forms kynurenine, nicotinic acid, nicotinamide adenine dinucleotide (NAD) [23]. Some of the enzymes involved in this pathway, also called the 'kynurenine shunt', which is dominant in tryptophan metabolism, are produced by aerobic bacteria in the gut microbiota [24]. Dysregulation of this pathway has been associated with many disorders in the central nervous system and gastrointestinal system. Serotonin, known to be effective in the etiology of many psychiatric disorders such as depression, anxiety, and obsessive-compulsive disorder, is mostly found in the gastrointestinal tract and synthesized by enterochromaffin cells [25]. Low levels of tryptophan in plasma affect immune system functions. This interaction may play a role in central nervous system functions and the development of mood disorders. In studies conducted to elaborate the anti-depressant properties of probiotics, it was observed that rats fed probiotics containing *Bifidobacterium infantis (B.infantis)* had decreased levels of pro-inflammatory cytokines, increased plasma tryptophan levels and decreased depressive behaviors of these rats [26]. It was also found that probiotic treatment caused a decrease in serotonin and dopamine destruction in the frontal cortex and amygdala [26].

2.4 Neurotransmitters and Neuropeptides: Neurotransmitters are chemical messengers responsible for signal transmission between neurons. Neuropeptides, which have different structures and properties than neurotransmitters, interact with different receptors in the brain and provide communication between neurons. These protein substances are responsible for specific

behavioral patterns [27]. Studies to date have shown that bacterial species in the gut microbiota such as *Lactobacillus, Bifidobacterium, Escherichia, Enterococcus,* and *Truchuris* produce neurotransmitters such as gamma-aminobutyric acid (GABA) and serotonin and some neuropeptides such as brain-derived neurotrophic factor (BDNF) [28]. These substances are associated with neuronal signal transduction and are thought to be involved in the regulation of brain function and behavior [28].

• **Gamma-Aminobutyric Acid (GABA):** GABA is an inhibitory neurotransmitter in the mature brain and an excitatory neurotransmitter in the developing brain [29]. Synthesized from glutamate, an excitatory amino acid, GABA acts as a second messenger in important body functions such as cellular development, homeostasis, and autophagy. The imbalance between GABA and excitatory and inhibitory neurotransmitters in the central nervous system is thought to be effective in the etiopathogenesis of various neuropsychiatric disorders such as memory loss, autism, epilepsy, anxiety, and depression [29]. Barett et al. realized that some bacterial species, such as *Lactobacillus* and *Bifidobacterium* synthesize GABA from glutamate in culture media [28]. Bravo et al. showed that *L. rhamnosus* regulates the production of central GABA receptors in some parts of the brain in animal models and suggested that this bacterial species may be useful in the treatment of depression and anxiety [30].

•**Serotonin:** Serotonin, a monoamine neurotransmitter, is involved in the regulation of many physiological processes in the central nervous system, such as mood, sleep, pain, aggression, sexual behavior, memory, and learning functions [31]. Serotonin in the gastrointestinal tract is responsible for the regulation of blood flow, motility, and secretory functions together with other intestinal hormones. It is known that serotonin, mostly synthesized by enterochromaffin cells in the gastrointestinal tract, is also synthesized by bacteria such as *Escherichia* and *Enterococcus* in the gut microbiota and that gut microbiota metabolites (short-chain fatty acids) trigger this synthesis [32].

• **Brain-derived neurotrophic Factor (BDNF):** BDNF is a neuroprotective peptide produced in the central nervous system. This structure, which can cross the blood-brain barrier, is responsible for the development and differentiation of neurons in childhood and ensures that neurons live a healthy life in adulthood [33]. In recent studies, plasma BDNF levels were found to be low in neurodegenerative diseases such as Alzheimer's, and Parkinson's and some psychiatric diseases such as depression and it was thought that BDNF synthesizing mRNA and protein levels are related to gut microbiota [34]. In experimental studies, increased hippocampal BDNF levels were found in mice free of specific pathogens [33]. It was observed that hippocampal BDNF m-RNA levels decreased in mice after infection with *Truchuris muris*, whereas BDNF levels increased to normal values after infection with *B.longum* [35]. On the other hand, Esworthy observed behavioral changes with increasing BDNF levels in male mice free of specific pathogens [36]. Given the role of BDNF in neuroplasticity and neuropsychiatric disorders, there is a need to elaborate on the relationship between BDNF and gut microbiota and to determine the conditions that affect this interaction.

3. The Relationship Between Gut Microbiota and Disease:

The gut microbiota is thought to play a role in the etiology of metabolic diseases such as obesity, diabetes, autoimmune diseases, neurodegenerative diseases, and psychiatric diseases such as autism spectrum disorder, depression, anxiety disorder, etc. Changes in the number, structure, and content of the gut microbiota cause the balance to be disrupted and 'unhealthy microbiota', also known as 'dysbiosis', to occur. This may result in local and systemic effects such as altered production of short-chain fatty acids, increased intestinal permeability, and decreased colonic resistance [8].

As a result of increased intestinal permeability, it is suggested that bacterial products pass into the systemic and local circulation, and as a result, they cause metabolic diseases such as obesity, metabolic syndrome, atherosclerosis, and diabetes by creating a low level of endotoxemia, or by affecting lipid and glucose metabolism or causing inflammation due to the change in short-chain fatty acid production [37]. Molecular similarity can be observed between products of the gut microbiota and cellular structures. It has been reported that dysbiosis may cause the production of some autoantibodies against these bacterial structures and may cause autoimmune diseases by negatively affecting healthy cells due to similarity [36]. However, decreased colon resistance is also said to predispose to infections with opportunistic pathogens or pathogenic bacteria.

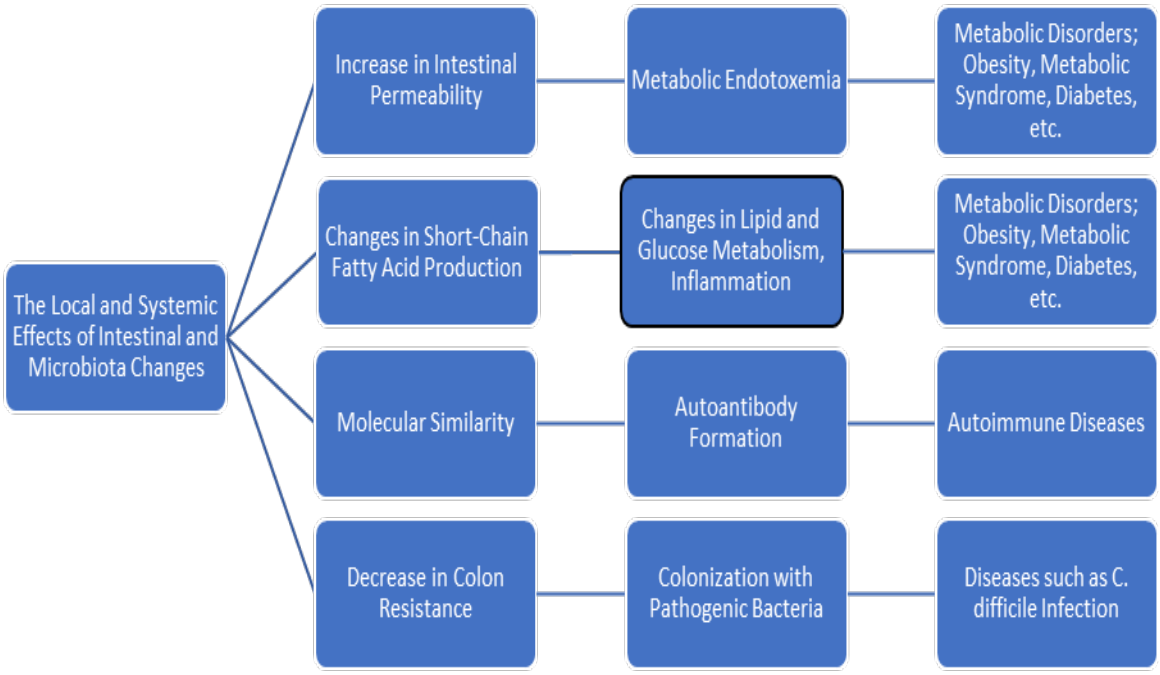

Figure 2. *Local and systemic effects of gut microbiota alteration* [39]

The positive neurochemical and physiological effects of chemical substances produced by healthy microbiota by binding to receptors on the intestinal surface or in different cells are thought to be negatively affected by dysbiosis [37]. Abnormal gut microbiota is thought to cause disruptions in the functioning of sulfur metabolism, increased oxidative stress, mitochondrial dysfunction, and neuroinflammation [38]. These changes may affect the structure and functioning of other systems, especially the central nervous system, and may cause diseases [38] (Figure 2).

4. The Gut-Brain Axis and Autism Spectrum Disorder (ASD)

The relationship between the gut microbiota and autism spectrum disorder, whose etiopathogenesis has not yet been fully explained and lacks a curative treatment, has been under investigation in recent years.In the literature, it has been shown that the majority of individuals with ASD are accompanied by gastrointestinal symptoms such as constipation, diarrhea, bloating, and indigestion and that these symptoms are associated with the degree of deficits in social relationships and social interactions and the severity of stereotypic behaviors, hyperactivity, and aggression in individuals with ASD [3, 4]. Furthermore, researchers have also reported that the gut microbiota content and distribution of individuals with autism differ from healthy children [4, 40].

Three main mechanisms have been proposed for the relationship between ASD and the gut-brain axis. The first is bacterial overload and/or abnormal bacterial diversity; the second is oxidative stress and disturbances in sulfur metabolism; and the third is increased intestinal wall permeability, called as “leaky gut hypothesis” [38].

4.1 Abnormal Intestinal Contents and/or Bacterial Overload

In the gastrointestinal tract, microorganisms are most abundant in the colon and these bacteria constitute the majority of microorganisms [41]. Bacterial colonization begins at birth. The intestinal flora of babies born vaginally is compatible with the vaginal flora of the mother and is predominantly *Lactobacillus*, while those born by cesarean section are predominantly *Clostridium*. In the first year, the flora is dominated by *Actinobacteria* and *Proteobacteria*, whereas around 2 years of age, the flora becomes similar to adult flora and *Firmicutes, Bacteroidetes,* and *Actinobacteria*, especially *Bifidobacterium*, predominate [42]. The majority of the intestinal microbial community is composed of five phyla: *Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria,* and *Verrucomicrobia*. The main members of the *Firmicutes* include the genera *Clostridium, Lactobacillus,* and *Ruminococcus*. *Firmicutes* and *Bacteroidetes* account for more than 90% of the known

phylogenetic categories [43]. Furthermore, the gut microbiota contains a balance of bacteria such as *Bifidobacterium* and *Lactobacillus* that produce anti-inflammatory cytokines and bacteria such as *Clostridium* and *Ruminococcus* that produce pro-inflammatory cytokines [38]. When the classes of bacteria colonized in the intestines were examined, the *Bacteroidetes* family was found at a higher rate in children with autism, while the *Firmicutes* family was found to be dominant in the control group [44]. Tomova et al. showed a significant decrease in *Bacteroidetes/Firmicutes* ratios and an increase in the amount of *Lactobacillus spp*. These results were consistent with the results of a study by Adams et al. showing that *Lactobacillus spp.* strains were significantly higher in individuals with autism [45]. In addition, an increase in *Clostridium spp*. strains was also found in a group of studies conducted at different times [46]. In different studies, *Actinobacter* and *Proteobacterium* branches differed in children with autism, and fewer *Bifidobacter spp*. were detected in individuals with autism [45]. In addition, *Desulfovibrio spp*. was found to be significantly overabundant [44]. Another remarkable result in the study by Tomova et al. was that the *Bacteroidetes/Firmicutes* ratio and the amounts of *Desulfovibrio spp.* and *Bifidobacterium spp*. were restored and returned to normal after giving probiotics containing *Lactobacillus, Bifidobacterium* and *Streptococcus* strains to children with ASD [47]. In a study conducted with children with autism who had gastrointestinal complaints, a high amount of *Sutterella spp*. was found in biopsy samples taken from individuals [48], while *Parasutterella excrementihominis*, a member of this family, was found in high amounts in stool samples [49]. In a 2000 clinical study by Sandler and Finegold, 8 out of 10 patients with late-onset autism who were treated with vancomycin for a short period showed transient improvement [50]. The researcher attributed this effect to the elimination of neurotoxin produced by the pathogens and stated that this improvement was transient as spores and toxins continued to multiply after vancomycin treatment was discontinued [50]. In a study conducted in Turkey in which ASD patients and their siblings were included, it was found that the total bacterial load decreased in both the ASD group and their siblings. In addition, in the same study, no statistically significant difference was found between *Bacteroidetes, Lactobacillus, Clostridium, and Desulfovibrio* species, but a difference was found between *Firmicutes* and *Bifidobacterium* species [51].

4.2 Oxidative Stress and Disorders of Sulfur Metabolism

Cysteine, which is a rate limiter in the synthesis of glutathione, the body's natural antioxidant, is synthesized from methionine, and disruption in this pathway leads to a decrease in cysteine and glutathione synthesis. It is thought that a deficiency in methionine synthesis may cause diseases such as autism by inhibiting gene expression [38]. Studies on gut microbiota and genetics suggest that genetic makeup may affect bacterial content. Furthermore, twins living in separate regions were found to have mostly similar bacterial content even years later [52]. These results showed that methionine deficiencies may cause alterations in the gut microbiota content in individuals with ASD [38]. In a different study in the literature, it was found that the amount of glutathione was lower and the ratio of oxidized glutathione to reduced glutathione was higher in individuals with ASD and this was associated with oxidative stress [53]. Recurrent infections, neuroinflammation, gastrointestinal inflammation, and metabolic disorders have been found more frequently in children with autism due to the important role of glutathione in the detoxification of heavy metals and toxic substances [53]. Disruptions in the transsulfuration mechanism along with increased oxidative stress have caused individuals with ASD to become more susceptible to the toxic effects of heavy metals, especially phenol-containing xenobiotics [54].

Desulfovibrio is a type of bacteria that reduces sulfate by consuming hydrogen gas in some chemical reactions. Another group of microorganisms that consume hydrogen in the absence of oxygen to form non-toxic methane are metagenic archaea [55]. Humans usually have one of two groups. If a sulfate-reducing bacterial group is present, it competes with archaea for hydrogen consumption and thermodynamically manipulates reactions in its favor. As a result, sulfate-reducing bacteria use hydrogen to form hydrogen sulfide, which is harmful to the human body [38]. *Desulfovibiro,* which differs from other bacteria in its ability to catabolize sulfur-containing compounds, can synthesize methionine and/or cysteine using sulfate as an electron acceptor [55]. It is thought that the deficiencies in sulfur metabolism seen in individuals with ASD may have

increased their need for *Desulfovibiro spp.* bacteria and that SAH, which increases due to disruptions in this pathway, may have increased by using *Desulfovibiro* to support growth and development [38].

4.3 Increased Intestinal Wall Permeability (the 'Leaky Gut' Hypothesis)

The term 'leaky gut' refers to the impaired barrier function that forms the wall of the small and large intestines [8]. It is thought that local endotoxemia and inflammation caused by disruption of this barrier, which is composed of tight junctions, epithelial cells, and various protein structures in the paracellular space, pass into the systemic circulation, reach the blood-brain barrier, and cause neuroinflammation, leading to neurodevelopmental disorders such as autism and attention deficit hyperactivity disorder (ADHD) [41]. Toxins produced by *Clostridia* and *Desulfovibrio* bacteria, lipopolysaccharides (LPS) found in the cell walls of gram-negative bacteria such as *Bacteroides*, heavy metals, and phenol-containing compounds that cannot be excreted from the body due to inadequate antioxidant and detoxification mechanisms are suggested to cause inflammation and impair intestinal wall permeability [56]. It is thought that the decreased amount of *Bifidobacteria,* despite the increased amount of *Clostridia* detected in the stool analysis of individuals with autism, shifts the balance between inflammatory cytokines in favor of pro-inflammatory cytokines, triggers an inflammatory response in the intestines, and the epithelial barrier exposed to this response for a long time is damaged and causes an increase in intestinal permeability [41]. At the same time, it has also been suggested in the literature that *Clostridium difficile* toxins cause rounding of intestinal epithelial cells through Rho-GTPase activity, increasing paracellular space, and impaired function of the intestinal epithelial barrier [57]. It is known that the lipopolysaccharide (LPS) structure, also known as endotoxin, found in the cell wall of Gram-negative bacteria can pass into the systemic circulation with impaired intestinal permeability. Serum endotoxin level is said to be an indicator of bacterial load passing from the intestines to the systemic circulation [51]. Long-term exposure to endotoxin, which can cross the blood-brain barrier, has been found to cause neuronal cell death and lead to chronic neuroinflammation. It is thought that the intestinal mucosal barrier with increased permeability in individuals with ASD allows the passage of high amounts of bacteria and metabolites; these structures reaching the central nervous system trigger immune reactions, initiate neuroinflammation, and cause autism [8]. In the study conducted by Yitik Tonkaz et al. investigating the leaky gut hypothesis, it was found that intestinal microbiota was similar between ASD and sibling groups; biological markers of bacterial translocation were significantly different in the sibling group, whereas fecal calprotectin levels indicating local inflammation did not differ between the groups. The authors stated that the findings of the study did not support the leaky gut hypothesis in the etiology of autism [51].

Conclusion

The central nervous system is a dynamic structure that develops through molecules and transmission pathways within itself and through interaction with external factors. The gut microbiota is one of the external factors affecting development. Although altered gut microbiota have been found in children with ASD, whether this is a cause or effect is still unknown. In studies investigating the relationship between ASD and gut microbiota, the heterogeneity of patients in terms of factors such as age range, diet, and probiotic use prevents generalization of the results. It should also be noted that most of the studies were cross-sectional and mainly investigated bacteria. The bidirectional nature of the microbiota-gut-brain axis makes it difficult to determine the first place where the problem started and to establish a cause-and-effect relationship. Double-blind, placebo-controlled, prospective studies with a homogeneous distribution of participants are needed.

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Ethical Declaration:

The study was conducted in accordance with the criteria of the Declaration of Helsinki.

Authors' Contributions: Concept: G.Y.T., B.S.O., Design: G.Y.T., B.S.O., Data Collection or Processing: G.Y.T., B.S.O., E.C., Analysis or Interpretation: G.Y.T., B.S.O., Literature Search: G.Y.T., B.S.O., E.C., Writing: G.Y.T., B.S.O., E.C.

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