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Gut-brain axis and the risk of autism spectrum disorders*

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ABSTRACT

Autism spectrum disorders (ASD) are a complex group of developmental pathologies characterized by the disorders of social interaction and communication, along with repetitive restrictive behavior. Many factors are associated with the development of ASD, including genetic and environmental factors such as nutritional deficiencies, infections, immune system dysfunctions, and allergies. The human gut microbiome is composed of communities of bacteria, viruses, and fungi that influence the central nervous system (CNS). Dysbiosis is defined as an imbalance or maladaptation in the gut microbial community which favors many pathological states and may be associated with some diseases. The changes in microbiota composition in children with ASD may contribute to both gastrointestinal and CNS symptoms. The disorders of the gut-brain axis signaling appear to affect neuropsychiatric disorders, including autism and ASD. The prevention and treatment of dysbiosis in ASD involves modification of the gut microbiome using the supplementation with probiotics – a live active culture.

Keywords: autism spectrum disorders; gut-brain axis; microbiome; dysbiosis; probiotics; postbiotics.

INTRODUCTION

Autism spectrum disorders (ASD) is a complex group of developmental pathologies characterized by disorders of social interaction and communication, along with repetitive restrictive behavior. Among these disorders, autism is the primary form [1]. Many factors are associated with the development of ASD, including genetic and environmental factors such as nutritional deficiencies, exposure to viruses, errors during embryonic neural tube closure, immune system dysfunctions, and allergies [2]. The genetic basis of ASD is complex and includes genes involved in the development of the central nervous system (CNS) [3]. Although the exact etiopathogenesis of ASD is poorly understood, recent studies indicate significant interactions between the intestinal microflora and the brain in patients with autism and other neuropsychiatric diseases. A significant number of patients with ASD suffer from gastrointestinal disorders and chronic abdominal pain that accompany their neurological changes. Gastrointestinal complaints seem to be closely related to the severity of ASD [4, 5]. Furthermore, recent studies suggest that changes in microbiota composition in children with ASD may contribute to gastrointestinal and CNS symptoms [6, 7]. Therefore, they are treated as risk factors that genetically predispose to ASD through their effects on the immune system and metabolism [8].

In recent years, studies have shown significant changes in the composition of the intestinal microflora in children with ASD. They suggest that gastrointestinal disorders may be a symptom of the underlying inflammatory process. Dysbiosis in ASD is associated with a disorder of the intestinal mucosa which leads to increased permeability of exogenous peptides of both food and neurotoxic peptides of bacterial origin and the production of inflammatory cytokines [9, 10]. The disorders of the nervous, hormonal, and metabolic mechanisms that play a key role in gut-brain axis signaling appear to affect neuropsychiatric disorders, including autism and ASD [8].

DYSBIOSIS IN PEOPLE WITH AUTISM SPECTRUM DISORDERS

Gut microbiome in humans

In adults, the human gut has 100 trillion microbes, which is estimated to be 10–100 times the number of all other cells in the human body, and the mass of the gut microbiome is about 2000 g [11, 12]. Similarly, there are nearly 3.3 million gut microbial genes, which is 150 times the number of genes in the human genome [13]. The microbiome is composed of communities of bacteria, viruses, archaea, fungi, and eukaryotes that inhabit the human body and are collectively referred to as the "second human genome" [14]. Bacteria live in each part of the digestive tract, with increased density and changed composition in distal parts. The entire mass of stool consists of bacterial biomass in 25–54% [15]. The composition of the gut microbiome is variable and depends on many

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factors, including the method of childbirth, age (other composition in children and other in adults), gender, diet, stress, infections, alcohol intake, diurnal variation, smoking, drugs (antibiotics), and physical activity [16, 17, 18]. Main gut bacteria in humans are of 6 types: *Firmicutes*, *Actinomycetes*, *Fusobacteria*, *Bacteroidetes*, *Proteobacteria*, and *Verrucomicrobia* [19].

Bacterial dysbiosis with autism spectrum disorders

Dysbiosis (also called dysbacteriosis) is defined as an imbalance or maladaptation in the gut microbial community [20, 21]. This imbalance favors many pathological states and plays a role in some diseases of the CNS. Increased microbiota abundance and reduced microbiological diversity characterize the intestinal biota of people with ASD. This combination of factors may lead to the overgrowth of harmful bacteria contributing to the severity of autistic symptoms [22, 23]. The analysis of the intestinal microbiome from feces of autistic children showed lower *Firmicutes* levels and a relatively higher amount of *Bacteroidetes.*

The product of *Bacteroidetes – short chain fatty acids* (SCFA) and their metabolites, especially propionic acid (PA), can affect CNS and autistic behavior through the modulation of the gut-brain axis [24]. In children with ASD, a decrease in the number of *Faecalibacterium* and *Agathobacter* was also observed, as well as a decreased level of 3-hydroxybutyric acid and melatonin with an increase in the level of serotonin. These changes may aggravate sleep problems and core symptoms in children with ASD [23].

A reduced level of *Bifidobacterium* has also been reported, which may play a protective role in autism through its anti-inflammatory properties. It was found that *Lactobacillus*, *Clostridium*, *Desulfovibrio*, and *Enterobacteriaceae* are more abundant in children with ASD and their siblings than in healthy children [22, 25]. The anaerobic *Desulfovibrio* bacterium, which is resistant to common antibiotics such as cephalosporins, is more common in people with ASD [22, 25]. A study of over 70 children with ASD showed a significant increase in the relative abundance of unidentified *Lachnospiraceae*, *Clostridiales*, *Erysipelotrichaceae*, *Dorea*, *Collinsella*, and *Lachnoclostridium*, and a decrease in the abundance of *Bacteroides*, *Faecalibacterium*, *Parasutterella*, and *Paraprevotella*. The presence of unidentified *Erysipelotrichaceae*, *Faecalibacterium*, and *Lachnospiraceae* was positively correlated with the severity of ASD [26]. In contrast, a meta-analysis of 18 other studies showed that children with ASD showed significantly higher numbers of *Bacteroides*, *Parabacteroides*, *Clostridium*, *Faecalibacterium*, and *Phascolarctobacterium*, and a lower proportion of *Coprococcus* and *Bifidobacterium* [27].

Autistic children underwent significantly more ear infections and received larger amounts of antibiotics than children tested in the control sample, which may lead to the hypertrophy of *Desulfovibrio* which have important virulent agents in their wall structure, i.e. lipopolysaccharide (LPS), involved in the pathogenesis of autistic social behavior.

Duodenal biopsies taken from patients with ASD with gastrointestinal problems showed an increased level of *Sutterella* that is associated with mucosal metabolism, and these data were confirmed by another feces study from children with ASD [28]. Because there is ample evidence of the numerous occurrences of ASD within individual families, it has been proposed that autistic children may transfer fecal microbiota to siblings or spouses, which is consistent with the development of autism in predisposed children [29].

The hypothesis of *Clostridium* as a potential risk factor for ASD was supported by a study in which children with regressive autism were treated with oral vancomycin for 6 weeks (an antibiotic used against the 1 type of *Clostridium*), which resulted in a significant improvement in both neurobehavioural and gastrointestinal symptoms. However, gastrointestinal and neurobehavioural symptoms began to gradually recur after the discontinuation of treatment, probably due to *Clostridium* spores which were vancomycin-resistant and could later develop into invasive forms [30, 31]. The level of environmental glyphosate pesticide in the environment can adversely affect the gut-brain axis and contribute to the pathogenesis of autism by changing the microbiome and producing toxins by *Clostridium* [32].

Fungal dysbiosis with autism spectrum disorders

Dysbiosis in ASD includes not only bacterial species but also yeast. *Candida albicans* of the gastrointestinal tract is twice as abundant in children with ASD than in healthy children and can release ammonia and other toxins, inducing autistic behavior [33]. Despite many studies, the specific microbiome range for people with ASD has still not been established. The microflora profiles differ depending on the age of the subjects and the section from which the samples were taken.

NEUROINFLAMMATION IN THE AUTISM SPECTRUM DISORDERS

Leaky gut syndrome

Many studies indicate a relationship between inflammation and immune dysfunction in children with ASD with associated gastrointestinal diseases [34]. The transcriptional profile of the ileum and colon tissues in children with ASD is similar to the profile of people with inflammatory bowel disease [35]. In addition, infiltration of lymphocytes, monocytes, eosinophilia, and natural killer cells has been observed in intestinal biopsy tissues from children with ASD, similar to children with immunodeficiency or food allergies [9].

Disruption of the intestinal epithelial barrier, which is physiologically responsible for controlling the passage of particles from the gastrointestinal tract through tight junctions, can lead to altered intestinal permeability [36]. Increased permeability of the intestinal epithelium facilitates the translocation of intestinal components such as gram-negative bacteria and LPS from the lumen to the mesenteric lymph and peripheral circulation. Damaged, inflamed, and therefore permeable epithelia are the main routes utilized by commensal bacteria to migrate to the bloodstream. In turn, the translocation of pro-inflammatory molecules across the intestinal barrier causes a low-grade systemic inflammatory response, which can alter the blood-brain barrier permeability [19]. Microbiota and its metabolites play a key role in maintaining the integrity of

the intestinal barrier, which is why dysbiosis in patients with ASD may increase its permeability [37]. This condition, called leaky gut syndrome, can allow bacteria, toxins, and metabolites to enter the bloodstream, activating the immune response and causing inflammation [36].

Neuroinflammation

An activated immune system releases inflammatory cytokines and chemokines that can modulate the CNS and contribute to the pathogenesis of autism by affecting early brain development [38]. Studies have shown the accumulation of advanced glycation end products in the brains of autistic people that can promote neuroinflammation, oxidative stress, and neuronal degeneration [39]. Moreover, astrocytes from children with ASD have a higher concentration of proinflammatory cytokines compared to control, which may contribute to the altered development of neurons and synapses in children with ASD [40]. Increased plasma inflammatory cytokines such as interleukins (IL): IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, tumor necrosis factor alpha (TNF-α), tumor necrosis factor beta (TNF-β), transforming growth factor beta (TGF-β), and interferon gamma (IFN-γ) and an excessive cellular immune response have been observed in children with ASD, and such a system may be associated with the severity of autistic neurobehavioral symptoms [41, 42].

GASTROINTESTINAL DISORDERS WITH AUTISM SPECTRUM DISORDERS

Constipation with autism spectrum disorders

Comorbidities, in particular gastrointestinal problems, are often seen in children with ASD. In a review of 144 studies [43] such symptoms were observed in 46.8% of patients on average. Patients with ASD with gastrointestinal symptoms have more problems with anxiety and other somatic complaints in addition to reduced social interactions compared to patients with ASD who do not have these symptoms [44]. It was found that constipation is the most common symptom observed in autistic children (22%) [43]. A relationship between rigid compulsive behavior and constipation has also been demonstrated [45]. In addition, gastrointestinal problems in autistic children lead to greater tantrums, aggressive behavior, and sleep disorders, which further aggravate autistic behaviors compared to children with ASD without gastric symptoms. It has been suggested that altered behaviors such as aggression, self-mutilation, and sleep disturbances seen in children with autism may be an expression of chronic abdominal pain and abdominal discomfort [30, 43]. Studies have shown that children with ASD have higher plasma zonulin levels than healthy children. Zonulin modulates intestinal barrier permeability and its increase appears to be associated with the severity of autistic symptoms [46].

Diarrhea with autism spectrum disorders

Autistic children show altered metabolism and absorption of disaccharides in the intestine [47]. It has been found that ileum disaccharidases such as lactase, maltase-glucoamylase and sucrase-isomaltase have lower levels of mRNA and therefore reduced gene expression. Sodium-dependent glucose cotransporter 1 and glucose transporter 2 actively transport glucose, galactose, and fructose across the basolateral mucosa of enterocytes. Autistic children show reduced mRNA levels of both hexose transporters in the ileum, which results in poor absorption in the small intestine and more monosaccharides and disaccharides entering the large intestine [44]. Therefore, the bacteria ferment low-molecular sugars, gaining and winning with bacteria that degrade polysaccharides, which leads to a changed microbiological composition in the digestive tract. Higher amounts of sugars in the large intestine can lead to osmotic diarrhea or can serve as substrates for gas production. Diarrhea and flatulence are symptoms observed in patients with ASD and correlate with the severity of autistic behavior [28, 43].

Other gastrointestinal disorders accompanying autism spectrum disorders

Patients with ASD have also exhibited other gastrointestinal problems such as nausea or vomiting, stool qualities or patterns (frequency, color, smell, presence of mucus), bloating, reflux or heartburn, food selectivity issues or allergies, soiling, incontinence or bedwetting, difficulty having a bowel movement, and colic [43].

THE ROLE OF BACTERIAL METABOLITES IN AUTISM SPECTRUM DISORDERS

Toxins of *Clostridium perfringens* and *Bifidobacterium difficile*

Intestinal bacteria produce toxins, metabolites, and co-metabolites that can cross the intestinal barrier and the blood-brain barrier, thus affecting the functioning of the intestines and brain, and, consequently, the occurrence of a characteristic behavioral pattern [48]. *Clostridium perfringens* bacteria, which are more common in children with ASD than in healthy children, produce toxins encoded by the *Clostridium perfringens* beta-2 (*Cpb2*) gene. The presence of *Clostridium perfringens* and its *Cpb2* gene is associated with gastrointestinal complications in ASD that may affect the severity of the disease [49]. Children with ASD have high levels of p-cresol and its metabolite sulfate p-cresol in feces and urine. They are phenolic compounds produced by *Bifidobacterium difficile*. Early exposure to p-cresol may contribute to intestinal infections and gastrointestinal disorders, which can translate into a change in behavior and impairment of the cognitive functions of young children with ASD [50].

Short chain fatty acids

Increased SCFA levels, including PA and acetic acid, have also been observed in children with ASD. These acids are the end products of the fermentation of undigested carbohydrates and take part in the pathogenesis of ASD [48]. The mechanisms by which SCFA affect the CNS include the systemic disorders of altered immunity, metabolism, gene expression, change in mitochondrial functions, and the epigenetic modulation

of ASD-associated genes [51]. Moreover, SCFA are capable of modulating blood-brain barrier permeability. It is known that SCFA are able to modulate gut permeability by upregulating tight junction proteins, which are also part of the blood-brain barrier. This conceivably raises the idea that barrier integrity of the gut and brain could be similarly affected by SCFA [52]. Propionic acid is produced by bacteria whose amount in the intestine has been associated with ASD (*Clostridia* and *Bacteroides*). This compound can modulate the synthesis and release of neurotransmitters, exhibit anti-inflammatory and antibacterial effects, and affect the metabolism of mitochondria and lipids, thus inducing autistic behavior as observed in the animal model [53]. Butyric acid (BA) is another endogenous SCFA produced by the enteric microbiome that modulates intestinal transmembrane transport and plays a role in mitochondrial function, stimulating oxidative phosphorylation and fatty acid oxidation [54]. Children with ASD also have disregulated free amino acid metabolism that comes from protein and peptide hydrolysis. Free amino acid concentration in fecal samples has been shown to be higher in children with autism than in healthy children, and these data are associated with the prevalence of proteolytic bacteria in children with autism [8].

Amino acids

Glutamate is an amino acid that acts as a neurotransmitter in the CNS. At the highest levels, it occurs in people with ASD and is involved in the etiopathogenesis of neurodevelopmental disorders [55]. Children with ASD excrete tryptophan, a 5-hydroxytryptophan (5-HT) precursor, and its degradation fragments in urine in larger amounts, which is also observed in other neuropsychiatric diseases [56]. These metabolic characteristics of ASD can be used in developing new diagnostic strategies as well as therapeutic options based on diet and restoration of physiological intestinal microbiota.

Recent studies point to the important role of the neurotransmitter serotonin as a link in the gut-brain axis in ASD. In addition, 5-HT has been shown to be involved in the development of both CNS and enteric nervous systems (ENS) [57]. It has long been known that hyperserotonemia is more common in ASD but has recently been shown to be associated with gastrointestinal symptoms [58]. Since almost the total amount of 5-HT in the blood is synthesized by enterochromaffin (EC), it is believed that its increased level in children with ASD results from excessive secretion in the gastrointestinal tract [57]. The causes of elevated serotonin levels in children with ASD seem to be both genetic and environmental. Recent studies indicate altered compositions of the microbiome in ASD mice that have been associated with gastrointestinal disorders and increased intestinal 5-HT production, which confirms its association with dysbiosis [59]. Due to the ongoing inflammation in the intestines, EC, mast cells, and platelets are stimulated to produce serotonin, which leads to intestinal dysfunction and tryptophan consumption [1, 60]. However, tryptophan is a precursor to many metabolites, especially kynurenine and serotonin. Consequently, less tryptophan is available in the synthesis of serotonin in the brain, which may explain ASD-related behaviors and cognitive impairment, as a reduction in the amount of tryptophan in the diet appears to worsen autistic behavior. Dysbiosis can directly affect the availability of tryptophan for the host by reducing the number of amino acids absorbed from the diet [1, 61].

MITOCHONDRIAL DYSFUNCTION

Dysbiosis and mitochondrial dysfunction

Mitochondrial disorders have been detected in children with ASD compared to rectal and cecum intestinal mucosa samples from children with Crohn's disease (representing non-autistic children with gastrointestinal symptoms) and healthy children [62]. Children with ASD showed lower activity of electron transport chain (ETC) IV and citrate synthase in mucosal samples from both the rectum and the cecum. In addition, a much larger amount of the ETC I complex has also been found in the mucous membrane of the cecum. The amounts of ETC enzyme belonging to complexes III, IV, and V were only significantly increased in the cecum. It is interesting that these changes occur only in the cecum and not in the anus. In addition to many other functions, the rectum acts as a passage while the cecum is inhabited by many bacterial species. Studies indicate that altered expression of those enzymes that are involved in energy production leads to mitochondrial dysfunction. Mitochondrial dysfunctions disrupt the normal functioning of enterocytes and therefore cause intestinal disorders and greater sensitivity to oxidative stress [44]. Increased oxidative stress causes damage to proteins and lipids in the cell and, consequently, weakens enterocyte function. This suggests that bacterial metabolites may affect mitochondrial function in the cecum. S*hort chain fatty acids* produced by *Clostridium species* can enter mitochondria and be used as substrates for energy production. Butyrate is converted to acetyl coenzyme A, which is used in the lemon cycle to produce nicotinamide adenine dinucleotide (NADH). In turn, NADH is a substrate for the ETC I complex, which may be a factor increasing its activity. In addition, the intestinal disorder caused by mitochondrial dysfunction would explain constipation observed in people with autism [62].

The occurrence of many gastrointestinal problems seen in children with autism can be partly explained by intestinal disorders. Interestingly, problems similar to those in children with autism have also been reported in children with mitochondrial dysfunction, which again suggests a link between mitochondrial dysfunction, gastrointestinal problems, and microbiota in people with ASD.

GENE EXPRESSION AND AUTISM SPECTRUM DISORDER-SUSCEPTIBLE GENES

Susceptible genes of autism spectrum disorders

The first common mechanism by which intestinal microflora and inflammation affect ASD neuronal origin is associated with the expression of genes and ASD-susceptible genes. Although

the specific role of genetics in the etiology of ASD is still unclear, there are more than 100 ASD-susceptible genes [63] that are associated with early brain development, synapse formation, brain connectivity, inflammation, and the markers of immunity and microglia [64]. These genes are influenced not only by their interactions with other genes but also by environmental factors [40].

The specific environmental impact on ASD-susceptible genes is due to factors such as pesticides, heavy metals, impurities, drugs, as well as neurotransmitters and hormones such as serotonin, dopamine, and noradrenaline, which selectively target many ASD-susceptible genes. Studies suggest that an increase in the incidence of ASD may be chemically induced in a gene-dependent manner. Many protein products of ASD-susceptible genes are involved in barriers such as the blood-brain barrier, and the skin or intestinal barriers. They can therefore affect the absorption, metabolism, and physiological effects of toxic environmental or endogenous factors [65].

Disorders of gene expression regulating the cell cycle, neurogenesis, and the digestive and immune systems have been observed in people with ASD [63]. Both studies of post mortem tissue gene expression in the brain and peripheral blood point to the varying expression of genes associated with the immune system in samples of individuals with ASD. Furthermore, the most important biological functions associated with the unique gene expression profile of children with gastrointestinal complaints and ASD are inflammatory diseases, the development and functioning of the endocrine system, and the development and functioning of the digestive system [40]. This suggests a link between inflammation and digestive problems in people with ASD experiencing gastrointestinal symptoms.

Relationships between genes, the gastrointestinal tract, and the immune system

Changed expression patterns of immune functions and genes of the digestive system in people with ASD can lead to the heterogeneity of the gastrointestinal tract and inflammatory diseases observed in people with ASD. There is evidence that intestinal microflora products and inflammatory cytokines can directly affect gene expression. As a result of epigenetic regulation of gene expression, microbial metabolites may increase cancer risk, whereas proinflammatory cytokines affect the expression of diabetes-related autoantigens [40]. The intestinal microbiota has the potential to combine genetic and environmental influences because its composition depends on both the genetic background and environmental factors [66].

Many environmental factors contributing to ASD coincide with changes in the immune response during prenatal or early postnatal development. Early encephalitis is a well-known risk factor for ASD, where the pathogenesis of ASD is associated with neuroinflammatory events in the developing brain that are affected by environmental factors, including maternal immune activation and intestinal microbiota [40]. Environmental toxins or endogenous factors, including inflammatory cytokines and gut microbiome products, may specifically target ASD-susceptible genes during prenatal and early postnatal development. This can affect the expression of genes involved in the development and functioning of the digestive and immune systems, thus causing continuous dysfunction in these systems.

Tensin homolog

One of the main genes associated with ASD, both in human and mouse models, is a phosphatase and tensin homolog (PTEN) [67, 68, 69]. Mutations in this gene are responsible for 5–17% of cases of autism. The current analysis shows that PTEN plays a crucial role in the mitochondrial biogenesis through the protein kinase B / glycogen synthase kinase-3 beta / peroxisome proliferator-activated receptor gamma coactivator 1-alpha signaling pathways (AKT/GSK-3β/PGC-1α). In the valproic acid induced autism mouse model, the PTEN protein level was significantly decreased while PGC-1α and cyclooxygenase IV levels were increased in the hippocampus and cortex [70]. A correlation between PTEN expression and mitochondrial dysfunction may be a potential mechanism of ASD.

Chromodomain helicase DNA binding protein

The literature data suggest a possible role of selected genetic factors in revealing both the phenotype of ASD and the severity of gastrointestinal problems in patients with ASD. The ENS plays a key role in controlling gastrointestinal function, therefore structural or functional modifications of the ENS may underlie gastrointestinal dysfunction in ASD [71]. In this context, an important role may be played by the gene encoding the chromodomain helicase DNA binding protein 8 (CHD8). Mutations of *CHD8* have been identified in ASD patients with gastrointestinal disorders characterized by periods of severe constipation followed by loose stools or diarrhea. The described phenotype is probably associated with impaired intestinal peristalsis resulting from a reduction in post-mitotic intestinal neurons [72]. These studies suggest the involvement of CHD8 in brain development and intestinal innervation.

Nitric oxide synthase

Another potential genetic predictor of the ASD phenotype is the nitric oxide synthase 1 (*NOS1*) gene, which encodes the neuronal nitric oxide synthase (nNOS) protein. Mutations in this gene can impair nitrogen transmission, which is strongly associated with esophageal achalasia in infants. Analysis of nNOS in autistic patients has revealed a mutation at position Y1202X, which causes termination of translation by premature codon STOP and impairs nitric oxide production. This mutation also causes cofactor binding by this truncated protein, leading to gastrointestinal motility disorders [73].

Neuroligin 3

There have also been reports on the role of genes encoding neuroligins in the genetic background of ASD. The R451C missense mutation in the neuroligin 3 (*Nlgn3*) gene may determine faster passage in the small intestine and colonic motility change, which has been described in the genetic animal models of ASD. These results suggest that the gut dysfunction of ASD patients could be due to mutations that affect neuronal communication [74].

Metalloproteases

Also noteworthy are the genes encoding the disintegrin and metalloproteases ADAM metallopeptidase domain 10 i metalloproteases ADAM metallopeptidase domain 17, widely expressed in the brain and intestines. ADAM10 and ADAM17 influence pathways that regulate intestinal permeability, homeostasis, and inflammation. These enzymes may be involved in microbiota-gut-brain interactions in the course of ASD by regulating immune and inflammatory responses in the gastrointestinal tract. Moreover, it seems likely that gut microbes can regulate ADAM10 or ADAM17 activity by producing bacterial metabolites, and at the same time ADAM10 or ADAM17 can alter the composition of the gut microbiome in ASD patients [75].

APPLICATION OF PROBIOTICS IN AUTISM SPECTRUM DISORDERS

Probiotics

The definition of probiotics has evolved from a live active culture which improves the balance of the gut microbiota composition to specific effects, in particular, the immunomodulatory potential of clearly defined strains [76]. Given the similarities in the gastrointestinal symptoms of irritable bowel syndrome (IBS) and ASD patients, as well as recent successes in the treatment of IBS with probiotics, one of the positive effects of using probiotics in people with ASD may be the improvement of gastrointestinal symptoms such as bloating, difficulty in defecation, and stomach pain [40, 77]. *Clostridium species* are elevated in children with ASD, and their high levels are associated with digestive problems. Treatment with a *Clostridium difficile*-targeted drug improves behavior and communication in children with ASD during treatment, but not after treatment discontinuation. This means that probiotics have a positive effect on maintaining the proper microbiological balance in the intestines. Increased intestinal permeability in ASD can be alleviated by probiotics, which are able to stabilize the mucosal barrier by reducing bacterial overgrowth, antioxidant synthesis, increasing mucin expression, and stimulating mucin immunity. Short-term exposure to probiotics improves the accuracy of the intestinal mucosal barrier [40]. The use of a probiotic containing 8 different bacterial strains (*Streptococcus thermophilus*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus paracasei*, *Lactobacillus delbrueckii subspecies bulgaricus*) also had a beneficial effect on the symptoms of the gastrointestinal tract, sensory functioning, and ASD profiles in children [77]. Probiotics can modulate the immune system in a species- and strain-specific manner through anti-inflammatory effects, effects on systemic cytokines, and interactions with other intestinal microbiota [78]. Mouse studies have shown that the offspring of immunologically activated mothers show increased intestinal permeability, abnormal cytokine levels and dysbiosis due to changes in *Clostridia* and *Bacteroidia*. Treatment of mice with probiotic *Bacteroides fragilis* corrected intestinal permeability in their offspring and restored IL-6 pro-inflammatory cytokine growth in the colon, but not other cytokines, revealing specificity for IL-6. The probiotic improved the communication, repetitive, sensorimotor, and anxiety behavioral disorders of the offspring of autistic mice [40].

Postbiotics

Butyric acid is one of the SCFA and is one of postbiotics – functional bioactive compounds generated in a matrix during fermentation that may be used to promote health [79]. Butyric acid is the basic source of energy for colonocytes. Its administration has therapeutic effects in gastrointestinal diseases, hepatic diseases, as well as heart diseases and several neurological conditions, such as dementia and depression, due to the normalization of physiological stress pathways [48]. Supplementation with BA has a positive effect on mitochondrial dysfunction observed in healthy and autistic children under the influence of physiological stress [34].

CONCLUSIONS

This review has shown that ASD is a complicated disease whose development depends on many factors. A better understanding of the relations between ASD and the gut microbiome could lead to new interesting perspectives in diagnosis and therapy research.

The probiotic and postbiotic treatment can help fight ASD symptoms by reducing inflammation, improving intestinal permeability, and restoring microbial imbalances. However, the exact mechanisms have not yet been fully explained and require further in-depth research.

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