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Potential Effect of Probiotics on the Modulating of Gut Microbiota in Autism Spectrum Disorders (ASD)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors WA and MMA managed the literature searches and wrote the draft manuscript. Author GAM edited the manuscript and finalized the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Microbiota is the summation of all microorganisms living in the body. The alteration in microbiota can lead to chronic diseases, however; colonization with different commensal bacteria can correct these deficits. Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by inadequate communication skills and social withdrawal and its etiology is uncertain. Typical gastrointestinal (GI) disorders symptoms are associated with ASD, in a prevalence range from 23% to 70%. The method of communication between the brain and the gut microbiota is likely the microbiota-gut-brain axis. Therefore, intervention studies have been published based on the use of prebiotics, probiotics and fecal microbiota transplantation (FMT). In this review, the possible correlation between gut microbiota and ASD is demonstrated. Additionally, how probiotics and microbial fecal microbiota transplantation (FMT) could modulate the gut microbiota and might represent a potential therapy for patients with ASD. Nearly all the GI functions postulated to be affected in ASD are improved by probiotics in animal studies. (FMT) ensures the transfer of several hundred bacterial strains, as opposed to probiotic therapy where only certain bacterial strains are supplemented. For ASD patients with dysbiosis, FMT is an interesting new therapeutic choice that could be considered.

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1. INTRODUCTION

The term microbiota describes the entirety of all microorganisms, including bacterial, archaeal and fungal, residing in the skin, mouth, respiratory, gastrointestinal (GI) and vaginal tracts [1]. In the large intestine, the greatest variety of microbiota is located [2]. By educating the immune system, defending against pathogenic bacteria, producing antimicrobial substances, and by the increased mucin production significantly strengthening the intestinal barrier, healthy intestinal microbiota interacts with human metabolism [3,4]. Factors that have been shown to affect the microbial composition are age, diet, diseases, geography and shared environment [5]. Approximately 1000 different bacteria species are reported to live in the GI tract [6]. Initial colonization usually starts at birth through the acquisition of maternal microbiota during vaginal delivery, although recent research shows that maternal microbiota may be acquired during birth7Infant microbiota supports breastmilk that is high in human oligosaccharides; however, the composition of early-life microbiota can be altered by delivery procedures, hygiene, and feeding practices such as formula feeding [7]. Initially, the healthy infant gut is dominated by Bifidobacterium and Lactobacillus; however, during weaning and food initiation, it is unstable for the first few years of life, then stabilizes to a more "adult-like" composition around age 3 [7]. In the healthy adult intestine, Firmicutes and Bacteroidetes are the dominant bacterial phyla, with a smaller proportion of the microbiota consisting of *Actinobacteria*, *Proteobacteria* and *Verrucomicrobia* [8]. The abundance of bacteria in the (GI) tract ranges from around colony-forming units (CFU) per gram measured in ileum to CFU / g measured in faeces [9], with the prevalent bacterial phyla Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria and Fusobacteria found in the mammalian GI tract [10].

Over the past decade, knowledge of the microbiota and its significance to health has flourished, and now it is understood that loss or alteration of microbiota can lead to chronic diseases [11,12]. Antibiotic therapy, dietary changes, immune disorders, and stress will cause microbiota disturbances7, and this could affect the gut balance between beneficial commensals and potentially pathogenic microbes. This disrupted balance is called dysbiosis [13]. However, these deficits can be reversed by colonization with various commensal bacteria, but this is age-dependent, suggesting that both the composition and timing of colonization are important for the instruction of the immune system [14–16]. Researchers have been able to investigate microbial communities in-depth and have made it possible to study human microbiota through improvements in essential molecular approaches. For example, DNA sequence data can now be obtained in a single reaction simultaneously from entire microbial communities using next-generation sequencing (NGS) technologies, which is the term that describes the collective approaches used to obtain parallel sequence data, at a fraction of the cost compared to conventional sequencing methods. Gut microbiota (GM) is currently primarily characterized by culture-independent techniques such as massive polymerase chain reaction (PCR) sequencing of 16S ribosomal RNA genes, as this allows easy identification of a wide proportion and diversity of bacteria and provides rapid results [17,18].

2. AUTISM SPECTRUM DISORDER (ASD)

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that occurs in the first years of life and is characterized by stereotyped expression and deficiencies in social communication. ASD is especially heterogeneous and its etiology is unclear. Previous research has shown many potential causes of this disorder, such as genetic disorders, dysregulation of the immune system, inflammation, and environmental factors [19–22]. There has been a marked increase in the incidence and prevalence of ASD in recent decades [23], and the disease affects more male than female individuals [24,25]. Autistic behavior is marked by communication difficulties, social withdrawal, repetitive or restrictive behavioral patterns, interests and activities, and the onset of these psychological features at the early developmental stage [26]. A subgroup of patients with initially normal development but with a gradual loss of acquired speech or social interaction abilities is identified as progressive autism or late onset autism4. Lastly, abnormal eating habits are commonly reported in autistic people, potentially leading to deficiencies in vitamins, minerals and fatty acids (Fig. 1) [27]. Early clinical treatment may alter the course of

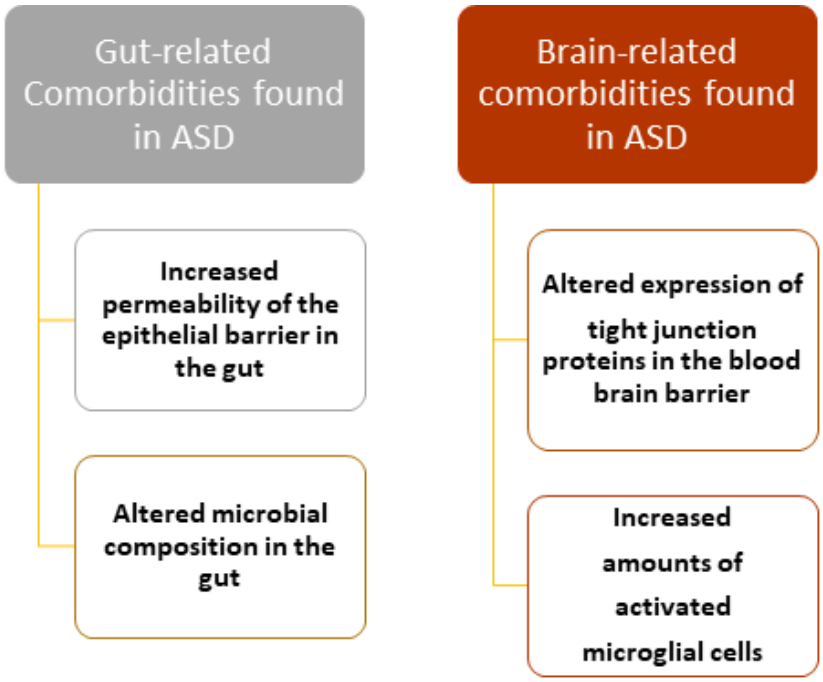

Fig. 1. Co-existing pathologies found in Autism Spectrum disorder (ASD). Left bar shows gut-related comorbidities found in ASD, right bar brain-related comorbidities. Figure adapted from Srikantha et al. [42]

symptoms, but it does not completely eradicate the symptoms of ASD, and pharmacological treatments are limited. Genetic factors account for about 10-20% of cases of ASD [28], leaving us with uncertainties about what could cause the etiology of these disorders. Air pollution, pesticide use, maternal diseases and/or inflammatory disorders or antibiotics during pregnancy are environmental factors that have been implicated in an increased risk of having a child with ASD [29,30]. ASD has a significant influence on the growth of children and on society. In 2012, The estimated prevalence of ASD was 14.6 per 1,000 children 8 years of age [22] and in the United States, the prevalence of ASD was one in fifty-nine children in 2014 [22,31]. The cost of caring for a child with ASD but without an intellectual disability is £0.92 million in the United Kingdom and $1.4 million in the United States. The heritability of ASD and autistic disorder among Swedish children was about 50%, confirming that both genetic and environmental factors play an important role in ASD development [32,33]. (GI) disorders, including constipation, abdominal pain, gaseousness, diarrhea, and flatulence, in a prevalence vary from 23% to 70% 20 [34–36], are common symptoms associated with ASD (Fig. 1). Although there is no conclusive evidence that there is a cause-effect relationship between GI symptoms and ASD, studies have shown that the gut plays an important role in the etiology of ASD [37]. Constipation has been identified as the most common symptom (85%) in children with ASD, according to parental reports and evaluations by pediatric gastroenterologists in a study by Gorrindo et al. [38]. The microbiota-gut-brain axis is likely the method of communication between the brain and the gut microbiota. Accumulating evidence suggests that the gut microbiota is associated with symptoms of ASD directly or indirectly, in part by affecting the immune system and metabolism [39-41]. The potential connection between the gut microbiota and ASD will be demonstrated in this review. Furthermore, how the intestinal microbiota could be modulated by probiotics and fecal microbiota transplantation (FMT) and may constitute a possible therapy for ASD patients.

3. CHANGES IN GI OF ASD

There is evidence that similar relationships exist between the microbiota, gut, and brain, and that cross-communication occurs frequently. For example, activities of the central nervous system

(CNS) influence the composition of the gut microbiome through peptides that are sent upon satiation and thus affect nutrient availability. In another scenario, the hypothalamic-pituitary-adrenal (HPA) axis releases cortisol, which controls intestinal motility and integrity. Mucin secretion from intestinal epithelial cells is mediated by immune and neural pathways and is known to maintain microbial communities in the gut [43]. In the other direction, the gut microbiota has been shown to control CNS functions across a number of neuronal, endocrine, immune and metabolic mechanisms [43]. The gut is thus considered a "second brain" (Fig. 2). Due to a large proportion of children with ASD have (GI) symptoms that may be related to irritable bowel syndrome (IBS) symptoms, the presumed correlation between ASD and microbiota may be partially due to recurrent GI symptomatology observations [44] including diarrhea, constipation, vomiting, reflux, stomach pain, flatus, and unusually foul-smelling stools [45]. Additionally, clinical trials have reported anomalies such as altered (GI) motility and increased intestinal permeability [46]. As the largest surface in the body, the (GI) contains trillions of intestinal barrier-separated microorganisms [47]. The intestinal barrier consists of the microbiota of the commensal gut, a mucus layer and epithelial cells connected by tight junctions [48,49]. The greater abundance of Clostridia species (spp) in autistic individuals indicates involvement in the pathogenesis of ASD [50]. A recent multicenter study of more than 14,000 ASD patients found a higher prevalence of inflammatory bowel disease (IBD) and other (GI) disorders compared to controls in ASD patients [51]. However, the exact prevalence of GI symptoms in children with autism is not clear, with previous estimates ranging widely from 9% to 70% [44]. These variations may be due to differences in the populations of the sample and GI symptomatology concept. What is not in doubt is that GI symptoms are a major issue in autism and may lead substantially to behavioral problems [44].

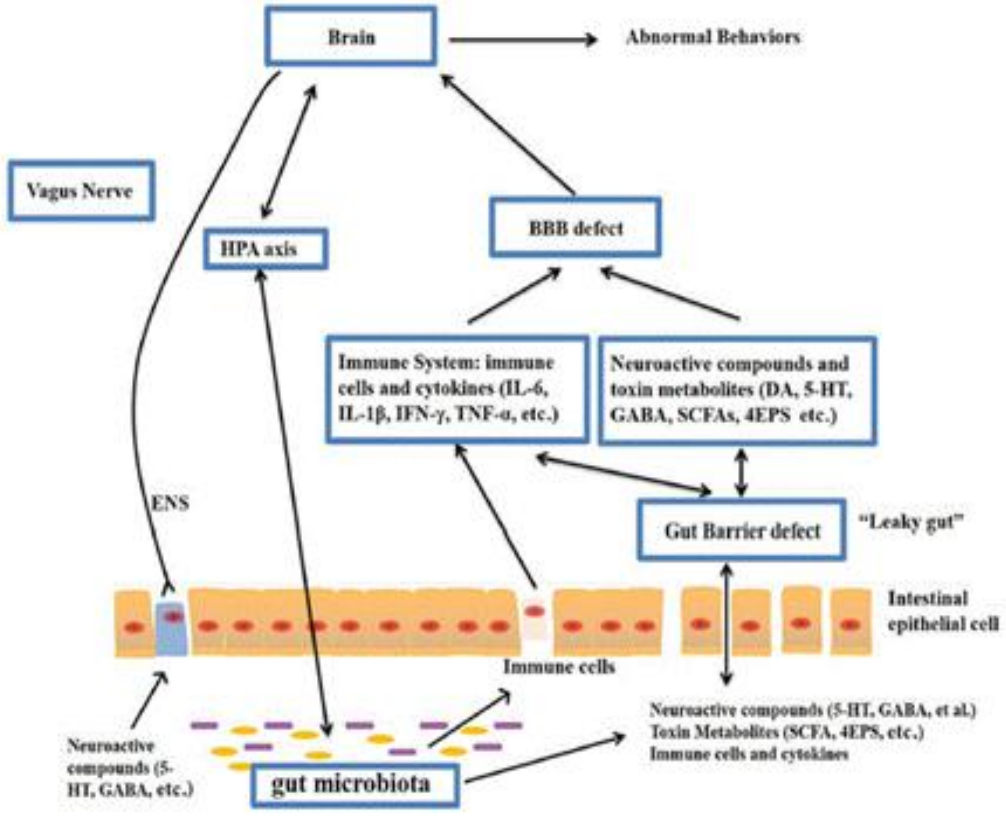

Fig. 2. Potential relationships between the microbiota and ASD (the gut-brain axis). Figure adapted from Li et al. [52]

4. GUT PERMEABILITY

Increased intestinal permeability was found in patients with ASD when lactulose was measured in the blood [3] however, the intestinal permeability increased in autistic children and their non-autistic siblings relative to controls after oral administration [53]. Increased gut permeability is likely to result from the reduced expression of barrier-forming proteins and the increased expression of pore-forming tight junction proteins. The examination of autistic children's mucosal samples from the small intestine compared to controls found these altered expressions [54]. Permeability variations in autistic patients may lead to lower Lactobacillus strain counts since they are related to the preservation of a tight junction in the intestinal epithelial barrier [3]. By changing cytokine levels, bacterial metabolites such as lipopolysaccharide (LPS), which is part of the gram-negative bacteria cell wall, can easily pass through the intestinal barrier and cause brain inflammation and is associated with impaired social behavior scores [55,56]. Increased intestinal permeability was also observed in some close non-autistic relatives of autistic individuals, suggesting that intestinal integrity is not a consequence of ASD. Instead, increased intestinal permeability should be considered a cause that may lead, at least in part, to ASD pathology in addition with other environmental factors [57]. Microbiota and their ligands are crucial in maintaining the cell-cell junctions necessary to barrier integrity, with GI barrier defects seen with dysbiosis [58]. The compromised integrity of the epithelial barrier has been termed a "leaky gut" and has been correlated to a wide range of intestinal and systemic disorders [59]. Furthermore, increased intestinal permeability may enable the passage of bacteria, toxins, and metabolites and can lead to immune system activation. A higher percentage of abnormal intestinal permeability was reported in 36.7% of ASD patients and their relatives (21.2%) compared to control children (4.8%) [46]. Fiorentino et al., found that the integrity of both the gut barrier and the blood-brain-barrier (BBB) were compromised in ASD individuals, as demonstrated by the increased levels of claudin (CLDN)-5, CLDN-12, CLDN-3, and MMP-9 in the brain of ASD patients and decreased levels of intestinal tight junction components (CLDN-1, OCLN, TRIC) in ASD individuals compared with controls [54]. In Hsiao et al. study, intestinal permeability was treated, and microbial composition was altered by oral administration of the commensal microbe Bacteroides fragilis in MIA mice, showing improvement in autistic symptoms as well as improvement in anxiety-like behavioral deficits. Interestingly, altered expression in the colon of the CLDN-8 and CLDN-15 tight junction proteins has also been reversed by probiotics. The authors demonstrated convincingly that 4-ethylphenylsulfate serum levels in MIA mice were corrected by oral administration of *Bacteroides fragilis* [58]. Collectively, these data have led to the hypothesis that ASD is a system-level condition that affects the gut and immune systems, in addition to the brain.

5. PROBIOTICS

Although the study GM in ASD is relatively new, intervention studies focused on the use of prebiotics, probiotics and fecal microbiota transplantation (FMT) have been reported. There are very few studies, however, that have studied the impact of probiotic administration on ASD symptoms and emotional symptoms. Probiotics contain living microorganisms and are administered in order to promote health by enhancing immunity, reinforcing the intestinal barrier, increasing mucin expression, minimizing pathogen overgrowth and producing vitamins and antioxidants [55,60]. Synbiotics are the possibly synergistic combinations of pro-and prebiotics [61]. The most commonly used probiotics are *Bifidobacterium* spp and Lactobacillus spp. [57]. Administration of the 'Children Dophilus' probiotic mixture containing strains of *Lactobacillus*, *Bifidobacterium* and *Streptococcus* three times a day for four months improved the Bacteroidetes/Firmicutes ratio to more Bacteroidetes [62]. Moreover, in autistic children, the abundance of *Desulfovibrio* and *Bifidobacterium* genera has also increased. Lactobacillus reuteri will, on its own, reverse inflammation of the intestine caused by LPS [63]. The posterior pituitary gland was impacted by the bacterium Lactobacillus reuteri to produce more oxytocin. Oxytocin is a hypothalamic hormone, which positively influences social behavior. It remains to be determined whether increased oxytocin levels in autistic children in a well-designed experimental setting will improve their behavior in social interactions [64]. In a four-week, randomized, double-blind, placebo-controlled study, the effects of Lactobacillus plantarum PS128 on boys with autism spectrum disorder (ASD) aged 7–15 in Taiwan were evaluated. The results showed that (PS128) strengthened opposition/defiance behaviors and

that the overall Swanson, Nolan, and Pelham-IV-Taiwan version (SNAP-IV) score for younger children (aged 7-12) improved substantially compared to the placebo group [65-68]. Another study, which investigated GI symptoms of autistic children before and after 3 months of probiotics with nutritional formula supplementation, each gram, contained 100 x colony forming units of three probiotic strains namely, (*Lactobacillus acidophilus*, *Lactobacillus rhamnosus* and *Bifidobacteria longum*). In autistic children, the stool PCR reported an improvement in the colony counts of Bifidobacteria and Lactobacilli after probiotic supplementation, with a significant decrease in body weight and a significant improvement in the incidence of autism (assessed by the ATEC) and gastrointestinal symptoms (assessed by the 6-GSI) [69]. It is noted that the microbial composition in the intestines of autistic children is enhanced by probiotics. Experiment results of positive behavioral changes seen in MIA mice after administration of Bacteroides fragilis raise a significant question as to whether the same result applies to humans as well [70]. Nearly all the GI functions postulated to be impaired by ASD have been shown to be improved by probiotics in animal studies. In a model of necrotizing enterocolitis [71] in newborn rat pups, another probiotic, *Bifidobacterium bifidum*, reduced intestinal permeability via tight junctions that seal together the epithelial cells. In order to further investigate abnormal behavior processes in autism, a recent study by Buffington et al [72] used a high-fat maternal diet to induce abnormal social (withdrawal) behavior in the offspring. It is should be noted that maternal obesity [73,74], and maternal diabetes [75] have also been shown to be correlated to autism in offspring in humans. A case-control study with a high risk of selection bias that showed improvements in mental (but not behavioral) concentration in *Lactobacillus acidophilus* treated patients with ASD [76]. A double-blind, placebo-controlled crossover study showed a reduction in aggressive behavior, anxiety, and communication impairment in children during probiotic use (*Lactobacillus plantarum*) [77]. Additional study reported that the 4-month combination probiotic therapy (consisting of 3 species of Lactobacilli, 2 species of Bifidobacilli and 1 species of Streptococcus) showed beneficial effects. The probiotic increased the ratio of fecal Bifidobacilli to Firmicutes and total qPCR measured Lactobacilli, thereby reducing the alpha levels of fecal Clostridia and fecal necrosis factor (TNF), indicating beneficial effects on the microbiota, although there were no reports of effects on autistic behaviors of this combination of probiotics [62]. The probiotic/prebiotic can normalize the gut microbiota in animal models or ASD patients, strengthen the gut barrier, and alleviate behaviors similar to ASD. The *Bacteroidetes*/*Firmicutes* ratio is normalized by supplementation with a probiotic containing *Lactobacillus*, *Bifidobacteria* and *Streptococcus,* and the amounts of *Desulfovibrio* spp. and *Bifidobacterium* spp. Similar to those found in samples from their non-autistic siblings or unrelated heathy controls in the feces of children with ASD. They also found that the limited/repetitive activity subscale score for the Autism Diagnostic Interview (ADI) was correlated with the amount of *Desulfovibrio* spp. [62]. However, following probiotic therapy, the authors did not determine the alteration of ASD actions. A cohort study found that oral supplementation with Lactobacillus acidophilus twice daily for 2 months decreased urinary D-arabinitol levels in children with ASD and improved their ability to follow directions. As seen in comparison with data obtained prior to therapy [76]. A case study showed that an ASD boy with severe cognitive disability was treated with VSL #3 for 4 weeks (a multi-strain mixture of 10 probiotics). The therapy improved the GI symptoms and enhanced the autism core symptoms [78].

6. FECAL MICROBIOTA TRANSPLANTATION

Fecal microbiota transplantation (FMT) ensures the transfer of several hundred bacterial strains, as opposed to probiotic therapy where only certain bacterial strains are supplemented [79]. A healthy person donates a stool sample 75, which is usually used fresh after donation within eight hours or within eight weeks when frozen directly [80]. (FMT) for recurrent Clostridium diffcile infection is a proven and effective therapy [79,81–83]. Microbiota transfer therapy (MTT) is a modified (FMT) treatment consisting of 14 days of antibiotic therapy followed by intestinal cleaning and 7-8 weeks of high initial dose administration of standardized human gut microbiota (SHGM). An open-label clinical trial showed that both GI symptoms (e.g., constipation, diarrhea, indigestion, and abdominal pain) and ASD-related symptoms were improved by (MTT) and that the ASD patients' microbiota was balanced [79]. Kang et al examined the influence of MTT on the microbial composition and course of GI and

autistic symptoms in eighteen autistic children [79]. After oral administration of vancomycin and bowel cleaning for 7-8 weeks, the transition occurred. The transition occurred after oral administration of vancomycin and bowel cleaning over a duration of 7-8 weeks. The findings were remarkable as GI symptoms such as constipation, diarrhea or abdominal pain decreased by 80%. The improvement persisted for at least eight weeks after. This study clearly shows an increase in the behavioral symptoms of not only GI but also seventeen ASD symptoms assessed by the "Parental Global Impressions-III" [79]. *Bacteroides fragilis* and *Bacteroides vulgatusin* were found to be more abundant in ASD. Therefore, in a randomized, placebo-controlled study of 24 ASD-children and 24 placebo-children, MTT was treated for 4 months under anesthesia by colonoscopy and gastroscopy. MTT treatment has been shown to continuously shift the GM of ASD patients to a stable status and decrease the abundance of *Bacteroides fragilis* [84].

FMT has been extended to the treatment of IBD and IBS based on the speculation that FMT will normalize gut microbiota in patients with IBD and IBS and minimize symptoms of constipation (100%) [85,86]. Accordingly, researchers are increasingly interested in the use of FMT to treat children with ASD. In the Nayeon Goo et al. study [87], it was hypothesized that an increase in specific gut microbiota through (FMT) will minimize autistic behaviors. The effects of FMT from normal mice to Fmr1 KO mice on autistic-like behaviors were tested using several behavioral experiments. Since the totals of A. muciniphila in Fmr1 KO mice were very low, the population of A. muciniphila was assessed, the expression of MUC2 was analyzed, and goblet cells in the gut were evaluated after treatment. FMT has been shown to enhance autistic-like behaviors, particularly memory deficits and social withdrawal, and A. muciniphila levels have been normalized to wild-type levels. Additionally, FMT reduced the increased levels of TNFα and Iba1 in the brains of Fmr1 KO mice. These results suggest that FMT could be a promising treatment tool for the symptoms of cognitive deficits and social withdrawal observed in ASD. However, the protection of FMT should be considered. FMT's possible adverse events include diarrhea, stomach cramps, short-term belching, mild abdominal discomfort / bloating, and low-grade intermittent fever [88]. In addition, donors may pass on opportunistic pathogens or infections to beneficiaries. A thorough pre-donation screening of donors minimizes the risk, although donors can be asymptomatic and spread infections unknowingly in the early stages of infection [89]. Despite these uncertainties, the main consequence of fecal transplantation was increased bacterial diversity and altered abundances of some bacteria, such as the genera *Bifidobacterium*, *Prevotella* and *Desulfovibrio*. This suggests that in autistic children, GI and behavioral symptoms are related and are likely to be traced back to lower microbial diversity by metabolite modulation [79]. Therefore, FMT is an interesting new therapeutic choice that should be considered for ASD patients with dysbiosis.

7. CONCLUSION AND FUTURE PERSPECTIVE

In this review, the evidence from several studies that an abnormal gut microbiota is associated with ASD was summarized. First, the relationship between the microbiota of the intestine and the ASD was reviewed. Second, the role of the gut microbiota in ASD was described. Finally, several potential therapies have been identified in patients with ASD to modulate the gut microbiota. Several recent clinical trials have shown that the symptoms of ASD are enhanced by therapies that control the gut microbiota. Various epidemiological, experimental human and in vivo studies were presented on the role of different microbes in the pathology of ASD, such as *Clostridia* spp, highlighting the evidence that several metabolites should be considered as possible biomarkers for ASD. In conclusion, additional microbiota-gut-brain-axis experiments aimed at an in-depth assessment of processes leading to autism pathology warrant the correlation between changes in various bacterial populations and behavioral changes linked to ASD. To further narrow down the mechanisms of disease, studies with larger sample sizes are required, as the studies mentioned in this review showed varying degrees of statistical significance and some results are based on small sample sizes. Wide ranges of methodologies and outcome measures have been developed, calling for standardization of treatment regimens with an aim of achieving to achieve greater statistical significance and better comparison of the results of the research. Additional independent research implementing standardized protocols and enrolling well-diagnosed larger cohorts is urgently needed to discover the interdependencies, the pattern of events leading to ASD, and to suggest unambiguous biomarkers

and effective therapeutic strategies. Selection of appropriate strains, dosage and timing of treatment are essential considerations that should be considered [90]. Due to the complexities of regulating the variability of the intestinal microbiome and the remaining questions about the complicated mechanisms of action, there is an inevitable limit to setting criteria for FMT. Nevertheless, well-designed, randomized, placebo-controlled clinical trials are needed to assess the efficacy of probiotics and microbial transfer therapies in the treatment of ASD.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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