Chapter

Could Gut Modulation through Probiotic Supplementation Be Beneficial in Autism Spectrum Disorder?

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Abstract

Evidence is mounting to a possible link between autism spectrum disorder (ASD) and gut microbiota through the well-known gut-brain axis. Numerous mechanisms have been suggested including bacterial metabolites that could involve in chemokines, antimicrobial peptides, or neuropeptides production. Hence, numerous studies reported dysbiosis in autistic patients. Antibiotic courses are known to more or less improve neurobehavioral symptoms; however, it could lead to side effects. Modulation of the gut microbiota using pro- and/or prebiotics is therefore an appealing way of treatment. Fecal microbiota transfer is suggested to be an alternative new approach that could be promising. The aim of our chapter will be first to briefly review the current data concerning the possible role of the gut microbiota and its mechanisms in ASD and second to review the interest and limits of the pre- and probiotic supplementations in ASD treatment. Lastly, we will discuss on the potential interest of the microbiota transfer in ASD.

Keywords: autism spectrum disorder, gut microbiota, dysbiosis, probiotics, prebiotics, fecal microbiota transplantation

1. The human microbiota

1.1 Definition and functions

The term microbiota describes the entirety of all bacterial, viral, fungal, protozoal, and archaeal microorganisms living on almost every cutaneous and mucosal surface of the body [1]. The gut microbiota (GM) inhabited by several trillion microorganisms that live in a symbiotic relationship with the host represents the most heavily colonized area of the human body. It is mainly dominated by organisms belonging to four major phyla that together account for more than 90% of the total bacterial population: *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, and *Actinobacteria*, followed by the minor phyla *Fusobacteria* and *Verrucomicrobia*. With a number of microorganisms being around 10 times higher than the number of human cells and a number of genes nearly 150 times greater than the human genome, the GM is now recognized as an environmental factor that affects normal host physiology, metabolism, immunity, brain function, and behavior.

During recent decades, the development of high-throughput sequencing technology has hugely contributed to increased understanding of host-microbe interactions in health and disease [2]. First, GM offers a barrier, protecting against external factors and proliferation of pathogenic microbes, through various mechanisms such as increasing mucus production, reinforcing intestinal epithelium permeability, and production of bacteriocins and antimicrobial peptides. GM is also involved in many fundamental metabolic functions such as synthesis of essential nutrients, hormones, vitamins, supply of energy from dietary sources otherwise unavailable to host and clearance of drugs, and toxins. Furthermore, GM is shown to be involved in the maturation of the host immune system, where specific strains such as *Bifidobacterium* and *Lactobacillus* produce anti-inflammatory cytokines, and others such as *Clostridium* and *Ruminococcus* produce pro-inflammatory cytokines. Hence, GM is essential for the development of innate and acquired immunity through stimulation of local and systemic immune responses.

Lastly, very recently there is mounting evidence of the significant influence of GM in the modulation of brain activity and behavior across the so-called "microbiota-gut-brain axis (GBA)" [3]. The GBA consists of bidirectional communication between the central nervous system (CNS), the enteric nervous system, and the gut linking emotional and cognitive centers of the brain with peripheral intestinal functions [4]. The exact mechanisms of signal transmission within this network are not completely elucidated. The CNS asserts its role over the GM through influencing gut motility patterns, altering the equilibrium in the gut permeability, and modulating mucus secretion which are known to exert control over gut microbial composition [5]. Conversely, the GM claims its influence over the CNS by regulating the hypothalamic-pituitary-adrenal axis and the production and turnover of cytokines and neurotransmitters. In addition to their effects on development and maturation of the enteric nervous system, these neuroactive metabolites can signal beyond the local gastrointestinal (GI) tract to the distant CNS potentially through signaling pathways that include the vagus nerve. Catecholamines can modulate important processes, including neurogenesis, myelination, microglia activation, brain plasticity, and blood-brain barrier permeability [6]. Indeed, recent studies demonstrated that, under extreme conditions (e.g., in a germ-free environment or during antibiotic (ATB) treatment), GM absence is associated with several abnormalities in brain gene expression and neurophysiology [7]. Interestingly, these aberrations are reversed after colonization with a conventional GM [8] or even specific bacterial species [7]. Thus, the disruption of neural, endocrine, immune, and metabolic mechanisms that are involved in gut-CNS signaling seems to be involved in neuropsychiatric, neurobehavioral, neurodegenerative, and mental disorders.

1.2 Colonization of intestinal ecosystem in early life and its evolution

The composition of the GM varies widely from fetal life to adult age. Until recently, babies were believed to be born sterile and only populated by microbes on exposure to their first postdelivery environments. Recent research suggests that the process of microbial colonization of the GI tract could begin prenatally as acquisition of maternal microbiota might occur during intrauterine life via placenta [9]. However, these findings are questionable, and recent data strongly suggest that bacteria isolated in utero are rather a contamination linked to the sampling methods than a specific microbiota [10]. Neonates show unstable and highly dynamic intestinal microbiota with a low microbial diversity. First colonizers in healthy neonates are enterobacteria, *Staphylococcus*, and *Streptococcus*, followed by strict anaerobes such as *Bacteroides*, *Bifidobacterium*, and *Clostridium* [11]. This pattern of microbial diversity provides an efficient means for adaptation to the variable circumstances over a lifetime such as changes in lifestyle, illness, puberty, and others. Over the first few years of life, GM matures and stabilizes to a more balanced "adult-like" composition at around the end of the third year [12].

Interestingly, the brain of neonates grows to approximately 90% of its future adult volume until the age of two, and the formation of new synapses in the brain peaks during this period [13]. Thus, the critical window for establishment of a healthy microbial composition falls into the same critical time window for brain development. Therefore, understanding GM establishment and its critical developmental window in early childhood is important because any perturbation during this period causes long-lasting effects on the development of the CNS. Being more flexible at infantile in contrast to the subsequent life, this temporal requirement may have important ramifications for potential preventative and therapeutic remediation strategies.

2. Autism spectrum disorder and the gut microbiota

2.1 Gut microbiota involved in the pathogenesis of autism spectrum disorders

Microbiota role in health and disease is as crucial as is complex. Alterations in normal commensal GM (known as dysbiosis) have been widely reported as a key contributor to the etiology and/or pathogenesis of various diseases including several neurobehavioral conditions, such as Parkinson's disease, schizophrenia, Alzheimer's disease, depression, anxiety, and most compellingly autism spectrum disorder (ASD) [14].

ASD refers to a group of heterogeneous and complex neurodevelopmental disorders characterized by impaired social interactions and reciprocal communication skills as well as restricted, repetitive, and stereotyped patterns of behavior, interests, and activities [15]. Over the last decades, a steady increase of ASD prevalence has been reported worldwide. ASD is currently estimated to affect about 1 in every 68 children, with greater incidence found among boys (4:1) [14]. To date, the etiology of ASD remains elusive, and it is thought to involve both genetic predisposition and different environmental triggers. Although several genetic factors are known to influence the etiology of different types of ASD, these only apply to a minor part of the autistic population. By estimate, the heritability accounts approximately for only 35–40% of the contributing elements and the remaining 60–65% results from the combination of prenatal, perinatal, and postnatal environmental factors as well as related medical disorders [16]. Besides, along with significant psychiatric symptoms, ASD is often characterized by a number of medical comorbidities, the most prominent of which implicates the GI tract. Children with ASD experience significantly more GI symptoms than children without ASD occurring nearly at a fourfold greater rate [17]. Symptoms include constipation, diarrhea, abdominal pain, bloating, gastroesophageal reflux, and food selectivity and seem to be strongly associated with the severity of ASD behaviors. Clinical abnormalities such as altered GI motility and increased gut permeability have also been reported [18]. The cause of ASD-associated GI problems is difficult to ascertain, but it appears to partly relate to the excessive use of oral ATBs which can alter GM. Indeed, several studies report increased use of oral ATBs in children with ASD compared to neurotypical children. By eliminating beneficial indigenous GM, long-term ATB use destabilizes microbial community and creates favorable environment for colonization by potentially harmful (toxin-producing) microorganisms. Thus, considering the potential interactions between intestinal microbes and the CNS, loss of the protective commensal microbiota along with the overgrowth of pathogenic microorganisms is hypothesized to cause or contribute not only to GI dysfunction but also to ASD-related behavioral symptoms. All these findings have gained an insight into the influence of GM in ASD as potential mediator of risk factors.

2.2 Disruptions of microbial colonization in autism spectrum disorder

Imbalances in GM composition at the first stages of life and concomitant behavioral changes have been related to various prenatal and early-life events [8].

Mode of birth, whether through natural birthing process or Cesarean section (C-section), greatly affects the initial microbial settlement. Vaginally delivered babies harbor bacteria similar to their mothers' fecal and vaginal microbiota with dominance of *Bifidobacterium,* whereas babies born via C-section would acquire an altered GM composition resembling to their mother's skin microbiota as well as microbes from the surrounding environment with a delayed colonization by bacteria from maternal origin, mainly enterobacteria, bifidobacteria, and *Bacteroides* [11]. Interestingly, these modifications can persist for several months [2]. Thus, concern has arisen that this reduced microbial diversity and altered bacterial profile in babies delivered via C-section could contribute to ASD. Indeed, studies employing animal models have revealed that, compared with vaginally delivered animals, those delivered by C-section suffer more frequently from behavioral abnormalities that have been associated with ASD [19]. Consistent with this, some clinical studies report that C-section births in comparison to natural vaginal delivery are associated with a significantly 23% higher risk of the child developing ASD [20]. Thus, the importance of C-section in conditioning negative effects on the CNS is debated.

In terms of gestational age at birth, preterm neonates (PT) are characterized by a delayed microbial colonization, missed or reduced acquisition of *Lactobacillus* and *Bifidobacterium*, greater abundance of *Proteobacteria*, and earlier acquisition of *Firmicutes* [11], hence increasing the risk to develop later disease. One study reported a relationship between premature birth and an increase risk of ASD development [21]. Interestingly, each week of shorter gestation was associated with an increased risk of ASD. The early development of infant at birth has also been recognized as a biomarker of future risk of neurodevelopmental disorders. Besides, gestational growth affects the neurodevelopment, and both infants born small or large for gestational age are associated with higher rates of ASD [22].

Early feeding pattern also interferes greatly in the regulation of the intestinal colonization, with breastfed infants harboring a less diverse but more stable and uniform GM. Specific biological markers of healthy GM, including early colonization of *Bifidobacterium* and greater prevalence of *Lactobacillus*, characterize microbial communities of breastfed infants [11]. By contrast, increased species richness accompanied by an overrepresentation of *Clostridium* prevails in bottle-fed ones. In fact, cognitive functioning and neurological development of children have been associated with the duration of exclusive breast-feeding and introduction of formula feed [23]. Remarkably, breast-feeding appears to be less frequent and, when present, occurs for a much shorter duration in children with ASD [24]. Further differentiation occurs after the introduction of solid foods. Feeding patterns and strong food preferences of ASD children for nutrient-poor starchy foods while rejecting fruits, vegetables, and proteins may lay the foundation of an abnormal GM. The high prevalence of ASD in some countries has been correlated with typical carbohydrate rich diet and consequently predominance of genera *Prevotella* and *Megasphaera* [25]. Therefore, understanding how these changes affect human GM suggests that early dietary habits have a more complex effect on the metabolic programming of a child than previously anticipated.

Additional modifications are induced by exposure to drugs either directly or indirectly from the mother. ATB-induced shifts in the gut microbial composition can persist several months after cessation of the treatment, inducing long-term dysbiosis [26]. An association between long-term ATB use, hospitalization, abdominal discomfort, and the onset of ASD symptoms has been shown [27]. Likewise, a population-based cohort study revealed the use of various ATBs during pregnancy as a potential risk factor for ASD [28]. Using animal models, periconceptional exposure to nonabsorbable ATB was shown to induce variations in GM composition in offspring associated to reduction in social interactions and increased anxiety [29]. Other drugs lead to similar observations. In mice, modeling maternal exposure to valproic acid, an antiepileptic drug induced lasting changes in the offspring GM composition, which was associated with neuroinflammation, abnormal GI physiology, and ASD-like behavioral abnormalities [30]. All these observations point to the common hypothesis that early exposure to drugs can modify GM composition transiently or permanently, possibly affecting the severity of non-GI-related symptoms of ASD patients.

Apart from exposition to drugs, another emerging explanation for the difference in GM between ASD and healthy individuals is immunological. Maternal infection during pregnancy was shown to alter microbial composition and is a primary environmental risk factor for ASD [31]. GM alterations (especially in the bacterial classes clostridia and bacteroidia) and higher gut permeability are seen in a maternal immune activation model of ASD [18]. Male progenies of pregnant mice injected during pregnancy with a viral mimetic develop abnormal communication and sociability, repetitive behaviors, and increased anxiety. Another well-established brain-gut connection is the role of stress and its mediators in altering the GM. Maternal separation is a typical model of early-life stress employed in animal studies. In rats, early maternal separation does not only lead to a dysbiotic state of the GM that persists into adulthood but also results in functional GI symptoms and long-term cognitive and behavioral deficits [32]. Likewise, psychological stress was shown to alter colonic mucosa-associated microbiota, with significant decrease in abundance of health-benefit bacteria, such as *Lactobacillus*, while abundance of clostridia increased [33]. Although one cannot extrapolate from mice to human without further evidence, these findings nonetheless suggest that the differences seen in the GM of ASD patients may be a result of immunological changes.

Further, metals and other contaminants have also been identified to increase the risk for ASD knowing their capacity to interfere with the composition and metabolic activity of the GM. In fact, results of a study evaluating the interaction between environmental chemicals and GI microorganisms suggest that alterations in the levels of seven elements (Pb, As, Cu, Zn, Mg, Ca, and Hg) and nine genera of GM (*Bacteroides*, *Parabacteroides*, *Sutterella*, *Lachnospira*, *Bacillus*, *Bilophila*, *Lactococcus*, *Lachnobacterium*, and *Oscillospira*) may be related to ASD [34]. Excessive accumulation of these typical neurotoxic elements leads to abnormally increased abundance of several genera and is reported to be closely related to poorer intellectual function [35].

Taken together, ASD is thought to be a result of a combination of different interacting mechanisms that each contributes a fraction of disease risk.

2.3 Microbial and metabolic dysregulations in autism spectrum disorder

Previously, much research effort on ASD focused on genetic, neurological, and behavioral aspects of disease. Recently, the evidence of the impact of dysbiosis on CNS raised interest in the analysis of the potential link between GM and ASD. So far, various studies demonstrated that children with ASD exhibit different compositions of GM compared to healthy controls [36–43]. Moreover, exciting work with animal models widely deepened the possible role of gut microorganisms in the pathogenesis of such disorders [44, 45]. These evidences have led to the hypothesis that GM alteration is not only associated with ASD but may play a key role in the exacerbation of ASD symptoms and/or its pathogenesis, at least for some ASD subgroups [14]. Overall, most studies agree that GM composition is distinctive in ASD compared to healthy controls, but results are often inconsistent as to the nature and/or extent of GI bacterial community differences, failing to generate a coherent picture. Microbiota analyses reported tenfold higher counts of pathogenic *Clostridium* spp. in children with ASD compared with healthy controls [46, 47]. *Clostridium* is known to produce neurotoxins and P-cresol, cause higher propionic acid levels, and promote conditions that favor inflammation and exacerbate ASD symptoms. On the other hand, there have been some consistent findings of decrease in certain beneficial bacteria, specifically *Bifidobacterium* [40, 48, 49] known for its health-benefit properties. Then, the composition of GM of ASD individuals has been characterized, showing a reduction of the *Bacteroidetes*/*Firmicutes* ratio which pointed to elevated numbers of *Firmicutes* in contrast to decreased levels of *Bacteroidetes* [36, 39, 41, 49–51]. *Bacteroidetes* are short-chain fatty acids (SCFA) producing bacteria, and their metabolites, especially propionic acid, may influence the CNS and autism behavior by modulating the GBA [36]. Moreover, species of *Desulfovibrio* were also isolated from the stool of patients with ASD, and, to a lesser extent, in non-affected siblings [52]. *Desulfovibrio* could be an important contributor to GI inflammation, as its major metabolic by-product—hydrogen sulfide—is cytotoxic to colonic epithelial cells. Furthermore, the presence of autistic symptoms in children with ASD has been correlated with a less diverse gut microbiome, with less carbohydrate degrading and fermenting bacteria of the genera *Prevotella*, *Coprococcus*, and the unclassified *Veillonellaceae* in ASD microflora samples as compared to the healthy controls [39]. This decrease in GM diversity can lead to a loss of key signals required for normal brain maturation. Additionally, increased *Sutterella* were found in significant numbers in intestinal biopsies and stools of ASD children [48, 50]. This genus is known to regulate mucosal metabolism and intestinal epithelial integrity. Lastly, there were still some conflicting results about the alterations of *Akkermansia*, *Ruminococcus*, and *Faecalibacterium* in ASD patients. *Akkermansia* and *Ruminococcus* are mucin-degrading bacteria [40], and *Faecalibacterium* is regarded as commensal or even beneficial due to its function of producing anti-inflammation butyrate [45].

Thus, the existence of a GI dysbiosis as an actor in the ASD etiopathogenesis remains a controversial topic. Indeed, other studies comparing children with ASD and their healthy siblings reported no meaningful difference in GM composition [53, 54]. According to the authors, other explanations for the GI dysfunction in this population should be considered, including elevated levels of anxiety and self-restricted diets. Therefore, given the higher incidence of ATB usage and often different diets compared with neurotypical individuals, both of which can alter the composition of the GM, such data should be interpreted with care.

Dysbiosis in ASD involves not only bacterial species but also yeasts, as reported in recent studies [40, 41, 55, 56]. One culture-based study showed significant presence of *Candida* species in the feces of children with ASD, mainly *Candida albicans*. It also identified hyphae formation, suggesting that the dimorphic yeast had switched to its invasive and adhesive form. However, another study did not report such overrepresentation of *Candida* in ASD children compared to control ones [49].

Moreover, correlations between ASD and GI disturbances may not alone be driven by the composition of the GM but also by differences in its functionality, such as the bacterial metabolites that could play a role in the GBA. Indeed, overproduction of bacterial metabolites (e.g., p-cresol) and SCFA (e.g., propionic acid) is frequently described in infants with ASD [30, 49, 57]. These compounds induce intracellular signaling and modulate host gene expression related to neurotransmission systems and behavior [58]. P-cresol seems to negatively affect the homeostasis of colonic epithelial cells in children with ASD. When tested *in vitro*, excessive concentration of p-cresol showed deleterious metabolic and genotoxic effects on colonocytes [59]. In addition, early exposure to p-cresol may contribute to the severity of behavioral symptoms and cognitive impairment in ASD toddlers. On the other hand, propionic acid is known to have a number of direct effects on gut physiology. It increases the contraction of colonic smooth muscle, dilates colonic arteries, stimulates serotonin release, and decreases gastric motility, which could be easily related to the GI abnormalities frequently observed in many ASD patients [60]. In fact, lower plasmatic levels of propionic acid have been reported in ASD children as an aspect of metabolic alteration in gut host-microbial co-metabolism. Authors related the occurrence of lower propionic acid in the plasma to elevated levels of propionic acid in the brain [61]. Additionally, administration of neurotoxic dose of propionic acid to animals was effective in inducing autistic features. Orally dispensed propionic acid was reported to induce oxidative stress. Elevated interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), and interferon γ (INF-γ) confirmed the neuroinflammatory effect of propionic acid [61]. When propionic acid was injected into the cerebral ventricles of rats, the rats showed biologic, chemical, and pathologic changes that are characteristic of ASD [62]. These chemicals can also alter the intercellular spaces between the cells, resulting in the "leaky gut syndrome" that can lead to detrimental neurologic effects.

Dysbiosis can also affect the functional intestinal barrier that can lead to an alteration in the intestinal permeability referred to as "leaky gut" state, a fundamental factor underlying the relationships between ASD and the GM [63]. Indeed, several reports show increased gut permeability in ASD patients [64]. Disrupted barrier function facilitates the translocation of bacterial metabolites from the gut into the bloodstream to possibly reach the otherwise sterile CNS inducing directly inflammatory reactions. One important bacterial component is the lipopolysaccharide (LPS) that was shown to increase the activity of areas deputed to the emotionalism control such as amygdale [65]. It also leads to the production of inflammatory cytokines that critically alter the physiological brain activity, modulating the neuropeptides synthesis [66]. Moreover, it has been demonstrated that the administration of low doses of LPS in healthy subject is associated to increased pro-inflammatory cytokines and plasma norepinephrine, with higher depression rates, fatigue, and apathy [67]. Consistent with this, a study showed LPS serum levels were significantly higher in autistic patients compared to heath individuals and correlated with socialization scores in an inverse and independent manner [68].

3. Restoring the gut ecosystem: therapeutic outlooks for autism spectrum disorder

Despite increased ASD diagnoses, there remains no US Food and Drug Administration (FDA)-approved pharmaceutical treatment to alleviate core symptoms of ASD [69]. Currently, recommended management strategies essentially involve rehabilitation, educational interventions, speech therapies, psychiatric medications, and specific treatments for individual comorbidities [70], all with limited success [71]. Considered the emerging role of gut dysbiosis in ASD, interest in rebalancing human GM as a possible therapeutic approach is growing [72]. Indeed, targeting the GM in children with ASD through administration of ATBs, pro- and prebiotics, and nutritional approaches or, more recently, through fecal microbiota transplantation (FMT) has been shown to improve not only GI disturbances but also behavioral and neurophysiological abnormalities associated with ASD [18, 24].

3.1 Antibiotics/antifungals

ATB therapy is used for the management of ASD and is routinely prescribed to treat ASD symptoms associated with several underlying disorders like pediatric autoimmune neuropsychiatric disorder associated with streptococcal infections (PANDAS) and pediatric acute-onset neuropsychiatric syndrome (PANS) [72]. Short-term administration of vancomycin was shown to provide significant improvement in both GI and behavioral disturbances in a subset of children with ASD [73]. Unfortunately, this attempt had only partial success since children relapsed, and benefits waned upon treatment termination. As vancomycin is a poorly absorbed ATB known to destroy Gram-positive anaerobes, observed improvement is believed to be the consequence of temporary elimination of toxin-producing clostridia population. Hence, symptomatic relapse was attributed to the spore-forming capacity of these bacteria, and spores resistant to ATB would later germinate into vegetative forms once treatment has stopped [46].

Children with ASD, particularly those with GI disease, are sometimes treated with antifungal agents, as they may have increased incidence of fungal infection. Despite the lack of evidence of fungal overgrowth in children with ASD, parents find that antifungal therapies can often be beneficial and may be a useful adjuvant for the treatments of ASD [74].

Thus, it is possible to speculate that anti-infectives, through modulation of GM, should be able to influence symptoms and expression of psychiatric disorders. However, ATB resistance (and to some extent antifungal resistance) is a major public health concern, making the safety of ATB/antifungal treatments ethically problematic, if they do prove to be beneficial. Therefore, extensive and prolonged use of ATB/antifungal treatments may not be advisable as a long-term therapy for ASD.

3.2 Probiotics

One of the most nutritional popular approaches selectively modulating the GM is probiotic supplementation due to ease of use, wide availability, and good safety profile. Probiotics are defined as live microorganisms that when administered in adequate amounts confer a health benefit to the host [75]. The main species used are one *Escherichia coli* strain, several lactic acid-producing *Lactobacillus*, a number of bifidobacterial strains, and a yeast *Saccharomyces boulardii*. Research on probiotics has shown efficacy in prevention or treatment of a wide variety of diseases associated with GI difficulties. Recently, some evidence has been accumulated regarding the possible role of probiotics in modulating symptoms of certain psychological diseases such as depression and anxiety as well as ASD [76–78]. Hence, a new class of probiotics, termed as psychobiotics, has emerged and refers to living organisms with beneficial effects on mental health [79]. In spite of the well-documented beneficial effects of probiotics, testing for ASD is still in its infancy, and the exact mechanism of their action has not been thoroughly elucidated to date, though there are several hypotheses. Implicating correction of both composition and/or activities of GM is the first mechanism of action of probiotics through several mechanisms including bacteriocins and metabolites, such as lactic, propionic, and acetic acids.

Hence, because ASD patients present GI dysbiosis, which may exacerbate the disease, these patients could benefit from GM modulation through probiotic supplementation. Conversely, other investigations have shown that probiotic administration could act independently from GM alterations by inducing a stabilization of the microbial communities making them less susceptible to perturbations from stressors such as ATBs, poor diet, and psychological stress [80]. Moreover, probiotics may correct the imbalance in the activity of the GM without changing its composition, via their metabolites released in the gut lumen or by potentially correcting the overproduction of harmful and underproduction of beneficial gut bacterial products [81]. The increased intestinal permeability in ASD may also be ameliorated by probiotics which are able to enhance the mucosal barrier. This supports the concept that probiotics can provide protective effect by preventing the metabolites of exogenous toxic substances from leaving the gut and entering the bloodstream to affect the brain [70]. Probiotics may therefore maintain or improve gut barrier integrity and aid in ASD rehabilitation by promoting "leaky gut" healing. Lastly, given the multiple findings of aberrant immune activation and higher levels of gut inflammation in a subset of individuals with ASD, that a major part of the immune system is concentrated in/around the intestinal mucosa, and that the GM plays an important role in the maturation and the regulation of the immune response, another mechanism of action of probiotics may be on the immune system [82]. Probiotics can downregulate gut and CNS inflammatory pathways in a species- and strain-specific manner [83], by promoting the production of regulatory T cells, diminishing the levels of LPS, providing tolerogenic signals, and boosting the brain-derived neurotrophic factor (BDNF). BDNF is a protein that promotes the survival of existent neurons in the developing brain, fosters the growth and differentiation of new neurons, and regulates the formation and plasticity of synaptic connections, thereby playing an essential role in the normal neurological development [6]. Impairment in BDNF signaling in early developmental phases is thought to be related to CNS abnormalities, the most severe forms of ASD, as well as intellectual disability [84]. Probiotics have been shown also to modulate the intestinal immune system by the production of secreted factors and metabolites that affect growth and function of intestinal epithelial and immune cells [85]. Moreover, probiotic immunomodulation may occur through inhibiting the production of pro-inflammatory cytokines such as IL-12, TNF-α, and INF-α or increase the expression of anti-inflammatory mediators such as IL-10 and transforming growth factor beta (β-TFGF). Thus, by alleviating gut inflammation and suppressing dysregulated immune functions, probiotic supplementation may be effective for improving both gut microbial and behavioral problems in ASD. Lastly, probiotics could act via the vagus nerve-mediated GBA to influence neurotransmission and mood states [86]. They can exert central actions by influencing several neuroactive metabolites such as gamma-aminobutyric acid (GABA) and serotonin which are also associated with neuropsychiatric disorders [87, 88]. Consequently, administration of probiotics regulates the behavior in a way that significantly reduces anxiety, depression, and stress and promotes positive emotional changes. Therefore, probiotic administration might be a useful new therapeutic option to restore normal GM, reduce inflammation, restore epithelial barrier function, and possibly improve some behavioral symptoms associated with ASD.

A role for probiotics has been suggested for children with ASD as well, as preliminary findings from experimental animals studies provide some evidence that administration of selected probiotics may be effective in reducing neurologic signs and symptoms associated with gut dysbiosis. In a summary of studies to date, several probiotic strains, i.e., *Bifidobacterium* sp. (*B. longum*, *B. breve*, *B. infantis*, and *B. bifidum*), *Lactobacillus* sp. (*L. acidophilus*, *L. helveticus*, *L. rhamnosus*, *L. plantarum*, *L. sporogenes*, *L. bulgaricus*, *L. delbrueckii*, *L. salivarius*, *L. casei*, and *L. paracasei*), *Streptococcus* sp. (*S. thermophilus* and *S. salivarius*), and *Bacteroides fragilis*, are presumed to be effective in ameliorating CNS functions related to mental disorders, as shown through several animal models [18, 89–92]. In a mouse model of ASD induced by maternal immune activation, oral administration of human commensal

B. fragilis corrected intestinal permeability, restored GM, improved GI physiology, and abolished ASD-like behavioral disruptions [18]. Interestingly, the probiotic also corrected altered expression of the tight junction proteins in the colon and restored the increase in the pro-inflammatory cytokine IL-6. Moreover, *B. fragilis* treatment mitigated elevations in several maternal immune activation-induced serum metabolites associated with ASD. One metabolite of particular interest was 4-ethylphenylsulfate (4EPS) which is chemically similar to p-cresol and is thought to be a possible putative urinary biomarker for ASD [59]. Hence, the administration of *B. fragilis* in a mouse model for ASD was able to reverse autistic symptoms and metabolic derangement. Similarly, reconstituting GM with a human breast milk and gut commensal *Lactobacillus reuteri* completely corrected social deficits and reversed aberrant neurotransmission in maternal high-fat diet offspring [89]. This is intriguing in light of reports that risperidone, an FDA-approved treatment for ASD, does not correct social abnormalities [93]. Treatment with *L. reuteri* resulted also in reduction of stress-induced corticosterone, and stimulation of the production of oxytocin, a key regulator of social behaviors, involved in the mesolimbic dopamine reward system, which is thought to be dysregulated in ASD [94]. On another hand, a recent study performed on hamsters in which autistic-like behaviors were induced by propionic acid and clindamycin administration studied the therapeutic effect of a 3-week oral treatment with a mixture of bifidobacteria and lactobacilli strains (Protexin®) [91]. Protexin® administration was effective in rebalancing GM, ameliorating oxidative stress, and counteracting behavioral deficits. Finally, in maternal separation-induced early-life stress mice model, oral administration of *L. plantarum* as a psychobiotic strain significantly reduced anxiety while improving locomotor activities and exploratory behavior [92]. According to various animal studies, *L. plantarum* can modulate the levels of neurotransmitters in the brain by influencing gene expression in the CNS and increasing dopamine level in the prefrontal cortex [92]. Summing up, by conducting behavioral tests in animal models, research provided convincing evidence for the efficacy of psychobiotic strains in improving ASD-like behaviors. However, the experimental evidence for the positive behavioral changes observed in animal models after administration of probiotics raises the interesting question whether the same result also applies to humans.

To date only few studies explored the effects of probiotics on ASD clinical features in humans. **Table 1** synthesizes available evidence on the efficacy and safety of probiotic supplementation as an adjunctive treatment for ASD [49, 51, 73, 95–106]. In these studies, probiotic interventions varied across all of the trials. Concentrations of the probiotic strains administered ranged from 10^7 to 10^{10} colony-forming units (CFU)/dose, and their usage by recipients differed. Strains were administered alone or as mixed strains with or without other additives (immunomodulators [99] and ATBs [73]). In summary, despite the variability in species, strains, dosages, and duration utilized, all studies pointed toward a similar trend of improvement in both caregiver-reported ASD and GI symptoms after probiotic therapies. Accordingly, a recent survey found that almost 20% of physicians treating ASD patients encourage probiotic use, and almost 60% accept their use [107]. In a recent study, the positive effect of probiotic treatment was represented not only by the ability to consistently normalize the *Bacteroidetes*/*Firmicutes* ratio and restore the amounts of *Desulfovibrio* and *Bifidobacterium* in children with ASD [51] but also by the tendency to reduce intestinal inflammation and permeability. Similarly, another study associated changes in the GM composition to improvements in GI symptoms and functioning [102]. After probiotic supplementation in the latter study, children with ASD experienced a significant increase in *Bifidobacterium* and *Lactobacillus* in their fecal stool samples with a simultaneous reduction of *Clostridium* species. Furthermore, in a third study, probiotic feeding of children

Authors	**Study design**	**Diagnosis**	**Probiotic therapy**
Blades M., 2000, UK [95]	Case report of a 6 y with ASD	Not reported	Strain and dosage not provided Administration for 2 mo
Sandler et al., 2000, USA [73]	Open-label with self-control study 11 ASD subjects (10 M, 3.5–7 y)	CARS	*L. acidophilus*, *L. bulgaricus*, and *B. bifidum*, 40×10^9 CFU/mL QD for 4 wk
Parracho et al., 2010, UK [96]	Randomized, double-blind, PBO-controlled, cross overdesigned feeding study 17 ASD subjects (14 M, 4–16 y)	Not reported	*L. plantarum* WCFS1 4.5×10^{10} CFU QD for 3 wk
Ray et al., 2010, USA [97]	Open-label with self-control study 10 ASD subjects (Gender not reported, 4–15 y)	ATEC	*L. rhamnosus*, *B. bifidum*, *L. acidophilus*, *B. infantis*, *B. longum*, *S. thermophilus*, *L. plantarum*, *L. salivarius*, *L. reuteri*, *L. casei*, *L. bulgaricus*, *L. acidophilus* DDS-1, and *L. sporogenes* Dosage not provided BID for 21 d
Adams et al., 2011 USA [49]	Retrospective case cohort study 57 ASD subjects: 19 PS vs. 38 no PS (50 M, 3–9 y)	ATEC	Strain and dosage not provided QD
Kałzuna-Czaplinska et al., 2012, Poland [98]	Open-label with self-control study 22 ASD subjects (20 M, 4–10 y)	DSM-IV	*L. acidophilus* (strain Rosell-11) 5×10^9 CFU/g BID for 2 mo
West et al., 2013, USA [99]	Open-label with self-control study 33 ASD subjects (Gender not reported, 3–16 y)	Not reported	*L. acidophilus*, *L. casei*, *L. delbrueckii*, *B. longum*, and *B. bifidum* 1×10^{10} CFU/capsule One capsule TID for 6 mo
Partty et al., 2015, Finland [100]	Randomized, double-blind, PBO-controlled prospective follow-up study 75 ASD subjects 40 PS (24 M, 13 y) vs. 35 PBO (16 M, 13 y)	ICD-10	*L. rhamnosus* GG 1×10^{10} CFU QD for 6 mo
Tomova et al., 2015, Slovakia [51]	Case control cohort study 10 ASD subjects (9 M, 2–9 y) 9 non-ASD siblings (7 M, 5–17 y) 10 non-ASD controls (10 M; 2–11 y)	ICD-10	3 strains of *Lactobacillus* (60%, one is *L. casei*), 2 strains of *Bifidobacterium* (25%, one is *B. longum*), and 1 strain of *Streptococcus* (15%, exact strain information not provided) Dosage not provided One capsule TID for 4 mo
Grossi et al., 2016, Italy [101]	Case report of a 12 y boy with ASD	DSM-V + ADOS-2	*B. breve*, *B. longum*, *B. infantis*, *L. acidophilus*, *L. plantarum*, *L. paracasei*, *L. bulgaricus*, *L. delbrueckii*, *S. thermophilus*, and *S. salivarius* 9×10^{10} CFU/g *Bifidobacteria* 8×10^{10} CFU/g *Lactobacilli*, and 20×10^{10} CFU/g *Streptococci* QD for 4 months

Authors	Study design	Diagnosis	Probiotic therapy
Shaaban et al., 2017, Egypt [102]	Prospective, open-label cohort study 30 ASD subjects (19 M, 5–9 y) 30 age/sex-matched non-ASD siblings	DSM-V + ADOS + ADI-R	*L. acidophilus*, *L. rhamnosus*, and *B. longum* 10 × 10^7 CFU/g 5 g QD for 3 mo
Liu et al., 2019, Taiwan [103]	A double-blind, randomized, PBO-controlled, parallel feeding study 71 ASD subjects: 36 PS (36 M, 7–15 y) vs. 35 PBO (35 M, 7–15 y)	DSM-V + ADI-R	*L. plantarum* PS128 3 × 10^{10} CFU/capsule 1 capsule QD for 4 wk
Kobliner et al., 2019, USA [104]	Case report of a 16 y child with ASD	Not reported	*S. boulardii* 3 × 10^9 CFU/capsule Week 1: 6 capsules QD. Weekly increases reaching a final dose of 12 capsules BID. After 3 mo, weaning down to 3 capsules BID
Sanctuary et al., 2019, USA [105]	Double-blind, crossover, randomized clinical trial 8 ASD subjects (7 M, 4–11 y)	ADOS	*B. longum* subsp. *infantis* 2 × 10^{10} CFU QD for 5 wk

y, years; M, male; PBO, placebo; PS, probiotic supplementation; ADI-R, Autism Diagnostic Interview-Revised; ADOS, Autism Diagnostic Observation Schedule; ATEC, Autism Treatment Evaluation Checklist; CARS, Childhood Autism Rating Scale; DSM-IV, Diagnostic and Statistical Manual-4th Edition; ICD-10, International Classification of Diseases-10th Edition; d, days; wk, weeks; mo, months; CFU, colony-forming unit; g, gram; QD, once a day; BID, two times a day; TID, three times a day; QID, four times a day.

Table 1.
Main clinical trials investigating the effectiveness of probiotic supplementation in autism spectrum disorders.

with ASD significantly increased levels of the beneficial bacteria, specifically, the amount of lactobacilli and enterococci, and reduced their fecal *Clostridium* counts [96]. Additionally, in the same study, the efficacy of probiotic supplementation seemed to be age-dependent, with better effects noticed on younger children, underscoring the importance of early interventions. Although all aforementioned studies showed changes in GM after the implementation diet with probiotics, only some of them analyzed the correlations with GI symptoms and reported improvement of GI function indices [96, 98, 101, 102, 105]. In fact, a survey conducted on caregivers showed that daily administration of a specific five-strain probiotic decreased the severity of comorbid GI problems [99]. Concurrent with increases in the proportion of normal stools, there was an increased appetite and willingness to consume novel foods that may be due to reduced abdominal pain or improved stool evacuation [105]. Besides, in one case study report, a multi-strain mixture of 10 probiotics not only relieved the GI disturbances but also improved ASD core symptoms [101]. However, benefits reversed after the term of the therapy. In addition to the alteration in GI dysfunction and GM, more than half of the studies also included assessment of change in behavior after probiotic therapy. Most of these studies recorded a reduction in the severity of ASD symptoms, although not all reached significance. Noted positive effects on mood and general behavior were (i) statistically significant differences in behavioral scores for disruptive/antisocial behavior, anxiety problems, communication disturbances, and self-absorbed behavior compared to baseline [96, 104]; (ii) decrease in the severity of speech/language/communication performance [97, 101]; (iii) progress in sociability, sensory and cognitive awareness, physical health, and behavior [99, 102]; and (iv) changes in adaptive, repetitive, and aberrant behaviors including irritability, lethargy, stereotypy, and hyperactivity [105]. Conversely, in other investigations, no statistically significant differences in behavioral scores were detected between probiotics and placebo control groups [73, 103]. Lastly, probiotic treatment reversed the metabolic disruptions and improved behavioral performance. In one of the studies, probiotics significantly lowered the concentration of myeloperoxidase (a marker of inflammation and oxidation) in ASD patients compared to other autistic individuals not taking probiotics [106]. Metabolic changes were also observed in another former study in which probiotics substantially diminished fecal levels of propionic acid in ASD individuals [49]. In a third study, probiotic supplementation significantly reduced metabolic products of the pathogenic *Candida* yeast species [98], which are remarkably elevated in the urines of individuals with ASD [108]. This biological improvement also corresponded to behavioral outcomes, where children showed significant ameliorations in their ability to concentrate and fulfill orders. In 2015, an intriguing randomized trial demonstrated that early postnatal probiotic administration has a preventive effect on ASD and may reduce the risk of developing further neuropsychiatric disorder [100]. Results showed that from children randomly assigned to probiotic or placebo groups during the first 6 months of life, 17% in the placebo group had a diagnosis of ASD at the age of 13, while none of the children in the probiotic group did.

At last, though probiotics are considered a relatively risk-free option for individuals with ASD, the current literature cannot confidently state their safety as there is a paucity of systematic reporting of adverse events. However, among the studies that monitored side effects, the reported ones (bloating, skin rash, worsening constipation or diarrhea, and weight loss) appear to be mild, transient, and infrequent [96, 99, 102, 105]. Conclusively, due to the large heterogeneity between trials, studies provide only suggestive but not conclusive evidence regarding the efficacy of probiotics on GI and behavioral symptoms among ASD patients. Thus, future probiotic research holds hopes for discovering the optimal species, strains, strength, and length of probiotic therapy for the particular comorbidity profile of different individuals with ASD.

3.3 Prebiotics

The International Scientific Association for Probiotics and Prebiotics (ISAPP) defines prebiotics as substrates "selectively utilized by host microorganisms, conferring a health benefit" [75]. Such benefits are not limited to gut homeostasis but can extend elsewhere in the organism, leading to improvements of the immune, metabolic, endocrine, or nervous functions. Fructans, comprising fructooligosaccharides (FOS) and inulin, and galactans (galactooligosaccharides (GOS)) are the most recognized prebiotics. Differently from most dietary fibers, which promote growth of a wide variety of microorganisms, prebiotics display a selective effect, being a substrate for beneficial strains only, while excluding metabolism by pathogenic bacteria [6]. Thus, the main reason for a potential influence of prebiotics on the treatment of ASD concerns the selective enrichment of *Lactobacillus* and/or *Bifidobacterium* spp. Besides, various other mechanisms have been identified through which prebiotics can act, including generation of SCFA that have an influence on gut energy metabolism, barrier function, water fluxes, motility [109], elongation of microvilli, increase in mucus layer thickness, and consequent protection of gut epithelium. However, as with probiotics, prebiotic studies regarding their impact in neurological problems are few and not conclusive. In rats, oral administration of GOS elevates BDNF levels [110], normalizes LPS-induced anxiety, and modulates cortical IL-1β levels [111], thus confirming the potential role for prebiotics in ASD where anxiety and neuroinflammation are prominent clinical features. Lastly, only two clinical trials examined the use of prebiotics in children with ASD [105, 112]. In the first study, GOS alone did not have a significant effect on GI symptoms, while when combined with bovine colostrum, GI symptoms improved as well as irritability scores and stereotypy [105]. Similarly, in the second trial, GOS intervention did not show a significant impact when provided alone. Nevertheless, when associating GOS treatment with an exclusion diet, notable increases occurred in beneficial bacteria supporting improvement in antisocial behavior [112]. GOS also affected SCFA production decreasing propionic acid as a result of normalized GM composition. Thus, it is sensible to postulate that combined intervention therapies might have a better impact on ASD individuals than single dietary approach and prebiotics could be a useful option for ASD children treatment in early life.

3.4 Fecal microbiota transplantation

The process of FMT consists in the delivery of feces from a healthy donor to a patient with gut dysbiosis, with the aim of replacing an impaired microbiota with a healthy one. Unlike probiotics, where only a restricted number of bacterial species are supplemented, FMT allows the transplantation of thousands of different components of the healthy GM. In fact, FMT is one of the most effective techniques recently considered in treating recurrent ATB-refractory *Clostridium difficile* infection [113] and has shown varying levels of success in patients with other GI diseases such as irritable bower syndrome [114], and non-GI diseases like autoimmune disorders, obesity, and insulin sensitivity [115]. Given the growing evidence for a role of GM disruption and GI symptoms in ASD, clinical trials are under way using FMT to treat children with ASD. In a recent open-label study, ASD children with chronic GI problems underwent a modified FMT protocol, termed microbiota transfer therapy (MTT), consisting of a 2-week vancomycin treatment followed by bowel cleansing, and administration of a high dose of standardized human gut microbiota [24]. After this 10-week treatment, MTT induced an 80% improvement of GI function and a slow but steady improvement in behavioral ASD scoring, both of them maintained at least 8 weeks after treatment stopped. In line with previous reports, few adverse events were described, and the most commonly reported include mild diarrhea, abdominal tenderness, flatulence, and nausea thus confirming treatment safety and tolerability in autistic patients. Coincident with these clinical improvements, FMT confirmed its ability in modifying GM composition by significantly increasing bacterial diversity and the relative abundance of *Bifidobacterium* and *Prevotella* [24]. Two years after this original clinical trial was completed, re-evaluation of the participants showed that GI benefits were mostly maintained and ASD symptoms were reported to have improved significantly since the end of treatment [116]. Changes in the GM also persisted for 2 years. According to these encouraging observations, intensive FMT intervention can be considered an effective and well-tolerated promising approach in treating children with ASD who have GI problems. However, the safety of FMT should be considered as it carries many risks including aspiration and transmission of opportunistic pathogens to recipients. Therefore, major efforts at refinement are necessary before FMT can be adopted widely.

3.5 Dietary interventions

A number of nutrition intervention strategies have been explored to treat behavioral symptoms and comorbid GI distress [117], but evidence is still relatively weak and sometimes inconsistent, as in the case with gluten-free and/or casein-free (GF/CF) diet and ketogenic diet (KD).

Various observational studies reported alleviation of GI problems and/or significant behavioral improvements in ASD children following an extended GF/CF diet [118, 119]. However, elimination diets for ASD patients should not be recommended as standard treatment and only be considered after reaching a diagnosis of an adverse food reaction since dietary restrictions are likely to limit variety of food intake and provoke nutritional deficiencies in developing children [120]. Moreover, GF/CF diet can also exacerbate the already disrupted gut microbial composition in ASD by reducing beneficial and increasing opportunistic bacteria [121].

Improvements in neurobehavioral symptoms have also been reported in ASD as a result of following a KD. KD is a high-fat and low-carbohydrate diet that, due to its beneficial effects on GM composition, has been suggested as a treatment for ASD. In humans, administration of KD to individuals with ASD results in increased sociability, improved communication, and decreased repetitive comportments [122]. In animal models, both improvement of behavioral symptoms and compositional remodeling of GM after KD have been described [123, 124]. However, KD causes an "antimicrobial"-like effect by significantly decreasing the total gut microbial abundance. Moreover, it results in an increase in clostridia species and may lead to adverse effects such as dehydration [125]. So, side effects and increase in harmful bacteria in the GM associated with limited number of positive results constitute insufficient evidence for the practicability of KD as a treatment for ASD.

4. Conclusion and perspectives

In the last few years, the importance of GM in the maintenance of physiological state into the CNS is supported by several studies that have shown qualitative and quantitative alterations of the intestinal flora in a number of neuropsychiatric diseases. Within neurobehavioral disorders, it seems that at least a subset of the cases comprising ASD are connected to, and perhaps dependent on, the health and well-being of the GM. In recent years the increased prevalence of ASD, along with the evidence of a significant link between ASD and GI disturbances, raised a special interest in exploring the reciprocal influences between GM and brain under the so-called GBA. Alterations of GM composition in children with ASD presented in the literature mainly consist in reduced levels of *Bifidobacterium* and increased levels of *Clostridium* spp. and *Desulfovibrio*. However, the available data do not allow to define a characteristic and unique profile of ASD. If dysbiosis is confirmed to be a precipitating factor in ASD, then several potential therapeutic approaches ranging from ATBs, probiotics, prebiotics, up to FMT and other nutritional strategies may be useful adjuvant treatments in these patients. Future research, together with the application of state-of-the-art "omics" methods, could address possible unequivocal microbial biomarkers for ASD. Further, as dysbiosis contributes to a significant subset of ASD, specific identification of ASD endophenotypes will allow patient stratification and personalized interventions. Addressing microbial processes could be the aim of the next pharmacological therapy of ASD that will potentially help to alleviate the burden of this disorder for the millions of people affected worldwide.

Abbreviations

4EPS	4-ethylphenylsulfate
ASD	autism spectrum disorders
ATB	antibiotic
BDNF	brain-derived neurotrophic factor

CFU	colony-forming unit
CNS	central nervous system
C-section	cesarean section
FDA	Food and Drug Administration
FMT	fecal microbiota transplantation
FOS	fructooligosaccharides
GABA	gamma-aminobutyric acid
GBA	gut-brain axis
GF/CF	gluten-free and/or casein-free
GI	gastrointestinal
GM	gut microbiota
GOS	galactooligosaccharides
IL	interleukin
INF	interferon
ISAPP	International Scientific Association for Probiotics and Prebiotics
KD	ketogenic diet
LPS	lipopolysaccharide
MTT	microbiota transfer therapy
PANDAS	pediatric autoimmune neuropsychiatric disorder associated with streptococcal infections
PANS	pediatric acute-onset neuropsychiatric syndrome
PT	preterm
SCFA	short-chain fatty acids
TNF	tumor necrosis factor
TFGF	transforming growth factor

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References

[1] Bik EM. The hoops, hopes, and hypes of human microbiome research. The Yale Journal of Biology and Medicine. 2016;**89**:363-373

[2] Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, et al. Dynamics and stabilization of the human gut microbiome during the first year of life. Cell Host & Microbe. 2015;**17**:690-703

[3] Dinan TG, Cryan JF. The microbiome-gut-brain Axis in health and disease. Gastroenterology Clinics of North America. 2017;**46**:77-89

[4] Carabotti M, Scirocco A, Maselli MA, Severi C. The gut-brain axis: Interactions between enteric microbiota, central and enteric nervous systems. Annals of Gastroenterology. 2015;**28**:203-209

[5] Kim Y-K, Shin C. The microbiota-gut-brain axis in neuropsychiatric disorders: Pathophysiological mechanisms and novel treatments. Current Neuropharmacology. 2018;**16**:559-573

[6] Campion D, Ponzo P, Alessandria C, Saracco GM, Balzola F. The role of microbiota in autism spectrum disorders. Minerva Gastroenterologica e Dietologica. 2018;**64**:333-350

[7] Desbonnet L, Clarke G, Shanahan F, Dinan TG, Cryan JF. Microbiota is essential for social development in the mouse. Molecular Psychiatry. 2014;**19**:146-148

[8] Sharon G, Sampson TR, Geschwind DH, Mazmanian SK. The central nervous system and the gut microbiome. Cell. 2016;**167**:915-932

[9] Collado MC, Rautava S, Aakko J, Isolauri E, Salminen S. Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. Scientific Reports. 2016;**6**:1-13

[10] Willyard BC. Could baby's first bacteria take root before birth. Nature. 2018;**553**:264-266

[11] Milani C, Duranti S, Bottacini F, Casey E, Turroni F, Mahony J, et al. The first microbial colonizers of the human gut: Composition, activities, and health implications of the infant gut microbiota. Microbiology and Molecular Biology Reviews. 2017;**81**:1-67

[12] Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography. Nature. 2012;**486**:222-227

[13] Diaz HR. Fetal, neonatal, and infant microbiome: Perturbations and subsequent effects on brain development and behavior. Seminars in Fetal & Neonatal Medicine. 2016;**21**:410-417

[14] Liu J, Zhang M, Kong X-J. Gut microbiome and autism: Recent advances and future perspectives. North American Journal of Medical Sciences. 2016;**9**:104-115

[15] American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders (DSM-5®). Washington, DC: American Psychiatric Publishing; 2013

[16] Modabbernia A, Velthorst E, Reichenberg A. Environmental risk factors for autism: An evidence-based review of systematic reviews and meta-analyses. Molecular Autism. 2017;**8**:1-16

[17] McElhanon BO, McCracken C, Karpen S, Sharp WG. Gastrointestinal symptoms in autism spectrum disorder: A meta-analysis. Pediatrics. 2014;**133**:872-883

[18] Hsiao EY, McBride SW, Hsien S, Sharon G, Hyde ER, McCue T, et al. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell. 2013;**155**:1451-1463

[19] Borre Y, Golubeva A, Crispie F, Scott K, Hyland N, Stanton C, et al. Mode of delivery at birth and behavioral outcomes: Rewiring of the brain-gut-microbiome axis? In: Society for Neuroscience. Washington, USA: Poster presentation; 2014:15-19

[20] Curran EA, O'Neill SM, Cryan JF, Kenny LC, Dinan TG, Khashan AS, et al. Research review: Birth by caesarean section and development of autism spectrum disorder and attention-deficit/hyperactivity disorder: A systematic review and meta-analysis. The Journal of Child Psychology and Psychiatry and Allied Disciplines. 2015;**56**:500-508

[21] Kuzniewicz MW, Wi S, Qian Y, Walsh EM, Armstrong MA, Croen LA. Prevalence and neonatal factors associated with autism spectrum disorders in preterm infants. The Journal of Pediatrics. 2014;**164**:20-25

[22] Grissom NM, Reyes TM. Gestational overgrowth and undergrowth affect neurodevelopment: Similarities and differences from behavior to epigenetics. International Journal of Developmental Neuroscience. 2013;**31**:406-414

[23] Dinan TG, Cryan JF. The impact of gut microbiota on brain and behaviour: Implications for psychiatry. Current Opinion in Clinical Nutrition and Metabolic Care. 2015;**18**:552-558

[24] Kang DW, Adams JB, Gregory AC, Borody T, Chittick L, Fasano A, et al. Microbiota transfer therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: An open-label study. Microbiome. 2017;**5**:1-16

[25] Kumbhare SV, Kumar H, Chowdhury SP, Dhotre DP, Endo A, Mättö J, et al. A cross-sectional comparative study of gut bacterial community of Indian and Finnish children. Scientific Reports. 2017;**7**:1-13

[26] Palleja A, Mikkelsen KH, Forslund SK, Kashani A, Allin KH, Nielsen T, et al. Recovery of gut microbiota of healthy adults following antibiotic exposure. Nature Microbiology. 2018;**3**:1255-1265

[27] Horvath K, Papadimitriou JC, Rabsztyn A, Drachenberg C, Tildon TJ. Gastrointestinal abnormalities in children with autistic disorder. Journal of Pediatrics. 1999;**135**:559-563

[28] Atladottir HO, Henriksen TB, Schendel DE, Parner ET. Autism after infection, febrile episodes, and antibiotic use during pregnancy: An exploratory study. Pediatrics. 2012;**130**:e1447-e1454

[29] Degroote S, Hunting DJ, Baccarelli AA, Takser L. Maternal gut and fetal brain connection: Increased anxiety and reduced social interactions in Wistar rat offspring following peri-conceptional antibiotic exposure. Progress in Neuro-Psychopharmacology & Biological Psychiatry. 2016;**71**:76-82

[30] de Theije CGM, Wopereis H, Ramadan M, van Eijndthoven T, Lambert J, Knol J, et al. Altered gut microbiota and activity in a murine model of autism spectrum disorders. Brain, Behavior, and Immunity. 2014;**37**:197-206

[31] Estes ML, McAllister AK. Maternal immune activation: Implications for neuropsychiatric disorders. Science. 2016;**353**:772-777

[32] O'Mahony SM, Marchesi JR, Scully P, Codling C, Ceolho AM, Quigley EMM, et al. Early life stress alters behavior, immunity, and

microbiota in rats: Implications for irritable bowel syndrome and psychiatric illnesses. Biological Psychiatry. 2009;**65**:263-267

[33] Galley JD, Nelson MC, Yu Z, Dowd SE, Walter J, Kumar PS, et al. Exposure to a social stressor disrupts the community structure of the colonic mucosa-associated microbiota. BMC Microbiology. 2014;**14**:1-13

[34] Zhai Q, Cen S, Jiang J, Zhao J, Zhang H, Chen W. Disturbance of trace element and gut microbiota profiles as indicators of autism spectrum disorder: A pilot study of Chinese children. Environmental Research. 2019;**171**:501-509

[35] Jacobson JL, Muckle G, Ayotte P, Dewailly É, Jacobson SW. Relation of prenatal methylmercury exposure from environmental sources to childhood IQ. Environmental Health Perspectives. 2015;**123**:827-833

[36] Finegold SM, Dowd SE, Gontcharova V, Liu C, Henley KE, Wolcott RD, et al. Pyrosequencing study of fecal microflora of autistic and control children. Anaerobe. 2010;**16**:444-453

[37] Martirosian G, Ekiel A, Aptekorz M, Wiechula B, Kazek B, Jankowska-Steifer E. Fecal lactoferrin and Clostridium spp. in stools of autistic children. Anaerobe. 2011;**17**:43-45

[38] Williams BL, Hornig M, Buie T, Bauman ML, Cho Paik M, Wick I, et al. Impaired carbohydrate digestion and transport and mucosal dysbiosis in the intestines of children with autism and gastrointestinal disturbances. PLoS One. 2011;**6**:e24585

[39] Kang DW, Park JG, Ilhan ZE, Wallstrom G, LaBaer J, Adams JB, et al. Reduced incidence of prevotella and other fermenters in intestinal microflora of autistic children. PLoS One. 2013;**8**:68322

[40] de Angelis M, Francavilla R, Piccolo M, De Giacomo A, Gobbetti M. Autism spectrum disorders and intestinal microbiota. Gut Microbes. 2015;**6**:207-213

[41] Strati F, Cavalieri D, Albanese D, De Felice C, Donati C, Hayek J, et al. New evidences on the altered gut microbiota in autism spectrum disorders. Microbiome. 2017;**5**:1-11

[42] Zhang M, Ma W, Zhang J, He Y, Wang J. Analysis of gut microbiota profiles and microbe-disease associations in children with autism spectrum disorders in China. Scientific Reports. 2018;**8**:13981

[43] Liu S, Li E, Sun Z, Fu D, Duan G, Jiang M, et al. Altered gut microbiota and short chain fatty acids in Chinese children with autism spectrum disorder. Scientific Reports. 2019;**9**:1-9

[44] Borre YE, O'Keeffe GW, Clarke G, Stanton C, Dinan TG, Cryan JF. Microbiota and neurodevelopmental windows: Implications for brain disorders. Trends in Molecular Medicine. 2014;**20**:509-518

[45] Kushak RI, Buie TM, Murray KF, Newburg DS, Chen C, Nestoridi E. Evaluation of intestinal function in children with autism and gastrointestinal symptoms. Journal of Pediatric Gastroenterology and Nutrition. 2016;**62**:687-691

[46] Finegold SM, Molitoris D, Song Y, Liu C, Vaisanen M, Bolte E, et al. Gastrointestinal microflora studies in late-onset autism. Clinical Infectious Diseases. 2002;**35**(Suppl 1):s6-s16

[47] Argou-Cardozo I, Zeidán-Chuliá F. Clostridium bacteria and autism spectrum conditions: A systematic review and hypothetical contribution of environmental glyphosate levels. Medical Science. 2018;**6**:29

[48] Wang L, Christophersen CT, Sorich MJ, Gerber JP, Angley MT, Conlon MA. Increased abundance of Sutterella spp. and Ruminococcus torques in feces of children with autism spectrum disorder. Molecular Autism. 2013;**4**:1

[49] Adams JB, Johansen LJ, Powell LD, Quig D, Rubin RA. Gastrointestinal flora and gastrointestinal status in children with autism—Comparisons to typical children and correlation with autism severity. BMC Gastroenterology. 2011;**11**:22

[50] Williams BL, Hornig M, Parekh T. Application of novel PCR-based methods for detection, quantitation, and phylogenetic characterization of. MBio. 2012;**3**:1-11

[51] Tomova A, Husarova V, Lakatosova S, Bakos J, Vlkova B, Babinska K, et al. Gastrointestinal microbiota in children with autism in Slovakia. Physiology & Behavior. 2015;**138**:179-187

[52] Finegold SM. Desulfovibrio species are potentially important in regressive autism. Medical Hypotheses. 2011;**77**:270-274

[53] Gondalia SV, Palombo EA, Knowles SR, Cox SB, Meyer D, Austin DW. Molecular characterisation of gastrointestinal microbiota of children with autism (with and without gastrointestinal dysfunction) and their neurotypical siblings. Autism Research. 2012;**5**:419-427

[54] Son JS, Zheng LJ, Rowehl LM, Tian X, Zhang Y, Zhu W, et al. Comparison of fecal microbiota in children with autism spectrum disorders and neurotypical siblings in the simons simplex collection. PLoS One. 2015;**10**:1-19

[55] Kantarcioglu AS, Kiraz N, Aydin A. Microbiota-gut-brain axis: Yeast species isolated from stool samples of children with suspected or diagnosed autism spectrum disorders and in vitro susceptibility against nystatin and fluconazole. Mycopathologia. 2016;**181**:1-7

[56] Iovene MR, Bombace F, Maresca R, Sapone A, Iardino P, Picardi A, et al. Intestinal dysbiosis and yeast isolation in stool of subjects with autism spectrum disorders. Mycopathologia. 2017;**182**:349-363

[57] Wang L, Christophersen CT, Sorich MJ, Gerber JP, Angley MT, Conlon MA. Elevated fecal short chain fatty acid and ammonia concentrations in children with autism spectrum disorder. Digestive Diseases and Sciences. 2012;**57**:2096-2102

[58] Andriamihaja M, Lan A, Beaumont M, Audebert M, Wong X, Yamada K, et al. The deleterious metabolic and genotoxic effects of the bacterial metabolite p-cresol on colonic epithelial cells. Free Radical Biology & Medicine. 2015;**85**:219-227

[59] Persico AM, Napolioni V. Urinary p-cresol in autism spectrum disorder. Neurotoxicology and Teratology. 2012;**36**:82-90

[60] Marteau P. Butyrate-producing bacteria as pharmabiotics for inflammatory bowel disease. Gut. 2012;**62**:1673

[61] El-Ansary AK, Ben Bacha AG, Al-Ayahdi LY. Plasma fatty acids as diagnostic markers in autistic patients from Saudi Arabia. Lipids in Health and Disease. 2011;**10**:62

[62] Macfabe DF, Cain N, Boon F, Ossenkopp K, Cain D. Effects of the enteric bacterial metabolic product propionic acid on object-directed behaviour, social behaviour, cognition, and neuro-inflammation in adolescent rats: Relevance to autism spectrum disorder. Behavioural Brain Research. 2011;**217**:47-54

[63] Kelly JR, Kennedy PJ, Cryan JF, Dinan TG, Clarke G, Hyland NP. Breaking down the barriers: The gut microbiome, intestinal permeability and stress-related psychiatric disorders. Frontiers in Cellular Neuroscience. 2015;**9**:392

[64] De Magistris L, Familiari V, Pascotto A, Sapone A, Frolli A, Iardino P, et al. Alterations of the intestinal barrier in patients with autism spectrum disorders and in their first-degree relatives. Journal of Pediatric Gastroenterology and Nutrition. 2010;**51**:418-424

[65] Haba R, Shintani N, Onaka Y, Wang H, Takenaga R, Hayata A, et al. Lipopolysaccharide affects exploratory behaviors toward novel objects by impairing cognition and/or motivation in mice: Possible role of activation of the central amygdala. Behavioural Brain Research. 2012;**228**:423-431

[66] de La Serre C, de Lartigue G, Raybould H. Chronic exposure to low dose bacterial lipopolysaccharide inhibits leptin signaling in vagal afferent neurons. Physiology & Behavior. 2015;**139**:188-194

[67] Grigoleit JS, Kullmann JS, Wolf OT, Hammes F, Wegner A, Jablonowski S, et al. Dose-dependent effects of endotoxin on neurobehavioral functions in humans. PLoS One. 2011;**6**:1-10

[68] Emanuele E, Orsi P, Boso M, Broglia D, Brondino N, Barale F, et al. Low-grade endotoxemia in patients with severe autism. Neuroscience Letters. 2010;**471**:162-165

[69] Neul JL, Sahin M. Therapeutic advances in autism and other neurodevelopmental disorders. Neurotherapeutics. 2015;**12**:519-520

[70] Santocchi E, Guiducci L, Fulceri F, Billeci L, Buzzigoli E, Apicella F, et al. Gut to brain interaction in Autism Spectrum Disorders: A randomized controlled trial on the role of probiotics on clinical, biochemical and neurophysiological parameters. BMC Psychiatry. 2016;**16**:1-16

[71] Liu J, Wan G, Huang M, Agyapong G, Zou T, Zhang X, et al. Probiotic therapy for treating behavioral and gastrointestinal symptoms in autism Spectrum disorder: A systematic review of clinical trials. Current Medical Science. 2019;**39**:173-184

[72] Williams KA, Swedo SE. Post-infectious autoimmune disorders: Sydenham' s chorea, PANDAS and beyond. Brain Research. 1617;**2019**:144-154

[73] Sandler RH, Finegold SM, Bolte ER, Buchanan CP, Maxwell AP, Vaisanen M-L, et al. Short-term benefit from oral vancomycin treatment of regressive-onset autism. Journal of Child Neurology. 2000;**15**:429-435

[74] Frye RE, Slattery J, Macfabe DF, Allen-vercoe E, Parker W, Rodakis J, et al. Approaches to studying and manipulating the enteric microbiome to improve autism symptoms. Microbial Ecology in Health and Disease. 2015;**26**:1-14

[75] Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, et al. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nature Reviews. Gastroenterology & Hepatology. 2017;**14**:491-502

[76] Srinivasjois R, Rao S, Patole S. Probiotic supplementation in children with autism spectrum disorder. Archives of Disease in Childhood. 2015;**100**:505-506

[77] Ng QX, Peters C, Yih C, Ho X, Lim DY, Yeo W. A meta-analysis of the use of probiotics to alleviate depressive

symptoms. Journal of Affective Disorders. 2018;**228**:13-19

[78] Messaoudi M, Lalonde R, Violle N, Javelot H, Desor D, Nejdi A, et al. Assessment of psychotropic-like properties of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rats and human subjects. The British Journal of Nutrition. 2011;**105**:755-764

[79] Dinan TG, Stanton C, Cryan JF. Psychobiotics: A novel class of psychotropic. Biological Psychiatry. 2013;**74**:720-726

[80] Sanders ME. Probiotics and microbiota composition. BMC Medicine. 2016;**14**:1-3

[81] Macfabe DF. Enteric short-chain fatty acids: Microbial messengers of metabolism, mitochondria, and mind: Implications in autism spectrum disorders. Microbial Ecology in Health and Disease. 2015;**26**:1-14

[82] Doenyas C. Gut microbiota, inflammation, and probiotics on neural development in autism spectrum disorder. Neuroscience. 2018;**374**:271-286

[83] Critchfield JW, Van Hemert S, Ash M, Mulder L, Ashwood P. The potential role of probiotics in the management of childhood autism spectrum disorders. Gastroenterology Research and Practice. 2011;**2011**:1-8

[84] Bercik P, Denou E, Collins J, Jackson W, Lu JUN, Jury J, et al. The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. Gastroenterology. 2011;**141**:599-609

[85] Preidis GA, Versalovic J. Targeting the human microbiome with antibiotics, probiotics, and prebiotics: Gastroenterology enters the metagenomics era. Gastroenterology. 2009;**136**:2015-2031

[86] Liu X, Cao S, Zhang X. Modulation of gut microbiota-brain axis by probiotics, prebiotics, and diet. Journal of Agricultural and Food Chemistry. 2015;**63**:7885-7895

[87] Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, et al. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. PNAS. 2011;**108**:16050-16055

[88] Israelyan N, Gross K. Serotonin as a link between the gut-brain-microbiome axis in autism spectrum disorders. Pharmacological Research. 2018;**132**:1-6

[89] Buffington SA, Di Prisco GV, Auchtung TA, Ajami NJ, Pestrosino JF, Costa-Mattioli M. Microbial reconstitution reverses maternal diet-induced social and synaptic deficits in offspring. Cell. 2016;**165**:1762-1775

[90] Wang X, Yang J, Zhang H, Yu J, Yao Z. Oral probiotic administration during pregnancy prevents autism-related behaviors in offspring induced by maternal immune activation via anti-inflammation in mice. Autism Research. 2019;**12**:576-588

[91] El-ansary A, Bacha A, Ben BG, Al-Orf N, Shafi Bhat R, Moubayed N, et al. Probiotic treatment reduces the autistic-like excitation/inhibition imbalance in juvenile hamsters induced by orally administered propionic acid and clindamycin. Metabolic Brain Disease. 2018;**33**:1155-1164

[92] Liu Y, Liu W, Wu C, Juan Y, Wu Y, Tsai H, et al. Psychotropic effects of Lactobacillus plantarum PS128 in early life-stressed and naïve adult mice. Brain Research. 1631;**2016**:1-12

[93] Accordino RE, Kidd C, Politte LC, Henry CA, Mcdougle C. Psychopharmacological interventions in autism spectrum disorder. Expert

Opinion on Pharmacotherapy. 2016;**17**:937-952

[94] Donaldson ZR, Young LJ. Oxytocin, vasopressin, and the neurogenetics of sociality. Science. 2008;**322**:900-904

[95] Blades M. Autism: An interesting dietary case history. Nutrition & Food Science. 2000;**30**:137-139

[96] Parracho HMR, Gibson GR, Knott F, Bosscher D, Kleerebezem M, Mccartney AL. A double-blind, placebo-controlled, crossover-designed probiotic feeding study in children diagnosed with autistic spectrum disorders. International Journal of Probiotics and Prebiotics. 2010;**5**:69-74

[97] Ray S, Sherlock A, Wilken T, Woods T. Cell wall lysed probiotic tincture decreases immune response to pathogenic enteric bacteria and improves symptoms in autistic and immune compromised children. Explore. 2010;**19**:1-5

[98] Kalzuna-Czaplinska J, Blaszczyk S. The level of arabinitol in autistic children after probiotic therapy. Nutrition. 2012;**28**:124-126

[99] West R, Roberts E, Sichel LS, Sichel J. Improvements in gastrointestinal symptoms among children with autism spectrum disorder receiving the Delpro probiotic and immunomodulator formulation. Journal of Probiotics & Health. 2013;**1**:1-6

[100] Partty A, Kalliomaki M, Wacklin P, Salminen S, Isolauri E. A possible link between early probiotic intervention and the risk of neuropsychiatric disorders later in childhood: A randomized trial. Pediatric Research. 2015;**77**:823-828

[101] Grossi E, Melli S, Dunca D, Terruzzi V. Unexpected improvement in core autism spectrum disorder symptoms after long-term treatment with probiotics. SAGE Open Medical Case Reports. 2016;**4**

[102] Shaaban SY, El Gendy YG, Mehanna NS, El-Senousy WM, El-feki HSA, Saad K, et al. The role of probiotics in children with autism spectrum disorder: A prospective, open-label study. Nutritional Neuroscience. 2017;**21**:676-681

[103] Liu Y-W, Liong M-T, Chung Y-C, Huang H-Y, Peng W-S, Cheng Y-F, et al. Effects of Lactobacillus plantarum PS128 on children with autism spectrum disorder in Taiwan: A randomized, double-blind. Placebo-Controlled Trial. Nutrients. 2019;**11**:820

[104] Kobliner V, Nutrition H, Mumper E. Reduction in obsessive compulsive disorder and self-injurious behavior with *Saccharomyces boulardii* in a child with autism: A case report. Integrative Medicine (Encinitas, Calif.). 2019;**17**:14-17

[105] Sanctuary MR, Kain JN, Chen SY, Kalanetra K, Lemay G, Rose DR, et al. Pilot study of probiotic/colostrum supplementation on gut function in children with autism and gastrointestinal symptoms. PLoS One. 2019;**14**:1-30

[106] Russo AJ. Decreased plasma myeloperoxidase associated with probiotic therapy in autistic children. Clinical Medicine Insights: Pediatrics. 2015;**9**:13-17

[107] Golnik AE, Ireland ÆM. Complementary alternative medicine for children with autism: A physician survey. Journal of Autism and Developmental Disorders. 2009;**39**:996-1005

[108] Shaw W, Kassen E, Ascp MT, Chaves E. Assessment of antifungal drug therapy in autism by measurement of suspected microbial metabolites in urine with gas chromatography-mass

spectrometry. Clinical Practice of Alternative Medicine. 2000;**1**:15-26

[109] Gibson G, Roberfroid M, De Louuain C. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. The Journal of Nutrition. 1995;**125**:1401-1412

[110] Savignac HM, Corona G, Mills H, Chen L, Spencer JPE, Tzortzis G, et al. Prebiotic feeding elevates central brain derived neurotrophic factor , N-methyl-D-aspartate receptor subunits and D-serine. Neurochemistry International. 2013;**63**:756-764

[111] Savignac HM, Couch Y, Stratford M, Bannerman DM, Tzortzis G, Anthony DC, et al. Prebiotic administration normalizes lipopolysaccharide (LPS)-induced anxiety and cortical 5-HT2A receptor and IL1-b levels in male mice. Brain, Behavior, and Immunity. 2016;**52**:120-131

[112] Grimaldi R, Gibson GR, Vulevic J, Giallourou N, Castro-mejía JL, Hansen LH, et al. A prebiotic intervention study in children with autism spectrum disorders. Microbiome. 2018;**6**:1-13

[113] Bagdasarian N, Rao K, Malani P. Diagnosis and treatment of *Clostridium difficile* in adults: A systematic review. Journal of the American Medical Association. 2015;**313**:398-408

[114] Cui B, Feng Q, Wang H, Wang M, Peng Z, Li P, et al. Fecal microbiota transplantation through mid-gut for refractory Crohn' s disease: Safety, feasibility, and efficacy trial results. Journal of Gastroenterology and Hepatology. 2015;**30**:51-58

[115] Vrieze A, ELSVAN N, Holleman F, Salojärvi J, Kootte RS, Bloks VW, et al. Transfer of intestinal microbiota from lean donors increases insulin. Gastroenterology. 2012;**143**:913-916.e7

[116] Kang D, Adams JB, Co DM, Pollard EL, Maldonado J, Mcdonough-means S, et al. Long-term benefit of microbiota transfer therapy on autism symptoms and gut microbiota. Scientific Reports. 2019;**9**:5821

[117] Doenyas C. Dietary interventions for autism spectrum disorder: New perspectives from the gut-brain axis. Physiology & Behavior. 2019;**194**:577-582

[118] Whitely P, Rodgers J, Savery D, Shattock P. A gluten-free diet as an intervention for autism and associated spectrum disorders: Preliminary findings. Autism. 1999;**3**:45-65

[119] Adams SJ, Burton N, Cutress A, Adamson A, McColl E, Hare A, et al. Development of double blind gluten and casein free test foods for use in an autism dietary trial. Journal of Human Nutrition and Dietetics. 2008;**21**:374

[120] Hediger ML, England LJ, Molloy CA, Yu KF, Manning-Courtney P, Mills JL. Reduced bone cortical thickness in boys with autism or autism spectrum reduced bone cortical thickness in boys with autism or autism spectrum disorder. Journal of Autism and Developmental Disorders. 2008;**38**:848-856

[121] Autism Speaks. Autism and Health: A Special Report by Autism Speaks. Advances in Understanding and Treating the Health Conditions that Frequently Accompany Autism. Autism Speaks; 2017, New York, USA.

[122] Castro K, Slongo L, Baronio D, Gottfried C, Schweigert I, Riesgo S. Research in autism spectrum disorders effect of a ketogenic diet on autism spectrum disorder: A systematic review. Research in Autism Spectrum Disorder. 2015;**20**:31-38

[123] Ruskin DN, Svedova J, Cote JL, Sandau U, Rho JM, Kawamura M, et al. Ketogenic diet improves core symptoms of autism in BTBR mice. PLoS One. 2013;**8**:4-9

[124] Newell C, Bomhof MR, Reimer RA, Hittel DS, Rho JM, Shearer J. Ketogenic diet modifies the gut microbiota in a murine model of autism spectrum disorder. Molecular Autism. 2016;**7**:1-6

[125] Ballaban-gil K, Dell O, Pappo SM, Moshc S, Shinnar S. Complications of the ketogenic diet. Epilepsia. 1998;**39**:744-748