Autism and Gut–Brain Axis: Role of Probiotics

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Abstract Characterized by a wide range of behavioural, social and language problems, autism is a complex developmental disability that affects an individual's capacity to communicate and interact with others. Although the real causes that lead to the development of autism are still unclear, the gastrointestinal tract has been found to play a major role in the development of autism. Alterations in macrobiotic compositions have been reported in autistic children. Irregularities in carbohydrate

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digestion and absorption could also explain some of the gastrointestinal problems reported in autistic patients, although their role in the neurological and behavioural problems remains uncertain. A relationship between improved gut health and decrease of symptoms in autism has been reported as well. Studies done to evaluate the gluten-free diets, casein-free diets, pre- and probiotic and multivitamin supplementation have shown promising results. Probiotics have been thought to alleviate the progression of autism and reduce cognitive and behavioural deficits.

Keywords Autism · ASD · Cognitive and behavioural deficits · Gut–brain axis · GI dysfunction · Barrier pathway · Microbiome · Probiotics

1 Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by disrupted social and communication interactions with stereotyped and repetitive behaviour of different levels of severity. ASD has traditionally been framed as a behavioural disorder. However, evidence is accumulating that ASD is characterized by certain physiological abnormalities, including oxidative stress, mitochondrial dysfunction and immune dysregulation/inflammation. The brain regions found to contain these physiological abnormalities in individuals with ASD are involved in speech and auditory processing, social behaviour, memory and sensory and motor coordination. Though genetics play a vital role in the cause of the disease, recent investigations have strongly suggested nutritional deficiencies and imbalances as also contributing to and aggravating autism. Studies have shown that people with autism often have abnormal digestive health conditions. Research indicates there is a strong link between the functioning of the brain and the gut where postnatal development of a child depends on the microbiome. Some experts claim that several types of food and diet interventions can treat (social and behavioural management) children and adults with ASD. In this chapter, we discuss various theories as well as the effectiveness of diets and probiotics, the so-called “friendly bacteria”, in helping ease the symptoms of autism. Also, we present some notable findings, demonstrating probiotic success in relieving GI symptoms in autistic kids as well as its efficacy in controlling the children’s anxiety and oversensitivity to stimuli.

2 Altered Gut–Brain Axis in Autism

The occurrence of gastrointestinal (GI) problems due to alterations in the gut microbiota has been documented in autism based on existing patient observations. This complexity in the crosstalk between the gut and the brain has been discussed broadly as the “gut–brain axis” or GBA. The triad of GBA, immune system and GI

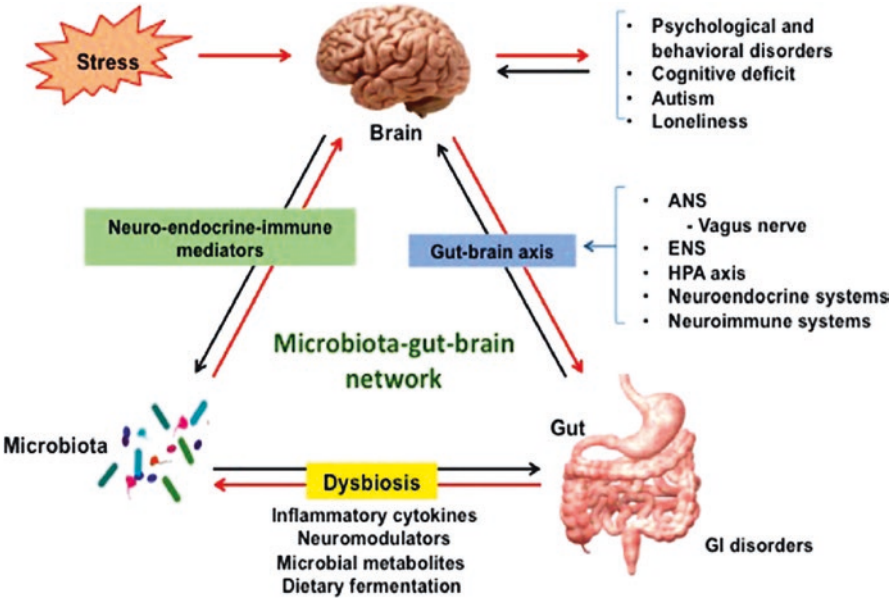

Fig. 1 The triad of gut–brain axis, immune system and GI microbiome. Adapted from Sirisinha [1] and Thakur et al. [2]

microflora cross-communication is illustrated in Fig. 1. Various studies involving animal models of different behavioural disorders such as autism, anxiety and cognitive dysfunction have shown that the constitution of the gut microbiota has an influence in the brain functions. It functions by monitoring and integrating the gut functions along with the emotional and cognitive centres of the brain and the peripheral intestinal functions and mechanisms such as immune activation, intestinal permeability, enteric reflex and entero-endocrine signalling [3]. Cognitive and behavioural alterations, induced due to various neuroactive compounds in the intestinal lumen crossing the blood–brain barrier [4]. The link between GI symptoms and neurodevelopmental disorders has been supported by the following observations:

- The onset of disease usually follows antimicrobial therapy.
- At the advent of the disease, frequent persistence of a number of gastrointestinal abnormalities has been observed.
- Autistic symptoms have sometimes been reduced by oral vancomycin treatment, while relapse occurs following cessation of treatment.

The gut–brain axis accesses the signal from the gut microbiota, influences the brain functions and vice versa. The bidirectional communication acts via the neuroendocrine and neuroimmune mechanism which involves both the autonomic nervous system (ANS) and the enteric nervous system (ENS). The fundamental morphologic components of the brain to gut microbiota signalling are the sympathetic and parasympathetic nerves of the ANS [5]. The sympathetic system inhibits

the intestinal motor functions and decreases gut secretion. Under conditions of stress, the sympathetic system is over activated, the integrity of the gut epithelium is destroyed and gut motility and secretions are changed [6]. Stress-induced changes of the gut alter the habitat of resident bacteria and promote alterations to microbiota composition or activity [7]. The hypothalamus–pituitary–adrenal (HPA) axis is another critical mechanism by which the brain influences the composition of the gut microbiota. When the HPA axis is overactivated, the levels of circulating cortisol and pro-inflammatory cytokines are significantly elevated [8].

The human GI tract contains approximately 10^4 bacteria belonging to approximately 1000 species. The healthy adult GI tract is most dominated by *Bacteroidetes* and *Firmicutes* phyla (both account for up to 70–90% of total bacteria), followed by *Actinobacteria, Proteobacteria* and *Verrucomicrobia* [9]. The symbiotic microbiota dwelling in the gut have long been appreciated for the various beneficial effects they offer to the host, including providing essential nutrients by metabolizing indigestible dietary compounds, defending the gut against opportunistic pathogen colonization by nutrient competition and antimicrobial substance production and contributing to the intestinal epithelial barrier. Moreover, studies on the immune defects in germ-free (GF) mice have suggested that gut microbiome is essential to the host immune system [10]. A recent review also indicated that gut bacterial colonization could drive maturation and functionality of the host's adaptive immune system [11, 12].

Bifidobacterium, a beneficial bacteria appeared to be reduced in children with autism [13], while other probiotics, i.e. *Lactobacillus*, *Bacteroides* and *Desulfovibrio*, were reported to be present in higher concentrations amongst them [14]. Consistent with this, the abundances of *Blautia*, *Dialister*, *Prevotella*, *Turicibacter* and *Veillonella* were all decreased [15]. Children with autism had much lower levels of *Bifidobacterium* (−45%, $p = 0.002$), slightly lower levels of *Enterococcus* (−16%, $p = 0.05$ per Wilcox) and much higher levels of *Lactobacillus* (+100%, $p = 0.00003$) [14].

Potentially harmful *Clostridium* species were observed to be abundant in faeces of children with autism [16]. Recent studies related to faecal microbial profiles of autistic patients have also indicated tenfold higher counts of *Clostridium spp*., which produce neurotoxins and contribute to the development of autistic behaviours, compared with healthy controls [17].

In addition, De Angelis et al. indicated that *Oscillospira* decreased and *Roseburia* increased in Alzheimer's Disease (AD). Meanwhile it was also observed that opportunistic pathogens like *Enterobacter* and *Shigella* were elevated in the case of AD patients [18]. *Faecalibacterium* [19] and *Ruminococcus* [20] were also reported to increase in patients with autism. Notably, these particular species are known to be versatile carbohydrate metabolizers [21]. *Blautia* plays an important role in nutrient assimilation and gut maturation in children [22]. The reduction of these beneficial bacteria in autism patients may be implicated in the pathogenesis of the disease. Short-chain fatty acids (SCFAs), the critical mediators within the microbiota–gut–brain axis, can cross the blood–brain barrier (BBB) and modulate brain activity directly [4].

Therefore, a number of possible mechanisms have been postulated relating the gut microbiome and the brain axis in autism. Few of these pathways are discussed below.

2.1 The Barrier Pathway

An increase in intestinal permeability was found in patients with autism, and this was measured by the lactulose/mannitol test [23]. One particular study showed that the impaired intestinal and blood–brain barrier function in autism decreased the level of intestinal tight junction (TJ) components and caused an increase in the Claudin level in the autism brain when compared to a group of controls [24]. The microbiota along with its metabolites contributes to the regulation of the intestinal barrier. The dysbiosis in the case of autism is a result of increased permeability of the gastrointestinal tract which is referred to as the "leaky gut". The "leaky gut" allows bacterial metabolites, metabolites that do not naturally cross this barrier and are potentially neuroactive, to readily cross the intestinal barrier. Studies have shown evidence of increased metabolites in urine and systemic circulation in autism [25]. Zonulin has structural similarities with several growth factors known to affect intercellular TJ integrity. This enzyme regulating intestinal permeability was seen to be significantly increased in subjects with autism bearing GI symptoms also showing hampered intestinal permeability in the disease condition [26]. Hence, a disrupted intestinal barrier allows endotoxins to enter the bloodstream. For instance, lipopolysaccharide (LPS) is a potent endotoxin which alters neuronal and microglial activity in the amygdala, a region involved in control of emotions [27]. In patients with autism, the serum LPS levels were significantly high when compared to healthy individuals, and this could be correlated with impairment in social behavioural scores [28]. Targeting improvement in the epithelial barrier in autism can reduce the entrance of the microbial endotoxins, thus normalizing the gut–brain pathway. The BBB acts as a shield against the infiltration of pathogens and other endotoxins entering the brain. In order to maintain brain functions and development, it is necessary to maintain the integrity of this barrier. BBB dysfunction can be caused by multiple prenatal and postnatal risk factors that are also evident in autism [29]. Several of these risk factors are detailed in Chap. "Overview and Introduction to Autism Spectrum Disorder (ASD)".

2.2 The Serotonin Pathway

The serotonin functions in the brain both for regulation of mood and cognition and for regulating intestinal secretion, motility and pain perception. Its synthesis in the intestine and the brain depends upon the intake of dietary tryptophan [30].

Serotonin synthesis in the brain is decreased in patients with autism. A recent study demonstrated the correlation between whole blood serotonin level and the intestinal symptoms in autism [31]. Inflammation in the intestinal tract leads to production of serotonin by the enterochromaffin cells and intestinal mast cells. This leads to alteration in motility, vasodilation and an increase in vascular permeability, causing functional intestinal dysmotility. During intestinal tract inflammation, there is increased consumption of dietary tryptophan, causing low concentrations to be available for the brain. Thus, brain serotonin levels will be reduced causing mood and cognitive dysfunction in autism. On depleting dietary tryptophan, indeed an increase in autistic behaviour was observed in autism patients. Also, the availability of tryptophan was seen to be affected in the case of intestinal dysbiosis in autism [32].

In a murine model of autism induced by prenatal exposure to valproic acid (VPA), impairments in social behaviour were associated with intestinal inflammation and a disturbed serotonergic system in the brain and intestinal tract [32]. In the prefrontal cortex as well as in the amygdale, reduced levels of serotonin and increased turnover were found in VPA-exposed male offspring. The reduction in intestinal serotonin in VPA-exposed mice was attributable to reduced number of serotonin-positive cells (possibly enterochromaffin cells) in the small intestine [33].

2.3 Immune System Pathway

The gut microbiota can also be related to cerebral dysfunctions by modulating the host immune response. Pathogenic and bacterial microbiota stimulate the secretion of pro-inflammatory cytokines like IL-1, IL-6 and IL-8 by the intestinal epithelial cells, dendritic cells and macrophages [34], which account for various neuropsychiatric disorders including anxiety, schizophrenia as well as autism [35]. Parents of autism children report more often food allergies than parents of healthy children [36]. The persistent default state of mucosal immune tolerance observed in food allergy is strongly associated with a changed microbiota composition such as enhanced *Bacteroidetes* and *Enterobacter*. The majority of allergies are characterized by a T-helper 2-type immune response with the characteristic cytokines interleukin (IL) 4, IL5 and IL13. Supporting the role of allergy in autism, children produced significantly higher levels of the mentioned cytokines [37]. In addition, less IL-10-producing T cells are present in the periphery and intestinal mucosa as well as reduced plasma levels of tumour necrosis factorβ in autism patients suffering from intestinal problems [38]. Taken together, there seems to be a disturbed T-cell balance in the intestinal tract of autism patients.

2.4 Neuronal Pathway

Another possible mechanism by which the microbiota–gut–brain axis mediates communication may be through the use of established neuronal circuits. Vagal afferents are critical neuronal pathways allowing information flow from the viscera to the CNS. Gut microbiota can deliver their signals to the brain via the vagus nerve [5]. In a study with autism patients suffering from epilepsy, besides reducing the seizure frequency, stimulation of the vagus nerve resulted in improved verbal skills, mood and alertness [39]. Epilepsy has been observed in about 30% of autistic patients [40].

It might be that microbial neurotransmitters affect the ENS and afferent nerve function directly or via the intestinal epithelium. Based on the fact that stress-related host neurotransmitter release increases the proliferation rate and the activity of intestinal microbiota [41], it has been postulated that microbiota-derived neurotransmitters have a primary role in the sustainability of the microbes themselves in the intestinal tract in stressful situations [29]. In fact, neurochemical and behavioural effects were not present in vagotomized mice, identifying the vagus as the major modulatory constitutive communication pathway between the microbiota and the brain [42]. These data suggest that vagus stimulation, possibly through a "healthy" microbiome, might be beneficial in autism. Taken together, the role of the ENS, the vagus nerve and bacterial neuroactive metabolites and molecular pathways in relation to the microbiome–gut–brain axis remains to be established in autism.

Recent research has indicated that the effect of the gut microbiota extends much beyond the modulation of the gut itself. Metabolites derived from the microbiota can be absorbed and transported by the blood before crossing the BBB to modulate cerebral function. For example, strains of *Lactobacillus rhamnosus* YS9 are able to produce gamma-aminobutyric acid (GABA), an important inhibitory neurotransmitter in the brain [26].

A large percentage of autism patients have a history of extensive antibiotic use. Oral antibiotics (i.e. β-lactams) disrupt the protective microbiota and cause the proliferation of anaerobic bacteria in the gut. For example, *Clostridia*, *Bacteroidetes* and *Desulfovibrio* are common bacteria that may promote GI symptoms and autistic behaviours in autism [43].

3 Probiotics in Autism

The internationally accepted definition of probiotics is "live microorganisms which when administered in adequate amounts confer a health benefit on the host". Probiotics are typically administered as a food supplement promoting various health benefits to the host by maintaining the stability and composition of the intestinal and gut microbiota and increasing resistance against various pathological infections [44].

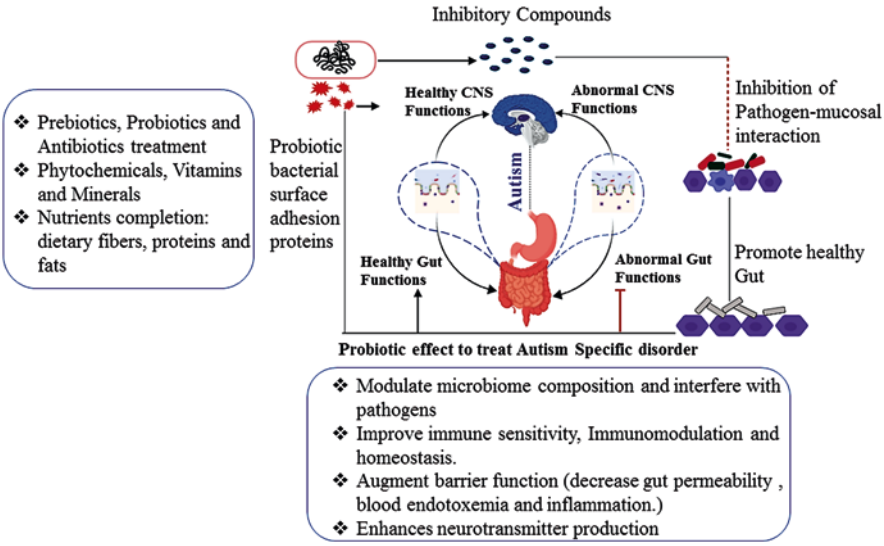

Fig. 2 Probiotic influence of gut microflora and ASD behavioural abnormalities. Adapted from Poornachandra Rao and Sreenivasa [47] and Sánchez et al. [48]

The potentially synergistic combinations of pro- and prebiotics are called symbiotics [45]. Various preclinical and clinical findings have suggested that treatment with probiotics can help improve gastrointestinal health, thereby stabilizing behavioural abnormalities in adults and children with autism [46]. The probiotic influence of gut microbiome and ASD behavioural abnormalities is depicted in Fig. 2.

Currently, the consumption of probiotic cells via food products has been categorized as functional foods, the worldwide market for which had been predicted to increase from 33 billion in 2000 to 176.7 billion in 2013. About 60–70% of the total food market comprises of probiotic foods [49]. There has been remarkable success in the past few decades in producing dairy products like ice cream, flavoured liquid milk, fermented milk, milk powder, baby food, frozen dairy products, buttermilk, cheese and many others which contain probiotics and can be safely administered in all these forms. One of the key aspects for probiotics to gain such rapid momentum is that they are safe, comparatively cheap and an accessible target for microbial infections. The World Health Organization (WHO) in 1994 considered probiotics to be used as an effective immune defence system in the cases of antibiotic resistance. This treatment was termed as microbial interference therapy [50].

The probiotic microbes are artificially introduced into the food at the time of its production. Most of the cultures are commercially available in extremely concentrated form as either freeze-dried powders or highly concentrated frozen cultures. Some of the popularly used probiotic microorganisms are *Lactobacillus rhamnosus*, *Lactobacillus reuteri*, *Bifidobacteria* and certain strains of *Lactobacillus casei* and *Lactobacillus acidophilus* group. Other microbes include *Bacillus coagulans*;

Escherichia coli strains; certain enterococci, especially *Enterococcus faecium* SF68; and the yeast *Saccharomyces boulardii*. Probiotic products may contain either a single strain or a mixture of two or more strains. The effect of probiotics is very strain specific and cannot be generalized. A single strain may exhibit different benefits when used individually and in combination. The benefits of a probiotic formulation also differ by the patient group [45]. Once probiotics are incorporated in the food, its effectiveness depends upon the total number of viable cells per ml and also the number of active cells present on being consumed by the individual [51].

It is also noted that on addition of the probiotic microbes, the aroma and the flavours in the food are modified due to the formation of metabolic components during fermentation such as the synthesis of acetic acid by *Bifidobacterium spp.* Therefore, in order to attain product quality and patient competence for its administration, necessary steps are taken in order to eradicate the smell and aftertaste [52].

The known mechanisms by which probiotic bacteria have an impact on the gut microbiota may be as follows:

1. Competition for dietary ingredients as growth substrates
2. Bioconversion of, for example, sugars into fermentation products with inhibitory properties
3. Production of growth substrates
4. Direct effect on pathogens
5. Competitive exclusion for binding sites
6. Improved barrier function
7. Reduction of inflammation, thus altering intestinal properties for colonization and persistence within and
8. Stimulation of innate immune response

In a recent study using a rodent model of autism, the alteration in the gut microbiota and the related alteration in serum metabolites were considered to play an important role in the behavioural manifestation of autism-like behaviour and subsequent GI function alteration. However, these changes were seen to be rapidly reversible by ingestion of probiotics [53].

There have been interesting findings made in human autism research where the main microbiota intervention in the clinical study is the probiotic administration. One such study used faecal transplantation. In this study, it was observed that on being treated with probiotics for over 6 months ($n = 6$), children diagnosed with autism showed a decrease in the severity of diarrhoea and constipation. Each participant received a 6-month supply of DelPro® containing 10 billion CFUs of different probiotic strains including *L. acidophilus, L. casei, Lactobacillus delbrueckii, Bifidobacterium longum* and *B. bifidum* and 8 mg of Del-Immune V® powder, containing peptidoglycan, muramyl peptides and nucleotide-containing components or DNA motifs that is derived from the *L. rhamnosus V* strain. Any other probiotics were advised to be discontinued. An Autism Treatment Evaluation Checklist (ATEC) score, which reflects the changes occurring in autism patients and accesses various domains like speech/language/communication, sociability,

sensory/cognitive awareness and health/physical/behaviour, was studied. Thus, in this study following probiotic treatment, an 88% improvement in the above-mentioned domains of autism was observed overall. The mean ATEC value decreased from 72.8 to 58.3 [54].

Parracho et al. carried out a randomized, double blind, controlled study with children from age 3 to 16 suffering from autism and divided them into a placebo and probiotic group. The probiotic group was given 4.5 × 1010 CFU *Lactobacillus plantarum* WCFS1 daily over a period of 6 weeks. Group I received placebo during the first feeding period (3 weeks) and probiotic during the second feeding period (3 weeks), and vice versa for group II (i.e. probiotic first). Improvements in destructive and antisocial behaviour, anxiety and communication problems were observed in the children with autism who were treated with probiotics [55].

A previous study showed that in the case of the offspring of an immune-activated mother, gut permeability was affected, an abnormal increase in the level of cytokines and gut dysbiosis was observed and hence changes in neuropathological and behavioural autism features due to changes caused by *Clostridia* and *Bacteroidia* in the gut environment were seen. On the treatment with probiotic *Bacteroides fragilis,* intestinal permeability was improved in MIA, and this specifically increased pro-inflammatory cytokine IL-6 in the colon. It was also seen that this treatment could restore 6 out of 67 bacterial species units which are compromised in the case of autism patients. An improvement in communication, repetitive sensorimotor and anxiety of the MIA offspring was achieved. However, they also found that the effect of *B. fragilis* on autism behaviour was seen when treated with *Bacteroides thetaiotaomicron* and not on treating with *Enterococcus faecalis.* Thus, from this study it was inferred that treatment with probiotics in autism relieved certain symptoms by reducing inflammation, improving the gut permeability, restoring microbial imbalances and ameliorating nonsocial autism symptoms [56].

In another clinical study, all the autism patients suffering from severe GI problems were grouped. The participants received probiotic capsules of *L. acidophilus* (strain Rosell-11, containing 5 × 109 CFU/g) orally, twice daily for 2 months. The use of antibiotics during therapy was restrained, and urine samples were collected for each of the participants. Prior to treatment, the level of D-arabinitol was significantly higher in the urine of children with autism and was seen to decrease thereafter. The autistic symptoms such as concentration and following out orders also improved after the probiotic therapy [57].

In a trial with autism patients ($n = 11$), an oral liquid dose of vancomycin 500 mg/day was given, thrice a day for 8 weeks. This was followed by probiotic therapy given orally for 4 weeks, comprising of a mixture of *L. acidophilus*, *L. bulgaricus* and *B. bifidum* (40 × 109 CFUs/ml). This suggested that multiple probiotic therapy led to short-term pre- and post-therapy improvement in communication as well as pattern behaviours [58].

From the results obtained by researchers on examining a group of children with autism ($n = 22$) of ages four to ten where the patients were administered a sugar-free diet and probiotic capsules of *L. acidophilus* (5 × 109 CFU/g) for a period of 2 months, twice daily, major changes were observed in the behavioural domains

with significant improvement in concentration and the ability to follow instructions. However, there was no improvement in other distinct behaviours and the ability to make eye contact [59].

4 Conclusion

Perturbation of GI tract bacterial microflora may play an important role in the pathophysiology of some digestive tract disorders. Probiotics have been used as a treatment modality for over a century. Microbial modification with the use of antibiotics, probiotics and faecal transplantation has been effective in the treatment of GI conditions. They may restore normal bacterial microflora and effect the functioning of the GI tract by a variety of mechanisms. Gut microbiome-related changes are seen in children with autism compared to normally developed children. Virtually all of the GI functions postulated to be impaired in ASD have been shown to be improved by probiotics in animal studies. Evidence suggests that probiotics can have beneficial effects for people with autism as well. However, many questions regarding the use of probiotics in GI disorders remain to be answered in future studies, such as most optimal doses, duration of treatment, physiological and immunological effects, efficacy of specific probiotics in specific disease states and safety in debilitated patients, since there is a complex interplay in these conditions between GI function (motility, secretion, permeability), the immune system and the microbiota.

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