The brain-gut axis allows bidirectional communication between the central nervous system (CNS) and the enteric nervous system (ENS), linking emotional and cognitive centers of the brain with peripheral intestinal functions. Recent experimental work suggests that the gut microbiota have an impact on the brain-gut axis. A group of experts convened by the International Scientific Association for Probiotics and Prebiotics (ISAPP) discussed the role of gut bacteria on brain functions and the implications for probiotic and prebiotic science. The experts reviewed and discussed current available data on the role of gut microbiota on epithelial cell function, gastrointestinal motility, visceral sensitivity, perception and behavior. Data, mostly gathered from animal studies, suggest interactions of gut microbiota not only with the enteric nervous system but also with the central nervous system via neural, neuroendocrine, neuroimmune and humoral links. Microbial colonization impacts mammalian brain development in early life and subsequent adult behavior. These findings provide novel insights for improved understanding of the potential role of gut microbial communities on psychological disorders, most particularly in the field of psychological comorbidities associated with functional bowel disorders like irritable bowel syndrome (IBS) and should present new opportunity for interventions with pro- and prebiotics.

**Introduction**

Neurogastroenterology is a research area in the field of gastroenterology which regards the interactions of the central nervous system (CNS) (brain) and the gut—the so-called “brain-gut axis”. The brain-gut axis is a bidirectional communication system and includes the CNS composed of the brain and spinal cord and the enteric nervous system (ENS), involving nerves, hormones and other molecules such as neuropeptides and cytokines.1 The roles of the brain-gut axis are to monitor and integrate gut function and to mediate environmental effects such as hunger, stress and emotions on gut functions. Stress and emotions may be reflected by changes in gut physiology and gut symptoms.

Microbes in the gastrointestinal (GI) tract are represented by a wide variety of bacterial species. They can exert numerous effects on the intestinal neuroimmune system and influence a variety of host functions such as metabolic activity, immune response and physiological function.2 The gut microbiota (whose genes represent the intestinal microbiome) composition and activity is subject to a variety of influence including host physiology, immunology, diet, antibiotic usage and enteric infection. Microbial dysbiosis is associated with gastrointestinal and metabolic disorders. A growing body of evidence suggests that the host-microbial interaction may result in dysregulated neuroimmune functions, impacting behavior.3,4 Probiotic bacteria, “live microorganisms which when administered in adequate amounts confer a health benefit on the host,” and dietary prebiotics, “selectively fermented dietary ingredients that result specific changes, in the consumption and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health” (www.isapp.net), have been used in...
animal studies and human clinical trials to improve peripheral (gastrointestinal) and central (psychological) symptoms.

This review highlights the reviewed research and discussions by the expert group on “Probiotics and prebiotics in neurogastroenterology” at the 2011 annual meeting of the International Scientific Association for Probiotics and Prebiotics (ISAPP). Some of the main questions that the experts addressed were: What are the effects of the gut microbiota on the brain-gut axis? What is the impact of the brain-gut axis on the gut microbiota? What is the relevance of data obtained from in vitro, animal studies and clinical studies for human health?

The experts summarized the evidence related to the role of the interactions between the gut microbiota and the host’s brain-gut axis communications. Data were gathered from recent observations in animal studies (summarized in Table 1) and in human studies involving patients with functional bowel disorders and associated psychological comorbidities. The experts also reviewed recent evidence of the impact of the neuroimmune system on the composition of the microbiota (Fig. 1). Finally, recommendations are provided for future research on this topic as well as for advancing the translation of the data of the effects of probiotics and prebiotics observed in vitro or in animal models to clinical interventions in the area of functional bowel disorders.

**Effects of the Intestinal Microbiome on the Brain-Gut Axis**

Effects of the intestinal microbiome and probiotics on the ENS. The ENS, often referred to as “the second brain”, is a part of the peripheral nervous system and a division of the autonomic nervous system which controls the function of the gastrointestinal tract. The ENS is embedded within the wall of the digestive tract and extends between the esophagus and anus. It contains thousands of ganglia and approximately 400 million neurons, more than any other peripheral organ and about the same number of neurons as the spinal cord. This system is capable of autonomous function, controlling the digestive system local physiological state. The ENS is involved in several functions including: control of gastrointestinal motility, sensation, regulation of fluid exchange, local blood flow, gastric and pancreatic secretion, gastrointestinal endocrine functions, defense reactions and entero-enteric reflexes.

Embedded within the wall of the GI tract, the ENS is “protected” from the luminal content by the intestinal barrier. The intestinal barrier function can be viewed in different ways. On one level barrier function is the prevention of diffusion of ions and small solutes across the single layer of epithelial cells that protects underlying compartments, including the ENS, from the noxious contents of the lumen. Tight junctions provide this level of protection as a result of the many transmembrane proteins including occludins, various claudins, junctional adhesion molecules (JAMs) and cingulin. Homotypic and/or heterotypic interactions between these proteins as well as charge differences protect movement across the paracellular space. Much of the inflammation-associated increase in intestinal permeability is now recognized to be driven by the impact of proinflammatory cytokines, including tumor necrosis factor-α (TNF), on signaling pathways, specifically myosin light chain kinase (MLCK), that govern tight junction permeability. In this case, MLCK phosphorylates myosin light chain, which interacts with actin, driving cytoskeletal contraction of the perijunctional actomyosin ring that underlies tight junctions thus, increasing paracellular permeability. On a more inclusive level, other factors contributing to intestinal barrier function including mucus layers, glycocalyx, secretory IgA, antimicrobial peptides, chloride and water secretion and the intestinal microbiota.

The gut microbiota and certain probiotics can promote the development of the intestinal barrier. Some specific probiotic strains altered the expression of tight junction proteins including occludin, cingulin, claudin-2 and ZO-2. Evidence also suggests that commensal and probiotic organisms bolstered additional aspects that contribute to intestinal barrier function including increased IgA production, mucin expression, prevention of intestinal epithelial cell apoptosis, inhibition of colonization by enteric pathogens and the immune response. Specific Lactobacillus strains upregulated the expression of key intestinal epithelial transporters such as the apical anion exchanger SLC26A3 (DRA) responsible for the bulk of Cl⁻ absorption by the gut suggesting a mechanism for therapeutic benefit in the treatment of diarrhea. Treatment with *Saccharomyces boulardii* reduced calbindin 28k myenteric neurones in pig’s jejunum receiving *S. boulardii*. (S. cerevisiae HANSEN CBS 5926).

In addition to their effect on the intestinal barrier, pathogens or probiotics can have an impact on the ENS. Microbes or their byproducts directly target intestinal sensory nerves. Cholera toxin releases 5-HT from the mucosa that acts on 5-HT3 receptors on sensory nerves. Conversely, certain probiotics can have opposite effects and reduced pain induced by colorectal distension in rats. *L. acidophilus NCFM* induced analgesic effect by increasing expression of intestinal mucosa opioid and cannabinoid receptors in rats. A variety of other probiotics, (i.e., *L. farciminosus*), prevented stress-induced hypersensitivity or reduced the excitability of enteric neurons in models of mice colitis or of gut dysfunction. *L. paracasei* NCC 2461 reversed rectal hyperalgesia in a model of maternal deprivation in rats. Interestingly, these effects have been observed not only with live strains, but also with dead (heat-killed) or even conditioned probiotic medium.

Effects of the intestinal microbiome and probiotics on brain-gut communications. The autonomic nervous system (ANS) consists of sensory and motor neurons that run between the CNS and different internal organs, including the GI tract. Its actions are largely involuntary. The ANS is divided into the sympathetic and parasympathetic system. The neurotransmitters of the pre- and post-ganglionic neurons are acetylcholine and noradrenaline [or norepinephrine (NE)] respectively. Activation of the sympathetic branch of the autonomic nervous system helps prepare and adjust the body for emergencies and acute change in homeostasis including the inhibition of gut peristalsis. The parasympathetic system returns the body functions to normal after they have been altered by sympathetic stimulation.
Table 1. Summary of studies performed in animal models evaluating the effect of the gut microbiota and/or changes induced by probiotic and prebiotic interventions

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Abbreviations: ANS, autonomous nervous system; CNS, central nervous system; ENS, enteric nervous system; EPM, Elevated plus maze; GF, germ-free; HPA, hypothalamic-pituitary-adrenal; IBS, irritable bowel syndrome; SPF, specific pathogen free.
Additionally, communication between the immune system (i.e., cytokines) and the nervous system cytokines play important roles in brain-gut communications.

Effect of probiotics on various mediators of the brain-gut axis have been demonstrated with several probiotics: *Bifidobacterium infantis* decreased pro-inflammatory cytokines in maternally deprived offspring rats, an animal model that has been used as a proxy for IBS.24 The same strain increased polyunsaturated fatty acids (PUFAs) and exhibited anti-inflammatory effects when co-administered with α-linoleic compared with α-linoleic supplementation alone.25,26 It is believed that the microbiota, as well as pre- and probiotics, can have a significant impact on the gut-brain axis, but further research in this area is needed to reveal the magnitude, mechanisms and clinical relevance of these effects.

**Effects of the intestinal microbiome and probiotics on the CNS.** The CNS consists of the brain and the spinal cord, with more than four hundred million neurons. Behavior is an excellent read-out of CNS function. Studies have been conducted with a view to establishing a behavioral phenotype and neurochemical profile that might be associated with the gut microbiota. To date, the most consistent data have been in relation to indices of anxiety. Neufeld and colleagues reported a less anxious phenotype for germ-free (GF) animals in the elevated plus maze test.27 This behavioral phenotype was replicated in another study with germ-free animals in both the elevated plus maze test and the light-dark box and was associated with an altered gene expression profile in relevant brain regions.28 Changes in CNS gene expression of plasticity-related genes have been reported; however the findings have been inconsistent from different laboratories, perhaps due to strain and sex differences in the animals. A decrease in brain derived neurotrophic factor (BDNF), a key neurotrophin involved in neuronal growth and survival and expression of the glutamate NMDA receptor subunit 2a (NR2a) were measured in the cortex and hippocampus of male GF Balb/C mice compared with SPF controls.29 More recently, Neufeld et al.27 demonstrated an increased expression of BDNF mRNA in the dentate gyrus of the hippocampus and reduced serotonin 1A receptor mRNA expression in the dentate gyrus of the hippocampus in female GF Swiss Webster mice,27 a molecular signature that is consistent with the observed reduced anxiety-like behavior in GF mice.27,28 If anxiety-like behavior in GF mice is considered in the context of inflammation, reduced inflammation leads to reduced anxiety-like behavior. Immunocompromised mice had reduced anxiety-like behavior, as observed in the recombinase activating

| Table 1. Summary of studies performed in animal models evaluating the effect of the gut microbiota and/or changes induced by probiotic and prebiotic interventions |
|-----------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| Balb/c mice and NIH mice                      | SPF + antibiotic vs. GF mice                    | Antibiotic decreased anxiety behaviors, Interspecies gut microbiota transplantation changed species specific associated phenotype | CNS                                             | 87                                              |
| CF1 mice                                      | *Citrobacter rodentium* infection               | Increase anxiety like in pathogen infected mice  | CNS                                             | 36                                              |
| C57BL/6 mice                                  | *Citrobacter rodentium* infection               | Increase memory dysfunction in pathogen infected mice | CNS                                             | 37                                              |
| Maternal deprivation in offspring’s rats      | *L. paracasei NCC 2461*                         | Probiotic reverse rectal hyperalgesia            | Animal model for IBS                            | CNS and brain-gut axis                          | 21                                              |
| Maternal deprivation in male offspring’s rats | *Citrobacter rodentium* infection               | Plasma PUFA different in maternally separated animal vs controls | Animal model for IBS                            | CNS and brain-gut axis                          | 88                                              |
| Maternal deprivation in male offspring’s rats | Lipopolysaccharide challenge                    | Plasma corticosterone increase in maternally separated animals. Increase in immune response after LPS challenge Alteration in fecal microbiota vs control group | Animal model for IBS                            | CNS and brain-gut axis                          | 89                                              |
| Maternal deprivation in offspring’s rats      | Swim test                                       | Probiotic treatment resulted in normalization of the immune response, reversal of behavioral deficits peripheral interleukin (IL)-6 release and amygdala corticotrophin-releasing factor mRNA level and restoration of basal noradrenaline concentrations in the brainstem | Animal model for IBS                            | CNS and brain-gut axis                          | 24                                              |

Abbreviations: ANS, autonomous nervous system; CNS, central nervous system; ENS, enteric nervous system; EPM, Elevated plus maze; GF, germ-free; HPA, hypothalamic-pituitary-adrenal; IBS, irritable bowel syndrome; SPF, specific pathogen free.
Studies with *Escherichia coli* suggest that NE acts by inducing de novo synthesis of various bacterial virulence factors or that cognate bacterial receptors utilize NE as a growth-activating ligand. Besides NE, other neurotransmitters (GABA, catecholamines, serotonin and acetylcholine) and hormones normally associated with the nervous and endocrine systems, could also play this role. An impact of stress on microbial colonization has been observed in monkeys exposed to stress in early life. The biochemical elements used by the nervous, endocrine and other systems for intercellular communication in vertebrates may originate from unicellular organisms and be conserved. A high affinity GABA system in *Pseudomonas fluorescences* with binding properties similar to those of a brain receptor has been reported. Conversely, stress response can be modulated by gut microbes. Microbes play a critical role in the development of an appropriate stress response later in life. A pivotal window in early life where gut microbial colonization takes place must occur to ensure normal development of the core stress axis, the hypothalamic-pituitary-adrenal (HPA) axis. Gut microbes also are able to modulate exaggerated stress responses: In GF mice, a mild restrained stress induced an amplified release of corticosterone and adrenocorticotropic hormone (ACTH) compared with specific pathogen-free (SPF) controls. This response was reversed by recolonization with fecal matter from SPF animals and by monoassociation with *B. infantis* in a time dependent manner. Other probiotic administration studies also support a role for the microbiota in alleviating anxiety-like behaviors. Administration of *L. helveticus* R0052 and *B. longum* R0175 gene (RAG-1) knockout model. Increased exploration in the open field and decreased open arm avoidance in the elevated plus maze test are characteristic of these mice. In contrast, increased inflammation leads to increased anxiety-like behavior in other animal models.

Probiotic treatment can also alter brain neurochemistry. Chronic treatment with *L. rhamnosus* (JB-1) induced region-dependent alterations in GABA<sub>agon</sub> mRNA in the brain with increases in cortical regions and concomitant reductions in expression in the hippocampus, amygdala and locus coeruleus, in comparison with control-fed mice. This strain reduced stress-induced corticosterone anxiety and depression related behavior. These changes were not observed in mice that had underwent vagotomy suggesting that the microbiota-brain changes observed were mediated by the vagus nerve.

**Effects of the Central and Peripheral Nervous Systems on the Intestinal Microbiome**

Although several studies in animals have demonstrated an effect of the brain on microbiota composition, investigators have focused primarily on changes for specific bacterial groups (i.e., lactobacilli, bifidobacteria and pathogens) observed in stressful conditions. Stressful conditions are known to impact gastrointestinal motility, permeability, immune function and release of specific hormones and mediators in the GI tract.

Stress hormones, such as NE, stimulate the growth of specific pathogens; however the underlying mechanisms remain poorly understood. Studies with *Escherichia coli* suggest that NE acts by inducing de novo synthesis of various bacterial virulence factors or that cognate bacterial receptors utilize NE as a growth-activating ligand. Besides NE, other neurotransmitters (GABA, catecholamines, serotonin and acetylcholine) and hormones normally associated with the nervous and endocrine systems, could also play this role. An impact of stress on microbial colonization has been observed in monkeys exposed to stress in early life. The biochemical elements used by the nervous, endocrine and other systems for intercellular communication in vertebrates may originate from unicellular organisms and be conserved. A high affinity GABA system in *Pseudomonas fluorescences* with binding properties similar to those of a brain receptor has been reported.

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![Figure 1. Interaction of the gut microbiome, probiotics and prebiotics on the brain gut axis. Modified from reference 85.](image-url)
An emerging area of interest is the role of the microbiota in autism spectrum disorders (ASD). ASD are a group of neurodevelopmental disorders including autism, Asperger’s Disorder, Rett’s Disorder, childhood disintegrative disorder, pervasive developmental disorder, not otherwise specified. GI disturbances are prevalent in children with autism and the number of GI symptoms is shown to be associated with the severity of autism. Several studies have now reported changes in microbiota profile in patients with autism. While this area of research is new and consensus across studies has not yet been established, several interesting observations are worth noting. In one recent study, an increased diversity of microbiota was observed in patients with severe autism compared with matched controls; and in particular Bacteroidetes was significantly increased in patients with autism, whereas Firmicutes was observed to be higher in controls. In contrast, another study that compared patients with autism and GI problems (ASD+GI) to patients with GI problems alone, showed reduced Bacteroidetes and increased Firmicutes and Betaproteobacteria (Sutturela) presence in ASD+GI patients. It has also been speculated that secretion of bacterial (neuro) toxins produced by clostridia may affect behavior and stress in autistic subjects. Furthermore, urinary metabolite phenotypes—mostly derived from gut bacterial fermentation—differentiated children with autism from their unaffected siblings. Clearly, more research is needed to clarify the gut microbial dysbiosis and determine the relationship between microbiota and autism.
Studies considering possible mechanisms for gut-brain communication in autism suggest that an altered metabolic phenotype in association with gut dysbiosis may contribute to ASD. Interestingly, short-term treatment with antibiotics has been reported to improve behavioral symptoms in some patients with autism. Probiotics and novel therapies targeted at gut dysbiosis show promise for treatment of ASD, however, more studies are needed to better define these novel therapeutic strategies.

Effects in functional gastrointestinal disorders. Functional gastrointestinal disorders (FGIDs) are defined as chronic or recurrent gastrointestinal symptoms not explained by structural or biochemical abnormalities. Multinational working teams known as the Rome Committees have defined and categorized FGIDs based on clusters of clinical symptoms and suggested symptom based criteria for their diagnosis in children and adults. It has been proposed that in some cases, FGIDs involve a continuum starting in infancy and continuing into adolescence and adulthood. A long-term follow up study has demonstrated that 25% of children who have FGIDs have symptoms as adults. FGIDs are heterogeneous disorders with pathophysiologic mechanisms that require better clarification. They are currently understood as an integration of multiple factors (genetic, physiological, psychological, microbial, environmental and dietary) that contribute to the overall clinical presentation and outcome. To illustrate this complexity, up to 60 potential genes thought to play a role in FGIDs have been identified in the aminergic pathway (serotonin, noradrenaline) and in alterations of intestinal immunity and barrier function.

Many reports associate intestinal dysbiosis and a common FGID, IBS, although a cause and effect relationship has not been demonstrated. Next generation sequencing technologies recently have revealed in more detail gut dysbiosis in different IBS cohorts in both children, teenagers and adults. In one study performed in the pediatric population, the IBS microbiome was characterized by greater abundance of the family Gammaproteobacteria, a family that includes several potential pathogenic bacteria. *Hemophilus parainfluenza* was found to be present in higher abundance in IBS patients. Additionally, bacterial genera that have not been previously linked with IBS, such as *Dorea*, were found to be associated with the disease in IBS adult subjects as well. Recent studies of the microbiota in adults with different IBS subtypes have also confirmed specific gut bacterial dysbiosis, with a decrease in bifidobacteria in IBS-D patients and increase in Gammaproteobacteria. Furthermore, several species have now been correlated with a lower or higher severity as well as frequency of pain in children (Fig. 3) and warrant further investigation. Although findings of compositional changes at the species levels are intriguing, individual species and the metabolic function of the gut microbiota may be more important, as most species of a genus may not contribute to disease.
The role of gut microbiota in IBS also is supported by the positive effect of the manipulation of gut microbiota on IBS symptoms with probiotics,64 prebiotics65 and antibiotics.66 More than 40 probiotic intervention studies performed in IBS patients have been published and recently reviewed.67–70 In most studies, a strong placebo effect was observed, underscoring the important role that subjective psychological factors play in the response to treatment in these patients. Nevertheless, many of these studies have demonstrated efficacy in improving functional GI symptoms in IBS patients.

Gut barrier function is vital in maintaining gut health, but this barrier is impaired in IBS. 15–50% of patients with IBS, depending on the cause and the particular subtype, have increased intestinal permeability as measured by sugar permeability tests and by changes in the expression of tight junction proteins.71 While in vitro work to identify important candidate effector molecules continues, the effects on the enteric nervous system in human intervention studies with probiotics are sparse. A short-term improvement in mucosal barrier function measured by a triple sugar test in patients with IBS was observed after administration of a probiotic fermented milk (Streptococcus thermophilus, Lactobacillus bulgaricus, L. acidophilus and Bifidobacterium longum) compared with a milk beverage containing no bacteria.72 As noted in other studies with prebiotics and probiotics, effects on gut barrier function varied among strains and effector molecules.73–75 While intestinal permeability may be altered in a subset of IBS patients, the relationship with dysbiosis requires further study. Commensal and probiotic organisms can regulate intestinal barrier function through a variety of mechanisms and hence contribute to the development or perpetuation of IBS. However, such a clear causative link has not been yet demonstrated.

Prebiotics are “selectively fermented food ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confer benefits upon host health”,65 Inulin-type fructans and lactulose modulate gut transit, decrease putrefactive activity within the gut lumen,76 prevent GI infections77 and mitigate inflammatory responses.78,79 Hypothetically, some of the alterations in the composition or function of the intestinal microbiota in IBS may be corrected or counteracted by prebiotics’ abilities to modulate the gut microbiota and their function. Few studies have investigated the effect of prebiotics in patients with IBS. The study by Olesen and Gudmand-Hoyer80 tested a large dose (20 g) of inulin during 12 weeks. The authors hypothesized that IBS symptoms may be provoked by large quantities of fermentable carbohydrates in the colon. After 4–6 weeks on treatment, IBS symptoms worsened, as expected, in patients on 20 g inulin per day and improved in patients on placebo. However, continuous treatment for 12 weeks resulted in adaptation and no difference was found between groups: symptoms improved in 58% of the inulin group vs 65% of the placebo group and symptoms worsened in 8% of the inulin group vs 13% of the placebo group. Large doses of highly fermentable carbohydrates should not be recommended to IBS patients.81 Nevertheless, two other published studies of adequate size have reported beneficial effects of a prebiotic in IBS.82,83 In the double blind placebo controlled study by Paineau et al.82 that included 105 IBS subjects, treatment with a short chain inulin-type fructan at 5 g/day over 6 weeks reduced the incidence and intensity of GI symptoms as compared with the placebo. Prebiotic treatment also improved functional digestive disorders related quality of life. Another study tested a galactooligosaccharide (GOS) in a double blind placebo controlled trial with 44 IBS subjects randomized into 3 groups, either receiving 7 g/day placebo, 3.5 g/day GOS and 3.5 g/day placebo or 7 g/d GOS for 6 weeks.85 The prebiotic significantly improved flatulence, bloating and the composite score of symptoms, as well as subjective global assessment. It also increased the proportion of bifidobacteria in faecal samples. In summary, evidence accumulated so far in clinical studies testing prebiotics in IBS subjects is limited, but suggests possible benefits of specific prebiotics (inulin, GOS) at moderate doses. Further studies with adequate methodology are warranted.84

**Conclusions and Future Research**

The knowledge gained in recent years about the ability of gut microbes to influence and contribute to host health and well-being opens new windows of opportunity for nutritional and pharmacological tools to improve host-microbe symbiosis, potentially using probiotics and prebiotics. The most innovative and exciting area for future applications derives from the evidence accumulated on the role of the gut microbiota in the brain-gut axis. Translation of the effects of microbiota/probiotics on the brain-gut axis found in laboratory and animal studies and further understanding of how these effects might improve physiological (e.g., GI) and psychological (e.g., anxiety and depression) disturbances are certainly a major challenge for research in forthcoming years.

Researchers need to better understand how to assess and manipulate the potential microbial impact on the brain-gut axis amidst a large number of bacterial, host and environmental influences. An integrated approach must consider a number of parameters including deep GI microbiome analysis, markers of immune and neural functions and responses to environmental conditions (e.g., stress and diet). A systemic characterization of the GI microbiome composition should be considered. New technologies, such as 16S rRNA metagenomics sequencing and bacterial metagenomics and metatranscriptomics combined with metabolomics likely will help to study segregate groups and disease subtypes. Characterization of the microbiome in other sites (oral cavity, small intestine) of the GI tract also may help to understand better these complex host-microbiome interactions. Finally, studies linking microbial colonization, brain development and behavior during early life in infants, children and adolescents are of paramount relevance to further develop this field. International initiatives such as the International Human Microbiome Consortium provide access to massive amounts of data linking microbial sequences with human phenotype. These bioinformatics tools certainly will assist researchers to segregate better different populations and target potential groups for interventions.
Investigators face numerous challenges including the demonstration and articulation of specific beneficial effects and associated mechanisms of probiotics and prebiotics. Additionally, the involvement of neuroimmune interactions in the GI tract is an understudied area with respect to probiotics and prebiotics. Before using probiotics in interventional studies, many factors remain to be considered. Continuing technological advances, enhancing knowledge of the immune system and state of the art investigation of the gut microbiota are essential to make human interventional studies with probiotics and prebiotics meaningful.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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References
36. Lyre M, Li W, Opitz N, Gaykema RP, Goehler LE. Induction of anxiety-like behavior in mice during the initial stages of infection with the agent of murine colonic hyperplasia Citrobacter rodentium. Physiol Behav 2010; 100:44-50; PMID:20079021; http://dx.doi.org/10.1016/j.physbeh.2010.06.019.


