The Gut Microbiome in Adult and Pediatric Functional Gastrointestinal Disorders

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The importance of gut microbiota in gastrointestinal (GI) physiology was well described, but our ability to study gut microbial ecosystems in their entirety was limited by culture-based methods prior to the sequencing revolution. The advent of high-throughput sequencing opened new avenues, allowing us to study gut microbial communities as an aggregate, independent of our ability to culture individual microbes. Early studies focused on association of changes in gut microbiota with different disease states, which was necessary to identify a potential role for microbes and generate novel hypotheses. Over the past few years the field has moved beyond associations to better understand the mechanistic implications of the microbiome in the pathophysiology of complex diseases. This movement also has resulted in a shift in our focus toward therapeutic strategies, which rely on better understanding the mediators of gut microbiota–host cross-talk. It is not surprising the gut microbiome has been implicated in the pathogenesis of functional gastrointestinal disorders given its role in modulating physiological processes such as immune development, GI motility and secretion, epithelial barrier integrity, and brain–gut communication. In this review, we focus on the current state of knowledge and future directions in microbiome research as it pertains to functional gastrointestinal disorders. We summarize the factors that help shape the gut microbiome in human beings. We discuss data from animal models and human studies to highlight existing paradigms regarding the mechanisms underlying microbiota-mediated alterations in physiological processes and their relevance in human interventions. While translation of microbiome science is still in its infancy, the outlook is optimistic and we are advancing in the right direction toward precise mechanism-based microbiota therapies.

Keywords: Microbiota; Brain-Gut; Abdominal Pain; Irritable Bowel Syndrome; Constipation; Dyspepsia.

The human gut is home to a complex microbial ecosystem with bacteria, fungi, viruses, and archaea that exist in a mutualistic relationship with the host in homeostatic conditions. The microbial members along with their genetic content often are referred to as the gut microbiome and can be viewed as a dynamic organ capable of mediating a wide variety of biochemical transformations that directly impact host physiology in health and disease.1,2 However, a disruption in this equilibrium can lead to alteration of host physiology resulting in disease states such as functional gastrointestinal disorders (FGIDs).

The role for gut bacteria in FGIDs such as irritable bowel syndrome (IBS) has been well described. An estimated 10% of IBS cases begin after an episode of infectious gastroenteritis.3 However, the study of intestinal microbial ecosystems has been limited by our inability to identify bacteria without cultivating them in the laboratory. At the turn of the century, ground-breaking advances in the genomics era and sequencing technologies4,5 gave way to culture-independent molecular approaches allowing us not only to identify and characterize microbial communities based on similarities in DNA sequences, but also provide knowledge that has significantly improved our ability to culture bacteria that previously were considered unculturable.6

These advances have led to extensive characterization of microbial communities in FGIDs over the past decade. Although no consistent microbial signature has been associated with FGIDs, several lines of evidence support a role for gut microbes in the development of FGID symptoms.7 There has been a significant effort to move beyond describing associations between the gut microbiome and FGIDs, to defining mechanisms underlying microbial contributions to the pathophysiology of FGIDs.
In this review, as part of our effort to define a path from bench to bedside, we summarize factors affecting the gut microbiome and describe a conceptual framework for the role of the gut microbiome in FGIDs. This foundation will allow us to identify gaps in our current body of knowledge and develop strategies to translate microbiome science into improved diagnosis, prognosis, and management of FGIDs.

**Factors That Shape the Gut Microbiota**

Gut microbial composition and diversity are largely the consequence of host selection pressures such as genetics, habits, sex, and location within the gastrointestinal (GI) tract, as well as environmental factors including diet (Figure 1). Gut microbial diversity varies with age, and substantial differences are seen at the extremes of life. At birth, assembly of the gut microbiota begins with colonization from environmental microbes (eg, maternal vaginal, fecal, skin microbiota). In the subsequent months to years, gut microbial communities continue to shift in response to key life events (eg, exposure to solid foods, illnesses, antibiotics), with gradual increases in diversity and convergence to an adult-like microbiota. The adult gut microbiota is relatively stable over time and surprisingly resilient to temporary perturbations, changing as we get older to a distinct and less-diverse microbiome.

Sex associations (Figure 1) with the gut microbiota have been characterized by increased relative abundance of Firmicutes and lower Bacteroidetes in women compared with men, and may be influenced further by body mass index. Host genetic influence on the gut microbiome is apparent from studies of monozygotic and dizygotic twin pairs that showed shared community structures between related individuals and temporally stable heritable taxa. However, the effect size is likely small given recent microbial–genetic association studies showing environmental factors to have substantially greater impact on the gut microbiome than genetics. The impact of both short-term and long-term dietary patterns on the gut microbiome cannot be overstated. The role of diet in microbial alterations is of significant interest in FGIDs because dietary intolerances are commonly reported in FGIDs and patients may alter or restrict their diets based on perceived associations between symptoms and food. The interaction of diet, gut microbiome, and symptoms in FGIDs (reviewed by Rajilic-Stojanovic et al) has not been well studied and the long-term consequences of current dietary interventions with reported benefit in IBS, such as supplementation with psyllium fiber and the low fermentable oligosaccharides, disaccharides, monosaccharides, and polyols diet, on the gut microbiome remain to be seen.

Other modifying factors (Figure 1) include psychological stress, physical activity, tobacco use, alcohol consumption, and antibiotic exposure. In a population-level analysis of gut microbiome variation, factors showing environmental factors to have substantially greater impact on the gut microbiome than genetics. The role of diet in microbial alterations is of significant interest in FGIDs because dietary intolerances are commonly reported in FGIDs and patients may alter or restrict their diets based on perceived associations between symptoms and food. The interaction of diet, gut microbiome, and symptoms in FGIDs (reviewed by Rajilic-Stojanovic et al) has not been well studied and the long-term consequences of current dietary interventions with reported benefit in IBS, such as supplementation with psyllium fiber and the low fermentable oligosaccharides, disaccharides, monosaccharides, and polyols diet, on the gut microbiome remain to be seen.

The distribution and composition of the gut microbiota changes along the length of the GI tract (Figure 2), and across the lumen, mucus layer, and the epithelium. Bacterial density increases from more proximal to distal sites. Microbial abundance and community structure in the proximal intestine are affected by gastric acid, pancreaticobiliary secretions, and fast transit. In turn, accelerated GI transit can alter the distribution and composition of microbial populations also can be a result of mucus from goblet cells and differential oxygen tolerance. Together, these factors lead to distinct microenvironments driving the biogeographic stratification of microbes across the GI tract.

**Effect of the Gut Microbiota on Host Physiology**

**Gut Microbiota and Gastrointestinal Motility**

Altered GI motility and transit have long been recognized in the pathobiology of FGIDs such as IBS and functional dyspepsia (FD), highlighting a bidirectional relationship (Figure 3). Gut microbes can accelerate GI transit. In turn, accelerated GI transit can alter the composition and spatial organization of microbial communities by creating luminal conditions suited for the growth of specific bacterial taxa or by affecting bacterial adherence. Interestingly, GI motility-related changes in the gut microbiome can further perpetuate the alteration in GI motility as a positive feedback effect. Several microbial mediators (Table 1) of GI motility have been identified, including short-chain fatty acids (SCFAs) and bile acids. SCFAs are produced by fermentation of dietary starches or complex carbohydrates by gut bacteria, while bile acid deconjugation and dehydroxylation by gut bacteria regulates the quantity and
derivatives of bile acids in the colon. SCFAs and bile acids may affect gut motility (Table 1). Prokinetic effects of bile acids can be mediated by the G-protein–coupled bile acid receptor Takeda G-coupled receptor 5, expressed by enteric neurons and enteroendocrine cells based on findings from animal models. Interestingly, microbial mediators of GI motility can vary by diet. For example, turmeric, a commonly used spice in Asian dishes, stimulates gallbladder contraction and increases intraluminal bile acids through its active ingredient of curcumin. Similarly, SCFA concentrations can vary based on dietary carbohydrate and protein intake. Other microbial products or metabolites (Table 1) that have been identified as potentially relevant in the microbial regulation of GI motility include bacterial lipopolysaccharide, which can improve survival of enteric neurons by activation of Toll-like receptor 4 (TLR4). Preliminary studies support the roles of other microbial metabolites such as hydrogen sulfide, tryptamine, and hydrogen gas in the regulation of human GI motility by their putative effects on GI smooth muscle and the enteric nervous system.

Gut Microbiota and Gastrointestinal Sensation

Abdominal pain in IBS and other FGIDs such as FD and functional abdominal bloating/distention has been attributed to visceral hypersensitivity to mechanical and chemical stimuli. Evidence for a role of the gut microbiome in regulating GI sensation (Figure 3) comes from gnotobiotic studies showing transfer of the visceral hypersensitivity phenotype after transplantation of gut microbiota from patients with IBS into germ-free (GF) mice. A recent study by Riba et al showed a correlation between visceral hypersensitivity and an increase of Escherichia coli abundance followed by induction of hypersensitivity in response to E. coli gavage in mice. Disruption of the gut microbiota in early life also has been associated with long-term changes in visceral sensitivity, emphasizing the importance of the gut microbiome in neurodevelopment of pain pathways. The exact mechanisms by which bacteria affect visceral perception and sensation still need to be determined. A few putative mechanisms include microbial induction of epithelial μ-opioid and cannabinoid receptors as shown with oral administration of Lactobacillus strains in rodents, regulation of central and peripheral neuronal pathways, antinociceptive effects from inhibition of transient receptor potential vanilloid as shown with administration of Lactobacillus reuteri in rats, microbial metabolites (eg, organic acids) or byproducts (eg, nitric oxide) altering sensation, and microbially derived bioactive molecules such as γ-aminobutyric acid (GABA) as shown with administration of GABA-producing Bifidobacterium dentium. The translation of findings from animal models to human beings, however, can be challenging. For instance, rectal administration of butyrate increases colonic hypersensitivity in rats, but decreases visceral sensitivity in healthy human beings. Hence, findings from animal studies, although valuable, need to be validated in human beings.
The intestinal epithelium and the overlaying mucus layer serve a key role in protecting the host by providing a physical and immunologic barrier against potentially harmful pathogens while also regulating fluid and nutrient absorption. Increased permeability or disruption of the epithelial barrier has been implicated in the pathophysiology of FGIDs. Microbes (Figure 3) can directly alter expression of tight junction proteins such as claudin-3 and zonula occludens-1, or enhance expression of genes involved in tight junction signaling. Microbial metabolites such as bile acids and SCFAs also can regulate intestinal permeability (Table 1). The mucus layer overlaying the epithelium is a reservoir of antimicrobial peptides and immunoglobulins and provides the first line of defense against gut bacteria. The mucus layer is compositionally rich in polysaccharides, which can serve as a nutrient source for subsets of bacteria. Hence, microbial starvation such as with decreased consumption of fiber can increase microbial reliance on the mucus polysaccharides, resulting in degradation of the mucus layer and increasing susceptibility to opportunistic pathogens and inflammation.

**Gut Microbiota, Immune Activation, and Inflammation**

Inflammation or immune activation involving both the innate and adaptive immune systems has been
described in subsets of patients with FGIDs. 87,88 There are several lines of evidence in support of the activation of mucosal and systemic immune responses by gut microbiota (Figure 3). Postinfectious FD is associated with increased numbers of duodenal CD68+ cells and eosinophils when compared with other subtypes of FD (epigastric pain syndrome, postprandial distress syndrome), and healthy states. 89 Increased expression of proinflammatory cytokines may be elicited through interactions between bacterial components and pattern recognition receptors including TLRs such as TLR2 and TLR4, which have been associated with IBS. 90 The gut microbiota also can influence immune activation via effects on lineage differentiation of T-cell subsets, 91 host-receptor-mediated signaling as seen with L. reuteri activation of histamine H2 receptor signaling 92 and production of microbial metabolites (Table 1).

### Gut Microbiota and Intestinal Secretion

Changes in small intestinal 93 and colonic secretion represents another pathophysiologic disturbance in FGIDs that may be influenced by the gut microbiome 48 (Figure 3). Secretory mechanisms are common therapeutic targets 94,95 of medications used to treat FGIDs. Microbial mediators of altered intestinal secretion include metabolites from breakdown of dietary polysaccharides as well as bile acids (Table 1). Specific bile acids, such as deoxycholate and chenodeoxycholate, can stimulate intestinal chloride secretion, 97,98 which is accompanied by water. SCFAs, similar to bile acids, are important intraluminal determinants of mucus and water secretion through effects on sodium and water influx, 99 duodenal bicarbonate secretion, 100 and colonic epithelial 5 hydroxytryptamine (5-HT3)-receptor expression. 101

### Gut Microbiota and Gastric Function

Disturbances in gastric motor and sensory function, including impaired gastric accommodation and increased intragastric pressure, may underlie FGIDs and often are related to food intake. 102 There are a paucity of data in support of microbial regulation of gastric function. The administration of the prebiotic arabinoxylanoligosaccharide in healthy volunteers was not associated with changes in gastric sensitivity, compliance, or accommodation despite increased colonic

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<td>Butyrate’s role in maintenance of intestinal barrier 195 may vary</td>
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<td>Immune activation</td>
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</table>

cAMP, cyclic adenosine monophosphate; CDCA, chenodeoxycholic acid; CFTR, cystic fibrosis transmembrane conductance regulator; DCA, deoxycholic acid.
fermentation.\textsuperscript{102} Reported associations between the presence of small intestinal bacterial overgrowth (SIBO) and delayed gastric emptying\textsuperscript{103} have not discerned whether associations are a result of microbial mechanisms or merely representative of underlying impairment in small intestinal motility and other confounding factors including chronic acid suppression and opioid analgesics.\textsuperscript{104} Recent evidence showing a similar gastric emptying time among patients with and without SIBO suggests that bacterial overgrowth does not necessarily predispose to impaired gastric emptying.\textsuperscript{105,106}

**Gut Microbiota and Central Nervous System Function**

The bidirectional microbiome–gut–brain axis (Figure 3) represents the reciprocal regulation of the gut microbiome and the central nervous system (CNS). Recent studies have highlighted the role of the gut microbiome in modulating brain–gut communication, which may significantly affect the pathophysiology of symptoms associated with FGIDs.\textsuperscript{107,108} Signals from the CNS can influence GI physiology while simultaneously shaping the gut microbial fingerprint as seen in early life stress rodent models, which show alterations in gut microbial community composition.\textsuperscript{109} Similar findings have been described in other rodent stress models.\textsuperscript{110} Conversely, microbial colonization and community composition are critical to the development of the hypothalamus–pituitary–adrenal axis as evidenced by the exaggerated adrenocorticotropic hormone and corticosterone release in GF mice. This exaggeration is attenuated after colonization with *Bifidobacterium infantis*.\textsuperscript{110} A different strain within the same genus, *Bifidobacterium longum* NCC3001, decreases anxiety-like behavior in mice through vagally mediated pathways.\textsuperscript{111} Microbial metabolites such as SCFAs and microbially derived neurotransmitters such as GABA and 5-HT may impact brain function and mental health further.\textsuperscript{112}

**Functional Gastrointestinal Disorders**

**Role of Gut Microbiota in the Pathophysiology of Irritable Bowel Syndrome**

The gut microbiota of patients with IBS is an area of considerable interest, and has been studied the most extensively among the various FGIDs (reviewed by Simren et al.\textsuperscript{113}). Despite the lack of a uniform IBS-microbiota pattern, key observations include a decrease in α-diversity and alterations in the relative abundance of specific taxonomic groups including an increased ratio of Firmicutes to Bacteroidetes, decreased *Lactobacillus* and *Bifidobacterium*, and increased *Streptococcus* and *Ruminococcus* species.\textsuperscript{112} Cross-sectional analysis of extensively phenotyped cohorts also has shown that although stool consistency is a significant contributor to gut microbiome compositional variation, the contribution imparted by IBS was much less.\textsuperscript{41} These data highlight the importance of investigating specific pathophysiologic disturbances beyond merely providing descriptive analyses of heterogeneous patient populations in elucidating the role of the gut microbiome in IBS. Correlative associations between the gut microbiome and IBS have been followed by efforts to better characterize the mechanistic link between the microbiome and pathophysiology of symptoms associated with IBS (Table 2). Among the various aforementioned aspects of gut physiology that are affected by the gut microbiome, many are implicated directly in the pathophysiology of IBS.

**Summary of Findings From Animal Studies**

Animal models, although imperfect correlates to IBS pathophysiology in human beings, have allowed us to explore putative interactions between the gut microbiome and mechanisms implicated in IBS such as altered motility, visceral hypersensitivity, increased permeability, immune activation, intestinal secretion, and disturbances in central mechanisms. De Palma et al.\textsuperscript{114} recently showed that GF mice colonized with the fecal microbiota of diarrhea-predominant IBS (IBS-D) patients showed faster GI transit, increased colonic permeability, increased anxiety-like behavior, and increased infiltration by CD3\textsuperscript{+} T lymphocytes compared with those colonized by microbiota from healthy controls. Studies of specific IBS pathways include reports describing microbiota-induced hypersensitivity to colonic distension in GF rats inoculated with fecal microbiota from IBS patients.\textsuperscript{116} Microbial regulation of host immune responses may be further relevant to IBS. An increase in mucosal immune cells including mast cells, macrophages or monocytes, T cells, and eosinophils has been reported in both pediatric and adult FGID populations.\textsuperscript{117,118,119} Mast cells contain biologically active substances including histamine, tryptase, cytokines, and membrane-derived arachidonic acid metabolites (eg, prostaglandins) that are released upon their activation. These mediators may alter nociceptive pathways in IBS\textsuperscript{115} or increase intestinal permeability.\textsuperscript{116} Macrophages and monocytes are important in modulating the adaptive immune responses and producing proinflammatory cytokines such as interleukin (IL)6 and IL8, which in some studies are increased in IBS patients.\textsuperscript{117} The role of gut microbes in these immune pathways remains unknown.

**Summary of Findings From Studies in Adult Irritable Bowel Syndrome Patients**

Numerous studies have examined microbiome-related effects on pathophysiological changes in IBS, building on work performed in animal models. Interventional studies investigating the use of probiotic and
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<th>Study</th>
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<th>Role of microbiota</th>
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<tr>
<td>Shin et al.</td>
<td>60 IBS-D</td>
<td><em>L. gasseri</em> BNR17 vs pcbo</td>
<td>Fecal</td>
<td>Transit</td>
<td>Transit significantly ↑ during 8 wk with <em>L. gasseri</em> BNR17</td>
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<td>Tap et al.</td>
<td>110 IBS, 39 HV</td>
<td>NA</td>
<td>Fecal, mucosal</td>
<td>Transit, GBA</td>
<td>↑ Transit with Clostridiales vs Prevotella and Bacteroides enterotypes</td>
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<td>Acosta et al.</td>
<td>24 nonconstipated IBS</td>
<td>Rifaximin vs pcbo</td>
<td>Fecal</td>
<td>Transit, permeability, SCFA and bile acid production</td>
<td>No association between HADS and enterotype</td>
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<tr>
<td>Dior et al.</td>
<td>15 HV, 15 IBS-C, 16 IBS-D</td>
<td>NA</td>
<td>Fecal</td>
<td>Fecal bile acids</td>
<td>No significant effects of rifaximin on permeability, bile acids, SCFAs</td>
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<td>Le Neve et al.</td>
<td>100 IBS</td>
<td>NA</td>
<td>Fecal</td>
<td>Sensation, transit</td>
<td>Rifaximin associated with ↑ ascending colon emptying, and colonic transit at 48 h</td>
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<td>Chumpitazi et al.</td>
<td>12 IBS children</td>
<td>LFSFD</td>
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<td>Transit, metabolite composition</td>
<td>Bacterial deconjugation of bile acids in IBS-D and IBS-C feces vs HV</td>
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<td>Jeffery et al.</td>
<td>37 IBS, 20 HV</td>
<td>NA</td>
<td>Fecal</td>
<td>Sensation, transit, GBA</td>
<td>Response to lactulose challenge associated with rectal sensitivity but not with fecal microbiota or transit</td>
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<td>Labus et al.</td>
<td>29 IBS, 23 HV</td>
<td>NA</td>
<td>Fecal</td>
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<td>Proteobacteria associated with ↑ mental component and pain threshold Actinomycetales associated inversely with depression</td>
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<td>Liu et al.</td>
<td>40 IBS, 15 depression, 25 IBS and depression, 20 HV</td>
<td>NA</td>
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<td>Desulfohalobiales and Methanobacteriaceae associated with transit</td>
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<td>Azpiroz et al.</td>
<td>79 IBS</td>
<td>scFOS vs pcbo</td>
<td>Fecal</td>
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<td>No correlations between anxiety or depression symptom scores and microbial parameters; Clostridia and Bacteroidia correlated with sensory integration regions</td>
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<td>Le Gall et al.</td>
<td>10 IBS, 13 UC, 22 HV</td>
<td>NA</td>
<td>Fecal</td>
<td>Fecal metabolites</td>
<td>↑ Bacteroidetes and ↑ Firmicutes in IBS-D, depression, and IBS-D</td>
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<td>Heitkemper et al.</td>
<td>93 IBS</td>
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<td>Colonic mucosa inflammation associated with ↑ Bacteroides or Prevotella</td>
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<td>Bednarska et al.</td>
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<td>Valentin et al.</td>
<td>15 IBS-D</td>
<td>SBI</td>
<td>Duodenal brushing, fecal</td>
<td>Immune, permeability, metabolism</td>
<td>No significant difference in rectal sensory threshold for scFOS vs pcbo</td>
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<td>Increased permeability to <em>E. coli</em> strain HS and <em>S. typhimurium</em> in IBS biopsy specimens vs controls</td>
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<td>↑ Plasma VIP in IBS vs HV</td>
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<td>↑ Trypptase and mast cells in IBS biopsy specimens vs HV</td>
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<td>Bile acid synthesis, tryptophan metabolism, permeability, and stool microbiome not significantly different with SBI</td>
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<td>Changes in β diversity analysis, increased ↑ Proteobacteria Burkhoderiales, Firmicutes Catonella, and unclassified genus organisms with SBI in duodenal microbiome</td>
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<td>Study/Authors</td>
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<td>Ko et al. (2013)</td>
<td>53 IBS-D</td>
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<td>GJS with DuoLac7, B lactis, L rhamnosus, L plantarum. No significant difference observed in permeability.</td>
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<td>3 IBS-C, 2 HV</td>
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<td>Fecal Rectal sensitivity</td>
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<td>Shulman et al. (2017)</td>
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<td>Fecal GBA, permeability</td>
<td>No differences in psychological symptoms, permeability, or microbiome between groups.</td>
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<td>Compare et al. (2017)</td>
<td>10 IBS-D, 10 HV (ex vivo)</td>
<td>LC-DG, postbiotic</td>
<td>Mucosal Immune</td>
<td>↑IL1α, IL6, and IL8 messenger RNA, TLR-4 protein expression with ↓ IL10 messenger RNA levels in PI-IBS-D vs HV.</td>
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<td>Hustoft et al. (2017)</td>
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<td>Sundin et al. (2015)</td>
<td>11 PI-IBS, 10 HV (ex vivo)</td>
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<td>IL1β ↑ in PI-IBS vs HV after stimulation with Subdoligranulum variabile; IL10 ↓ in HV vs PI-IBS after stimulation with Eubacterium limosum.</td>
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<td>13 PI-IBS, 19 IBS, 16 HV</td>
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<td>Pinto-Sanchez et al. (2017)</td>
<td>44 IBS</td>
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<td>Parthasarathy et al. (2017)</td>
<td>25 CC, 25 HV</td>
<td>NA</td>
<td>Fecal Transit</td>
<td>No difference in fecal microbiota, serum markers of inflammation, neurotrophins, and neurotransmitters.</td>
<td></td>
</tr>
<tr>
<td>Parthasarathy et al. (2017)</td>
<td>25 CC, 25 HV</td>
<td>NA</td>
<td>Fecal, mucosal Transit</td>
<td>Reduced urine methylamines and aromatic amino acid metabolites with BL.</td>
<td></td>
</tr>
<tr>
<td>Tian et al. (2017)</td>
<td>60 STC</td>
<td>FMT</td>
<td>NA</td>
<td>Reproducibility of fecal microbiota lower in normal transit vs slow-transit constipation.</td>
<td></td>
</tr>
</tbody>
</table>

BL, Bifidobacterium longum NCC3001; cc, chronic constipation; FODMAP, fermentable oligosaccharides, disaccharides, monosaccharides, and polyols; GBA, gut-brain axis; HADS, Hospital Anxiety and Depression Scale; HV, healthy volunteer; IBS-M, irritable bowel syndrome mixed subtype; LC-DG, Lactobacillus casei DG; LFSD, low fermentable substrate diet; LPS, lipopolysaccharide; pcbo, placebo; PI-IBS, postinfectious irritable bowel syndrome; SBI, serum-derived bovine immunoglobulin/protein isolate; scFOS, short-chain fructooligosaccharide; TFF3, urine trefoil factor 3.
antibiotic therapy in IBS have led to the identification of potential microbial effects on transit (Table 2). Treatment with a probiotic containing *Bifidobacterium lactis* accelerates whole-gut transit and improves symptoms in patients with constipation-predominant IBS (IBS-C), whereas treatment with the nonabsorbable antibiotic rifaximin is associated with increases in both ascending colonic emptying and overall colonic transit rate at 48 hours in nonconstipated IBS patients. A role for the gut microbiome in immune modulation was suggested by findings from the clinical trial wherein *B infantis* 35624 alleviated symptoms and was associated with normalization of abnormal IL10/IL12 ratios in IBS patients. Colonic mucosal gene expression profiling of IBS patients also found differential expression of genes associated with host immune responses against microbial invasion, further suggesting that immune activation may be shaped by microbial interactions. Alterations in mucus-associated bacteria that may influence mucus integrity and intestinal secretion (eg, *Akkermansia muciniphila*, *Ruminococcus gravis*, and *Ruminococcus torques*) also have been associated with IBS. Microbially mediated effects on intestinal secretion in IBS may be a consequence of differential bile acid biotransformation by the gut microbiome. This concept is supported by the decreased concentrations of fecal unconjugated bile acids known to stimulate colonic secretion (deoxycholate and chenodeoxycholate) in IBS-C. Regarding the role of the microbiome–gut–brain axis in IBS (reviewed by Quigley and Mayer et al), probiotic therapy has been shown to modulate CNS function in healthy volunteers through effects on brain regions controlling the processing of emotion and sensation. More recently, in a recent placebo-controlled trial in IBS patients, treatment with the probiotic *B longum* NCC3001 was associated with improved symptoms of depression and changes in brain activation patterns measured by functional magnetic resonance imaging.

**Summary of Findings From Studies in Pediatric Irritable Bowel Syndrome Patients**

Similar to adult studies, the composition of the gut microbiome differs between children with IBS and age-matched healthy controls, despite the lack of a uniform IBS-microbiota signature across studies. One study enrolling children ages 7 to 12 years found pediatric IBS to be associated with decreased relative abundance of *Bacteroides* species and increased relative abundance of the class Gammaproteobacteria, including *Haemophilus parainfluenzae*, along with increased abundance of novel taxa related to the genus *Ruminococcus*. In this cohort, microbiota composition correlated with abdominal pain severity and frequency, and could be used to distinguish IBS-C from unsubtyped IBS. Another study of children ages 11 to 18 years found IBS-D to be associated with increased abundance of the genera *Veillonella*, *Prevotella*, *Lactobacillus*, and *Parasporobacterium*, and with decreased abundance of *Bifidobacterium* and *Verrucomicrobium*. By adding fecal metabolomic profiling to microbiome signatures, stool from children with IBS-D could be discriminated from that of healthy controls more accurately, with formate, pyruvate, and glucose being the most predictive metabolites. Probioic bacterial community composition also might be used to predict which children with IBS are more likely to respond to a low fermentable oligosaccharides, disaccharides, monosaccharides, and polyols diet: in 2 separate studies, responders had distinct baseline microbiome signatures compared with nonresponders.

Among the most studied probiotics in pediatric IBS is *Lactobacillus rhamnosus* GG, which was found in a meta-analysis of 3 randomized, placebo-controlled trials (RCTs) to confer a modest but significantly increased rate of treatment response vs placebo. Two multicenter, randomized, double-blind, placebo-controlled, cross-over studies provided further evidence of microbiota involvement in pediatric IBS. One study found that VSL#3 improved GI symptoms, while the other reported that a combination of 3 bifidobacteria resolved abdominal pain and improved quality of life to a greater extent than placebo. On the other hand, psyllium fiber reduced pain episodes in an RCT enrolling children with IBS without altering the composition of the gut microbiota based on 16S ribosomal RNA analysis. RCTs in children with functional abdominal pain have shown that *L reuteri* DSM 17938 is effective in treating abdominal symptoms. Jadresin et al showed a reduction in days with pain and pain severity in children with IBS and functional abdominal pain. In studies focused specifically on functional abdominal pain, Romano et al reported reduced pain severity and Weizman et al and Maragkoudaki et al both reported reduced pain severity and frequency in those treated with the probiotic compared with placebo.

In summary, both animal and human studies underscore the importance of the gut microbiome in mediating peripheral and central mechanisms implicated in IBS. Moreover, factors affecting gut microbiota composition are akin to the etiologic factors in IBS and probiotic interventions have a generally beneficial effect. However, given the heterogenous nature of the disease with multiple putative mechanisms, our broad nontargeted approach without consideration for the underlying physiological disturbance likely dilutes the overall impact and makes it difficult to ascertain the precise benefit of microbiota modulation. As we move forward, it will be important to phenotype patients based on the underlying physiological alterations so that we can develop targeted approaches directed toward specific microbes driving the host phenotype.
Role of Gut Microbiota in the Pathophysiology of Functional Dyspepsia

Similar to IBS, multiple pathogenic mechanisms including altered gastric function, visceral hypersensitivity, low-grade inflammation or immune activation, increased duodenal permeability, and abnormal CNS function have been postulated to contribute to symptoms in FD. As summarized earlier, gut microbiota have shown to modulate the majority of these physiological functions. Although data on the gastroduodenal microbiome and its particular role in FD are sparse, there are a few studies that lay the groundwork for future work investigating the role of microbial community alterations in FD.

Summary of findings from animal studies. In general, animal studies investigating the microbial effects on putative pathophysiologic mechanisms in FD are lacking given the absence of reliable models. The described effects are attributed to fermentative end products such as SCFAs. Bacterially derived or ingested SCFAs can alter duodenal bicarbonate secretion. In addition, the absorption of SCFAs also can influence the luminal bacterial population, which may be relevant in FD.

Summary of findings from studies in adult patients with functional dyspepsia. There are few human studies describing the gut microbiome in patients with FD, hence the precise role of the microbiota remains unknown. SIBO has been proposed to trigger symptoms in FD, although studies examining the role of SIBO in FD are limited by the relative inaccessibility of the more distal regions of the small intestine and concerns regarding the accuracy and interpretation of available testing methods for the diagnosis of SIBO. Recently, Zhong et al found the relative abundance of the anaerobic genera Prevotella, Veillonella, and Actinomyces were decreased significantly in the duodenal mucosa of 9 patients with FD compared with controls. Interestingly, the severity of symptom responses to a standardized meal correlated positively with mucosal bacterial load, which in turn correlated inversely with bacterial diversity. Igarashi et al found that gastric fluid samples from patients with FD were characterized by an increased Bacteroidetes to Proteobacteria ratio and an absence of Acidobacteria. In contrast, healthy volunteers had a decreased Bacteroidetes to Proteobacteria ratio and the presence of Acidobacteria. Nonblinded probiotic therapy with Lactobacillus gasseri OLL2716 subsequently was associated with shifts in gastric fluid microbial community composition similar to that found in healthy controls. In another RCT among patients with FD, rifaximin treatment was associated with significant improvement in global dyspeptic symptoms, belching, and postprandial fullness/bloating, further suggesting a potential role for the microbiome in FD.

Summary of findings from studies in pediatric patients with functional dyspepsia. Relatively little is known regarding the gut microbiome in pediatric FD. Although the previously highlighted multicenter, randomized, double-blind, placebo-controlled, cross-over study reported that a combination of 3 probiotic bifidobacteria improved pain scores and quality of life among 48 children with IBS, no benefit was observed among the 25 enrolled children who had FD, perhaps owing to the small number of patients treated. Likewise, the moderate overall benefit associated with L rhamnosus GG treatment in an RCT of children with IBS or FD was not observed in the subset of children with FD. However, it would be premature to make definitive conclusions given the small sample size (n = 20 with FD vs n = 37 with IBS).

In summary, the gut microbiome can affect mechanisms underlying FD similar to IBS, but the microbial community composition of the stomach and small bowel remain elusive and much work is needed before we can target specific microbial mediators that drive symptoms in FD. The overall positive impact of probiotics is encouraging and highlights the need for better mechanistic understanding to develop more precise microbiota-based therapeutics.

Role of Gut Microbiota in the Pathophysiology of Functional Abdominal Bloating

Abdominal bloating and distension are common complaints among patients suffering from FGIDs, and are among the most challenging symptoms to treat. The pathophysiologic mechanisms contributing to bloating are poorly understood, although SIBO and alterations in gut microbial communities have been hypothesized to play a role through microbial fermentation of dietary nutrients. Because this is predominantly a subjective sensation, there are no animal models to mimic these symptoms.

Summary of findings from adult patients with functional abdominal bloating. The majority of clinical studies investigating symptoms of bloating have been performed in IBS patients, with bloating and distension evaluated as secondary end points. A recent study showed depletion of operational taxonomic units within Subdoligranulum and Anaerovorax, belonging to the families Ruminococcaceae and Eubacteriaceae, respectively, in IBS patients without bloating compared to those with bloating and to healthy controls. Placebo-controlled studies of antibiotic (rifaximin) treatment in FGIDs and IBS have shown a significant reduction in bloating scores with rifaximin compared with placebo. Efficacy of probiotic administration for symptoms of bloating have been reported less consistently, although some studies in IBS patients have suggested benefit with specific probiotic strains including B lactis DN-173, Bifidobacterium animalis DN-173 010, and VSL#3. Little is known regarding the microbiome in functional abdominal bloating in children. The trial noted previously by Weizman et al, which reported benefit for abdominal
pain with the probiotic *L reuteri* DSM 17938, also reported a lower incidence of perceived abdominal distention and bloating. Similarly, patients in the VSL#3 trial had decreased abdominal bloating/gassiness compared with placebo. 134

In summary, although gut microbes potentially can impact these symptoms both via fermentative end products and by their effect on visceral sensation, we need to better characterize the potential microbial mediators to develop relevant therapeutics.

**Role of Gut Microbiota in the Pathophysiology of Functional Constipation**

There is evidence supporting an association between the altered mucosal and fecal microbiota and chronic constipation. 156,157 Most of our knowledge regarding the effects of the gut microbiota on peripheral mechanisms associated with constipation, such as GI motility, comes from animal studies. However, in recent years, several studies have been published exploring the gut microbiome in patients with constipation (Table 2).

**Summary of findings from animal studies.** An investigation of the causal relationship between alterations in gut microbial communities and constipation was described in a recent study 158 reporting up-regulation of 5-HT transporter and decreased 5-HT content in the colonic tissue of germ-free mice that received fecal microbiota from constipated patients. 5-HT was correlated negatively with transit time and changes were accompanied by decreased relative abundance of the phylum Firmicutes and increased Bacteroidetes in mice receiving fecal microbiota from constipated patients. Genus level analyses further showed decreased relative abundance of *Clostridium*, *Lactobacillus*, *Desulfovibrio*, and *Methylobacterium*, and increased relative abundance of *Bacteroides* and *Akkermansia*. The findings suggest a potential role for gut microbiota in the pathogenesis of chronic constipation via increased expression of the 5-HT transporter. 158 Interestingly, gut microbiota changes resulting from constipation can further impact GI motility, suggesting a more complex interaction with feedforward regulation rather than a simple cause–effect relationship. 53 The potential role of microbially derived metabolites is supported further by findings of delayed GI transit and altered SCFA and bile acid profiles after transfer of fecal microbiota from patients with slow-transit constipation to antibiotic-treated mice. 159

**Summary of findings from adult patients with functional constipation.** Several studies have reported a positive relationship between prolonged colon transit times, with increased richness and diversity of the fecal microbiome in adults without a prior history of GI disorders. 160,161 However, the association between constipation and the gut microbiome may involve mechanisms beyond that of slow transit. In a study of adults with chronic constipation, overall composition of the colonic mucosa-associated microbiota could discriminate patients with constipation from control subjects independent of transit time. 157 Taxonomic profiling of the fecal microbiome from patients with functional constipation (FC) and healthy volunteers has shown decreased abundance of *Bacteroides*, *Roseburia*, and *Coprooccus* in FC patients. Furthermore, healthy volunteers were found to have a gut microbiome enriched in genes involved in carbohydrate, fatty acid, and lipid metabolism whereas FC patients harbored a high abundance of genes involved in methanogenic pathways, hydrogen production, and glycerol. 162 Analysis of functional gene targets in constipated and healthy females also has shown increased abundance of hydrogenogenic (hydrogen-producing) and hydrogenotrophic (hydrogen-utilizing) genes by quantitative polymerase chain reaction in the colonic mucosa of constipated individuals. 163

**Summary of findings from pediatric studies.** In a cross-sectional study of 8 constipated obese children and 14 nonconstipated obese children, FC was associated with a decreased abundance of the phylum Bacteroidetes, including a significant reduction of the genus *Prevotella*, and an increased abundance of multiple genera within the phylum Firmicutes, including *Blautia*, *Coproccoccus*, and *Ruminococcus*. 164 A recent systematic review included 7 RCTs, enrolling a total of 515 children, that investigated the effects of probiotics in pediatric FC. Although 2 of the included studies, those evaluating *L reuteri* DSM 17938 165 and *B longum*, 166 reported significantly increased defecation frequency in the treatment arm, the meta-analysis concluded that there currently is insufficient evidence to support the use of probiotics for pediatric FC. 167 Finally, although a low-fiber diet is a known risk factor for FC in children, 168 there is currently little evidence to support the use of fiber for pediatric FC. Multiple systematic reviews note the sparse data and high risk of bias among the current evidence base. 169–172

In summary, the reciprocal interactions between GI transit and gut microbiota suggest that even if changes in gut microbiota are initiated by a change in transit, the altered microbial community can perpetuate the alteration in GI transit, highlighting the adaptability of the gut microbial community. Consequently, we need to think beyond the simple cause–effect paradigm as irrespective of the inciting event that alters the microbial community, these changes still can perpetuate a disease phenotype. The effect of gut microbiota on the host serotonergic system provides a plausible target for altering GI transit.

**Role of Gut Microbiota in the Pathophysiology of Infant Colic**

Infant colic, a characteristic group of behaviors featuring prolonged crying, is present in up to 25% of infants at 6 weeks of life 173 and is associated with an increased risk of recurrent abdominal pain and allergic
disorders later in childhood. Underlying mechanisms are unclear, in part owing to a lack of small animal models. Multiple pathophysologies, including gut microbiome alterations, have been proposed to promote abdominal pain. Early culture-dependent studies by Savino et al.\textsuperscript{175,176} showed that colicky infants were colonized more frequently by anaerobic gram-negative proinflammatory bacteria and colonized less frequently by lactobacilli when compared with noncolicky infants. Subsequent molecular studies confirmed enrichment of proinflammatory and gas-producing taxa within Proteobacteria in stool from colicky infants.\textsuperscript{177–179}

Given these observations, the probiotic \textit{L reuteri}, one of the few endogenous lactobacilli in the human GI tract, was proposed as a means of normalizing these gut microbial community alterations and potentially reducing crying times in infant colic and has become the most extensively studied microbiome-targeting therapy for colic. \textit{L reuteri} has been tested in 6 prospective RCTs: 2 meta-analyses that included more than 400 infants found that \textit{L reuteri} significantly reduced crying time in formula-fed infants by a mean of nearly 1 hour per day.\textsuperscript{180,181} Of note, the only other therapy to show efficacy in infant colic was fennel oil, while often-recommended interventions including simethicone and maternal diet manipulation produced mixed results.\textsuperscript{181} Interestingly, \textit{L reuteri} also has shown benefit in prevention trials, reducing the risk of developing colic at 3 months of life.\textsuperscript{182,183} Finally, a number of small studies have tested other microbiome-targeting therapies, including \textit{L rhamnosus} \textit{GG},\textsuperscript{184,185} and a symbiotic combination of fructooligosaccharide and 7 probiotics;\textsuperscript{186} these small studies generated mixed results.

\textbf{Modulating the Gut Microbiota for Treatment of Functional Gastrointestinal Disorders}

Targeting the gut microbiota for therapeutic intervention in FGIDs remains an area of significant interest for patients and clinicians. Probiotics have been studied extensively in adult and pediatric FGID populations as previously discussed and summarized in Table 2. A prior systematic review of probiotics in IBS suggested evidence for efficacy on global IBS symptoms, abdominal pain, bloating, and flatulence; however, there remain many unanswered questions regarding strain-specific effects, mechanisms of action, mode of administration and dosing, and patient selection. Despite their relative accessibility and general safety, clinical recommendations regarding specific probiotic use in FGIDs are limited by a lack of rigorous clinical trial data. Rifaximin has been studied in functional dyspepsia,\textsuperscript{146} abdominal bloating, and flatulence,\textsuperscript{150} and is approved for treatment of adults with IBS-D.\textsuperscript{151} The exact mechanisms by which rifaximin exerts its effects in IBS, however, remain uncertain, with a recent study of patients with non-constipated IBS showing borderline effects on microbial richness and increased rates of proximal colonic emptying, but no clear effects on bowel function, permeability, or production of intraluminal metabolites.\textsuperscript{119} More recently, results of several trials investigating the efficacy of fecal microbiota transplantation (FMT) for IBS have been reported. In 1 RCT among patients with moderate-to-severe IBS, higher response (\(P = .049\)) rates at 3 months, defined as a 75-point improvement in the IBS severity scoring system, were observed in patients receiving FMT (65%) compared with those receiving placebo (43%). However, differences were no longer significant at 12 months of follow-up evaluation.\textsuperscript{168} On the other hand, a separate multicenter RCT\textsuperscript{189} comparing FMT capsules with placebo in patients with diarrhea-predominant IBS was unable to show significant symptom relief at 3 months with FMT, although subgroup analysis suggested patients with postinfectious IBS experienced greater improvement with FMT compared with placebo (\(P = .09\)). The role of FMT in IBS needs to be better defined because there may be specific features in the donor microbiome as well as additional recipient characteristics that predict clinical outcomes. FMT, however, represents a stop-gap measure and it is imperative that we determine which specific microbes, microbial consortia, or microbial products yield benefit in FGIDs to provide precision care without unwanted effects.

\textbf{Translating Microbiome Research: Where Are We, and What Do We Need?}

The role of the gut microbiome in FGIDs must be considered in the context of the environment, the host, and host-specific factors. For us to advance the field and develop novel microbiota-based diagnostic and therapeutic targets in FGIDs, we will need to move from simple taxonomic associations to functional phenotypes and mechanism-based studies. In animal studies, we need to determine the specific microbes or microbial products as well as the mechanisms that alter host physiology. The use of gnotobiotic models allows inclusion of heterogeneity among gut microbiome and diet similar to human subjects, phenotype transfer to better understand cause–effect relationships, and complex reciprocal interactions among the host and microbiome. In terms of human studies, we need well-controlled longitudinal studies incorporating functional genomic, transcriptomic, metagenomic, and metabolomic analyses, as well as robust clinical metadata for the evaluation of mechanism-based phenotypes. There are several factors that can affect the gut microbiome including diet, demographics, body mass index, medication, and so forth as described earlier and hence these should be controlled before linking the microbiome with host outcomes. In addition to understanding the role of the microbiome in the pathophysiology of symptoms in FGIDs, assessing the impact of the microbiome on the efficacy of dietary and
pharmacologic therapy in conjunction with host features will allow for better treatment stratification compared with the current one-size-fits-all approach. Finally, we need to move the needle from empirically selected prebiotic and probiotic therapies to the next generation of precise mechanism-based diagnostic and therapeutic interventions. The use of genetically engineered bacterial strains to assess the gut environment, release metabolites of interest at specific locations within the GI tract, and optimize drug metabolism appears to be on the horizon. Rapid advances in these areas provide an optimistic outlook for microbiota-based interventions in FGIDs.

**Perspective**

It is now apparent that the gut microbiome is an integral player in the pathophysiology of FGIDs through its effects on host physiological processes even though the precise mechanisms underlying microbial regulation remain an area of active investigation. The improved understanding of factors that shape the gut microbiome allow us to better identify confounding effects in human studies, including physiological development through childhood and adolescence to adulthood, and at the same time, appreciate the adaptation of this resilient microbial ecosystem to short- and long-term perturbations in the host environment. A comprehensive view of the gut microbiome in both pediatric and adult FGIDs is important to account for the dynamics of the gut microbiome because it shows a continuum across the lifespan, with hallmark characteristics in different phases of life.

The expanding ecosystem of microbiome-based startups and industry funding, the shift away from compositional changes toward functional products of the microbiome, better integration of clinical metadata, and genetic engineering and synthetic biology tools to make designer probiotics targeting specific host functions together instill confidence in our ability to move microbiome science from the bench to the bedside.

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cystic fibrosis transmembrane conductance regulator phospho-


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