**REVIEW ARTICLE**

**Gut microbiota dysbiosis in functional gastrointestinal disorders: Underpinning the symptoms and pathophysiology**

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**Abstract**

Functional gastrointestinal disorders (FGIDs), currently known as disorders of gut–brain interaction, are emerging microbiota–gut–brain abnormalities that are prevalent worldwide. The pathogenesis of FGIDs is heterogeneous and is intertwined with gut microbiota and its derived molecule-modulated mechanisms, including gut dysmotility, visceral hypersensitivity, gut immune abnormalities, abnormal secretion, and impaired barrier function. There has been phenomenal progress in understanding the role of gut microbiota in FGIDs by underpinning the species alternations between healthy and pathological conditions such as FGIDs. However, the precise gut microbiota-directed cellular and molecular pathogeneses of FGIDs are yet enigmatic. Determining the mechanistic link between the gut microbiota and gastrointestinal (GI) diseases has been difficult due to (i) the lack of robust animal models imitating the various aspects of human FGID pathophysiology; (ii) the absence of longitudinal human and/or animal studies to unveil the interaction of the gut microbiota with FGID-relevant pathogenesis; (iii) uncertainty about connections between human and animal studies; and (iv) insufficient data supporting a holistic view of disease-specific pathophysiological changes in FGID patients. These unidentified gaps open possibilities to explore pathological mechanisms directed through gut microbiota dysbiosis in FGIDs. The current treatment options for dysbiotic gut microbiota are limited; dietary interventions, antibiotics, probiotics, and fecal microbiota transplantation are the front-line clinical options. Here, we review the contribution of gut microbiota and its derived molecules in gut homeostasis and explore the possible pathophysiological mechanisms involved in FGIDs leading to potential therapeutics options.

**Introduction**

Functional gastrointestinal disorders (FGIDs), currently known as disorders of gut–brain interaction, including irritable bowel syndrome (IBS) and functional dyspepsia (FD), are common conditions in clinical practice and community.¹ The prevalence of FGIDs is approximately 40% in the world based on a recent survey using the Rome IV diagnostic criteria.² The spectrum of FGID symptoms includes abdominal pain/burning, bloating, nausea, fullness, vomiting, and altered bowel habit.³ The pathophysiology of FGIDs is complex, but mounting studies indicate that gut microbiota plays a central role in the development of FGID and modulation of the symptoms.⁴ Understanding of the pathogenesis of FGIDs is evolving with the elucidation of pathophysiological mechanisms at the cellular and molecular levels.⁵ ⁶ Not long ago, “functional” conditions, such as FGIDs, were explained as idiopathic, and the patients were suspected by physicians and researchers to be neurotic and/or healthy subjects with an imaginary illness.⁷ By far, with enhanced pathophysiological knowledge, the best approach to examining patients with FGIDs would be a holistic view of the patients’ genetic predisposition, epigenetics, brain connectome, enteric nervous system (ENS), lifestyle and environmental factors, and their interface with gut microbiota.⁸ ⁹

Gut microbiota changes quite rapidly in humans because of variations in diets, medication, mode of birth, age, gender, and psychological state.¹⁰ The pathogenic potentials of gut microbiota have been well documented as up to 10% of IBS patients had gastroenteritis followed by gut microbiota dysbiosis, resulting in the development of IBS (postinfection IBS).¹¹ The advent of modern technology broadened our understanding of the gut microbiome.¹² Next-generation sequencing has expanded our knowledge of characterizing gut microbial alternations using culture-independent techniques and functional changes in patients with FGID, although the findings between studies are inconsistent.¹³ ¹⁵ Notably, the most convincing evidence of the role of microbiota comes from the findings of alterations in gut transit, intestinal barrier function, and visceral sensation observed in germ-free (GF) mice after fecal microbiota transplantation.
(FMT) from patients with FGIDs.

Extensive research identifies a knowledge gap regarding whether gut microbiota dysbiosis is a cause or consequence of gut diseases. However, to ensure significant progress and to effectively incorporate gut microbiome science in the clinic, robust longitudinal studies in humans and animal models are warranted to underpin the precise functional roles of microbiota and its derived molecules that link to the specific pathophysiological mechanisms of FGIDs.

This review aims to assess the impacts of gut microbiota and its derived molecules on the pathophysiological mechanisms of FGIDs, including gastrointestinal (GI) dysmotility, impaired barrier dysfunction, GI immune dysfunction, and visceral hypersensitivity. Besides, we summarize the potential therapeutic strategies for gut microbiota dysbiosis in FGIDs, highlighting the gaps and areas for further explorations.

Pathophysiological mechanisms of FGIDs with special emphasis on gut microbiota dysbiosis

The human gut is home to a heterogeneous microbial system termed the gut microbiome, mediating a wide array of biochemical transformations that modulate host physiology and pathophysiology. Disruption of gut microbial equilibrium can trigger mucosal immune activation, leading to breach in the epithelial barrier, which further results in visceral hypersensitivity and gut motility abnormalities, which are the hallmarks of FGIDs, reinforcing intestinal barrier dysfunction as the core pathophysiology. Currently, the majority of gut microbiota-mediated mechanistic studies related to FGIDs have been performed using animal models. The implications of the animal studies enable us to extend the understanding of the crosstalk between the gut microbiota and the underlying pathogenies implicated in FGIDs.

Gut microbiota dysbiosis is an emerging pathophysiology of FGIDs. Gut microbiota alterations in the small bowel and colon have been evidenced extensively in patients with FGIDs. Several studies showed that the gut microbiota profile of IBS patients significantly differs from that of healthy subjects. A recent meta-analysis showed that IBS patients presented higher levels of pathogenic bacteria of the genera Enterobacter, Clostridium, and Enterococcus compared to healthy controls. A microbiome signature from patients with IBS was found to be decrease in microbial diversity, and the severity of the symptoms was associated with Clostridiales species and/or methanogenic microbes. Another study demonstrated the overrepresentation of Methanobrevibacter smithii in fecal samples from patients with functional constipation, and constipation-predominant IBS (IBS-C). One study showed an increased ratio of Firmicutes to Bacteroidetes, increased Streptococcus and Ruminococcus, and decreased Lactobacillus and Bifidobacterium species in patients with IBS.

Gut microbiota alterations in the small bowel include an increase in small intestinal bacteria on quantitative culture (≥10^5 colony-forming units/mL of jejunal aspirate), defined as small intestinal bacterial overgrowth (SIBO), and low-grade SIBO (≥10^3 but <10^5 colony-forming units/mL of jejunal aspirate), which have clinical significance. Several studies found a strong association between the IBS and SIBO. A meta-analysis showed a higher relative risk of the SIBO among IBS patients compared with controls. A recent study on patients presenting with bloating, abdominal pain, or diarrhea, thought to be suffering from SIBO, found alterations in small intestinal microbial composition. Another study showed a significant reduction of the anaerobic genera Veillonella, Prevotella, and Actinomyces in the duodenal mucosal biopsies from patients with FD compared with healthy controls. One study demonstrated the absence of Acidobacteria and an increased ratio of Bacteroidetes to Proteobacteria in gastric fluid samples from FD patients. However, skepticism about gut microbiota dysbiosis causing IBS still continues, partly because what constitutes a healthy microbiome is unclear. Taken together, this breakthrough evidence indicates that gut microbiota alteration is a common finding in FGIDs and has provided the impetus to whether reforming the gut microbiota composition might be a potential treatment for FGIDs.

Gut microbiota alterations modulating gut motility. Abnormal GI muscular movements driving fast or delayed gut transit is a major pathophysiological mechanism of FGIDs. Alterations in gut microbiota due to GI hypomotility can further perpetuate GI dysmotility resulting from gut dysbiosis. Gut microbes could alter gut transit; similarly, an irregular gut transit could modify the spatial organization and proportion of the microbiome by creating a luminal microenvironment for the growth of specific bacterial taxa or by affecting bacterial colonization. Patients with IBS-C tend to present slow gut motility, while patients with diarrhea-predominant IBS (IBS-D) have relatively faster motility. One proof-of-concept study demonstrated that GF mice colonized with the fecal samples from IBS-D patients had accelerated gut transit, impaired intestinal barrier function, gut immune dysfunction, and increased anxiety levels compared to those colonized with fecal samples from healthy controls. Another proof-of-concept study showed that GF mice colonized with the fecal samples from patients with IBS-C had slowed gut transit, upregulated 5-HT transporter, and reduced serotonin (5-hydroxytryptamine, 5-HT) levels in the colonic biopsy, which were supported by decreased relative abundance of gut microbiota such as Firmicutes and increased Bacteroidetes. These findings support the potential regulatory functions of gut microbiota dysbiosis in gut motility.

Gut microbiota-derived molecules and gut motility. In addition, several studies showed that gut microbiota-derived molecules directly regulate gut motility. These molecules relay signals modulating the function of targeted host cells and subsequently alter the GI functions. Short-chain fatty acids. Short-chain fatty acids (SCFAs) are primary metabolites produced from gut microbial dietary fiber fermentation. SCFAs, particularly butyrate, acetate, and propionate, are versatile molecules modulating host physiology through various mechanisms, including interactions via specific G-protein-coupled receptors. SCFAs affect GI physiology, including contractility, visceral sensation, secretion, and intestinal barrier integrity. These effects suggest considering the dynamic functions in FGIDs based on microbial activity. One study demonstrated that antibiotic-treated mice colonized...
with fecal samples of patients with slow-transit constipation had altered SCFAs profiles and delayed gut transit. The authors further showed that butyrate supplementation reversed the delayed gut motility in mice colonized with fecal samples of patients with slow-transit constipation (Table 1). A recent meta-analysis compared the differences in SCFAs in patients with IBS and found that butyrate and propionate were decreased in patients with IBS-C, whereas butyrate was increased in patients with IBS-D compared to healthy controls. One study showed increased gene expression of \textit{tryptophan hydroxylase 1} (Tph1) and serotonin production in colon biopsy through stimulation of enterochromafﬁn (EC) cells, a specialized subtype of enteroeendocrine cells, via butyrate. Taken together, these findings support the fact that SCFAs regulate gut motility.

**Serotonin and tryptamine.** A study reported that indigenous spore-forming bacteria from human and mouse promote biosynthesis of serotonin from colonic mucosal EC cells. The host as well as the microbes appear benefit from serotonin signaling. A state-of-the-art study suggested that increased levels of gut luminal serotonin increased the relative abundance of spore-forming members of the gut microbiota. For instance, \textit{Turicibacter sanguinis} expresses a eukaryotic neurotransmitter sodium symporter-related protein-sharing sequence and structure with the mammalian serotonin transporter. Another group of researchers detected the mechanism of crosstalk between enteric neurons and the gut microbiota as the stimulus signal for serotonin release and the subsequently activated serotonin type 4 (5-HT4) receptor, which regulates smooth muscle contraction and relaxation. This study demonstrates direct crosstalk between the microbiota and the ENS mediated via serotonin and indicates a potential mechanism linking microbial dysbiosis to FGIDs.

Tryptamine is produced by gut bacteria, particularly \textit{Clos- tridium sporogenes} and \textit{Ruminococcus gnavus} from tryptophan. One study examined whether tryptamine in the gut can function by facilitating the 5-HT4 receptor. The authors showed that tryptamine increased the ionic flux across the colonic epithelium, as well as fluid secretions in colonoids from GF and humanized mice, validating the physiological function of tryptamine for gut secretion. Furthermore, improved gut motility was observed in

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**Figure 1** Gut microbiota-directed pathophysiological mechanisms of functional gastrointestinal disorders (FGIDs), currently known as disorders of gut–brain interaction. 5-HT4R, 5-hydroxytryptamine receptor; BAs, bile acids; E-cadherin, epithelial cadherin; GLP-1R, glucagon-like peptide-1 receptor; ICCs, interstitial cells of Cajal; IPAN, intrinsic primary afferent neuron; NGF, nerve growth factor; PDGFRα, platelet-derived growth factor receptor alpha; SCFAs, short-chain fatty acids; SMCs, smooth muscle cells; TLRs, toll-like receptors; TNF-α, tumor necrosis factor-alpha; TRPA1, transient receptor potential ankyrin 1; TRPV1, transient receptor potential cation channel subfamily V member 1; ZO, zonula occludens.
GF mice that were colonized with *Bacteroides thetaiotaomicron* microbes, which were genetically engineered to produce tryptamine.\textsuperscript{17}

**Bile acids.** Bile acid (BA) pool alternations induced by microbial dysbiosis have been proposed in FGID pathogenesis (Fig. 2).\textsuperscript{51,52} In humans, the primary BAs cholic acid (CA) and chenodeoxycholic acid (CDCA) are deconjugated from their gly-cine or taurine conjugate by microbial bile salt hydrolases through the microbial biotransformation process.\textsuperscript{53} Microbiota-mediated effects on intestinal fluid secretion are the consequences of differential BA biotransformation.\textsuperscript{11} BA malabsorption is known to be involved in IBS-D.\textsuperscript{7} BA malabsorption is observed more in patients with IBS-D than those with IBS-C.\textsuperscript{54} A meta-analysis demonstrated that approximately 10% patients were IBS-D severe BAs malabsorption (425 patients in 5 studies, SeHCAT retention <5% of baseline), 32% patients presented moderate BA malabsorption (1,073 patients in 17 studies, SeHCAT retention <10%), and 26% patients showed mild BA malabsorption (618 patients in 7 studies, SeHCAT retention <15%).\textsuperscript{55} One study showed that the quantity of BAs (CDCA and CA) were noticeably higher in stool samples from IBS-D patients and lower amounts in IBS-C patients. Another study showed that patients with IBS-D had elevated BA levels, and it correlated with bowel movements, colonic transit, and visceral pain.\textsuperscript{35} One study on patients with FD showed reduced duodenal BA concentrations, which was associated with the impaired intestinal barrier function.\textsuperscript{52,56}

**Gut microbiota alterations modulate intestinal gas profile.** Carbohydrate fermentation leads to the formation of hydrogen (H\textsubscript{2}) and carbon dioxide (CO\textsubscript{2}), which constitute intestinal gases produced by gut microbiota (Fig. 2).\textsuperscript{38,39} Variations in the intestinal gas profile have been consistently associated with FGIDs.\textsuperscript{57} A few studies, using computed tomography scans, showed increased luminal gas in patients with FGIDs during a flare of abdominal distension that might or might not result from consumption of a “high-flatulence” diet.\textsuperscript{58} Numerous studies highlighted the contribution of gut microbiota to the alterations of intestinal gases.\textsuperscript{51,59}

Gut microbiota dysbiosis, which includes a quantitative alteration called SIBO and qualitative variations, is common among FGID patients and is known to be associated with abdominal distension, flatulence, and bloating.\textsuperscript{60} Dysbiotic gut microbiota may cause these symptoms due to carbohydrate fermentation followed by substantial gas production.\textsuperscript{60} The intestinal gases diffuse into the systemic circulation and are exhaled.
| Gut microbiota derived molecules | Gut microbes | FGID/animal model | Key findings | Pathophysiological mechanism | References |
|---------------------------------|--------------|------------------|--------------|-------------------------------|------------|
| Propionate and butyrate         | *Bifidobacterium adolescentis*, *Roseburia-E. rectale* | Constipation-predominant irritable bowel syndrome (IBS) (IBS-C) | *Bifidobacterium adolescentis* increased and *Roseburia-E. rectale* decreased in fecal samples of IBS-C, possibly regulating the levels of butyrate and propionate, resulting in slowed gut transit | Altered gut motility | Sun et al. [32] |
| Butyrate                        | *Fecalibacterium prausnitzii* | Diarrhea-predominant IBS (IBS-D) | *Fecalibacterium prausnitzii* increased in IBS-D, which is a major producer of butyrate, resulting in accelerated gut transit. Butyrate might be considered a biomarker for IBS-D | Altered gut motility | Sun et al. [32] |
| Butyrate                        | *Blautia obeum ATCC 29174* | IBS-C | *Blautia obeum ATCC 29174* were decreased in IBS-C and correlated with butyrate levels | Altered gut motility | Bhattarai et al. [17] |
| Tryptamine                      | *Ruminococcus gnavus*, *Clostridium sporogenes* | IBS, slow transit constipation (STC) | *Ruminococcus gnavus* and *Clostridium sporogenes* identified as Tryptophan decarboxylase Tryptamine accelerates gut transit and increases colonic secretion by activating epithelial serotonin type 4 (5-HT4) receptor | Altered gut motility | Abnormal secretion | Bhattarai et al. [17] |
| BAs (CDCA, CA)                  | *Blautia wexlerae DSM19850* | IBS-C | Reduced bile acid (BA) levels correlated with delayed gut transit | Altered gut motility | Abnormal secretion | Mars et al. [34] |
| BAs (CDCA, CA)                  | *Blautia wexlerae DSM19850* | IBS-D | Increased BA levels correlated with bowel movements, colonic transit and visceral pain Elevated CDCA levels increased water content in stools from IBS-D | Altered gut motility | Mars et al. [34] |
| Methane                         | *Methanobravibacter smithii* | IBS-C, STC | Methane (CH₄) in the depletes gut serotonin resulting in slowed gut transit and constipation | Altered gut motility | Visceral hypersensitivity | Triantafyllou et al. [26] |
| Hydrogen                        | Hydrogen-producing bacteria | Small intestinal bacterial overgrowth (SIBO), IBS-D | Hydrogen gas correlated with SIBO condition in IBS-D patients | Altered gut motility | Visceral hypersensitivity | Gut immune dysfunction Pimentel et al. [38] |
| Hydrogen sulfide                | *Escherichia coli*, *Salmonella enterica*, *Clostridia*, and *Enterobacter aerogenes* | Diarrhea, SIBO, IBS-D? | Probably due to formation of sulfuric acid in the cells? Hydrogen sulfide (H₂S) might be considered a biomarker for SIBO | Altered gut motility | Visceral hypersensitivity | Abdominal bloating and distension Kalantar-Zadeh et al. [23] |
| Lactic acid                     | *Lactobacillus*, *Bifidobacterium* | SIBO, IBS-D | Possible link between SIBO, brain foginess, and D-lactic acidosis. Patients with brain fogness presented a higher SIBO prevalence with bloating, pain, and constipation | Altered gut motility | Visceral hypersensitivity | Dysregulated microbiota-gut-brain axis Rao et al. [39] |
| Alcohol (Ethanol)               | *Klebsiella pneumonia* | Need to explore in FGIDs and SIBO? | Alcohol abuse triggers gut microbiota dysbiosis, mucosal inflammation, and intestinal barrier derangement which corroborates liver damage | Impaired barrier dysfunction, gut immune dysfunction, dysregulated microbiota-gut-brain axis | Meroni et al. [41] |
in the breath, such as H₂ and methane (CH₄) (Table 1). An increase of ≥20 parts per million (PPM) of H₂ or >10 PPM of CH₄ above the baseline after glucose and within 90 min after lactulose ingestion on breath tests suggest the presence of SIBO, are associated with GI symptoms, for instance, abdominal bloating and/or distension. A few studies showed an association between high breath CH₄ and constipation. Methanogenic microbes in the colon produce CH₄, which depletes serotonin, resulting in slowed gut transit and constipation. Another study showed the association of H₂ gas mainly with IBS-D. Some gut microbes such as Escherichia coli, Salmonella enterica, Clostridia, and Enterobacter aerogenes produce hydrogen sulfide (H₂S) in the colon. H₂S is quickly absorbed in the colonocytes and forms sulfuric acid, which is highly toxic to the cells, resulting in altered gut motility, abnormal secretion, visceral hypersensitivity, and gut immune dysfunction (Table 1). One recent study showed that there was “brain fogginess” due to D-lactic acidosis in patients with SIBO. Another study showed that gut bacteria such as Klebsiella pneumonia produces alcohol, which might modulate brain and intestinal barrier functions. Thus, the aforementioned findings suggested that gut microbes and their derived molecules are essential to maintain proper GI function. These signaling molecules further enlighten the understanding of host and gut microbiota crosstalk, alluding to potential therapeutic options for FGIDs.

**Gut microbiota alterations modulating gut immune function.** Gut immune dysregulation is implicated in the pathogenesis of subsets of patients with FGIDs. A few studies showed an increased number of gut immune cells such as macrophages, mast cells, eosinophils, and T cells in patients with FGIDs. Mast cells, upon activation, release tryptase, histamine, cytokines, and prostaglandins, which have been implicated in altering nociceptive pathways and intestinal barrier functions in FGIDs. A breach of the intestinal barrier allows infiltration of luminal antigens, activating the immune response, and contributes to the severity of the symptom in FGIDs through neuroimmune dysregulation.

There is a plethora of evidence supporting gut immune system activation triggered by the gut microbiota. Several studies showed increased expression of proinflammatory cytokines following interactions between bacterial components and pattern recognition receptors, including toll-like receptors (TLRs), which play essential roles in the innate immune system’s ability to recognize structurally conserved molecules derived from microbes. One study on patients with IBS showed that the expression levels of proinflammatory cytokine IL-6, TLR4, TLR5, C-X-C motif chemokine ligand-11, and CXC-3 receptor (CXCR) were elevated in colonic biopsies of patients compared to controls; on the contrary, the expression levels of anti-inflammatory cytokine IL-10 were downregulated. The authors confirmed a positive correlation between the number of gene copies of the commensal bacteria such as Bifidobacteria, Lactobacillus, and IL-10. This study further demonstrated a positive correlation between weekly stool frequency and the expression levels of TLR4 and CXCR3; in contrast, it was negatively correlated with IL-10. Another study showed that IBS patients with SIBO presented higher mucosal IL-1 α and β levels than those without SIBO, and the elevated IL-1 β levels were associated with abdominal bloating and loose stool. Studies on mucosal T cell alterations in FGIDs are scant. One study showed reduced expression of lymphocyte marker, Fas cell surface death receptor (FAS), and human leukocyte antigen-DR isotype (HLA-DR) in patients with FD, while duodenal eosinophilia has been implicated in dyspeptic symptom generation. Another study showed increased small bowel-homing CCR9 T lymphocytes and eotaxin, which correlated with the symptom severity in patients with FD. This evidence strongly suggests that patients with FGIDs experience gut immune activation influenced by microbiota alternations, reinforcing low-grade inflammation as a potential mechanism of FGIDs.

**Gut microbiota alterations modulating intestinal barrier function.** The gut luminal–mucosal interface represents the place where food particles, immunogenic and toxic molecules, and gut bacteria and their molecules challenge the scrutiny of the gut mucosa-associated immune system. As a core pathophysiological mechanism, intestinal barrier dysfunction leads to mucosal immune response activation, which modulates GI motility in relation to the severity of symptoms of FGIDs. The gut epithelium is a single layer of cells that acts as a selective barrier with astonishing dynamics, preventing the passage of detrimental luminal contents while allowing the absorption of water, electrolytes, and dietary nutrients.

The mucus layer overlaying the gut epithelial cells serves as a primary factor in protecting the host by providing a strong immunologic and physical barrier against pathogens. The mucus layer is a reservoir of antimicrobial immunoglobulins and peptides and is enriched in polysaccharides, a major nutrient source for different populations of bacteria. Furthermore, the intestinal barrier is protected by the epithelial tight junction proteins, which are multifunctional complexes that form a seal between adjacent intestinal epithelial cells near the apical surface and seal the paracellular space, thus preventing gut luminal contents from entering the subepithelial space. The starvation of microbiota, for instance, with decreased fiber consumption might increase the reliance of microbiota on the mucus polysaccharides, leading to mucus layer degradation and increasing pathogen susceptibility, which further leads to immune activation. A few studies showed that alterations in mucosa-associated bacteria, for instance, Ruminococcus gravis, Akkermansia muciniphila, and Ruminococcus torques, were associated with mucus integrity and intestinal secretion. Furthermore, in susceptible conditions, a harmful pathogen or the consumption of certain foods impairs intestinal barrier function by altering tight junctions, resulting from proteasome-mediated degradation triggered by inflammatory mediators including proteases, eicosanoids, and histamine. Several studies showed that the expression of adhesion proteins, zonula occludens-1 and occludin, was significantly lower in duodenal biopsy samples with decreased transepithelial resistance and impaired intestinal barrier function in FGID patients compared to controls. Recently, a new endoscopic technology, confocal laser endomicroscopy and endocytoscopy, enables us to carry out a high-resolution assessment of GI mucosal histology at cellular and subcellular levels and obtain “optical biopsies” of the endoluminal surface. Exploiting the endomicroscopy, one study reported duodenal mucosal epithelial breaks and increased intervillous spaces when the mucosa was challenged with foods.
in patients with IBS.79 In the setting of impaired barrier function, the pathogens or food antigens cross the intestinal barrier and trigger a T helper 2 (TH2) immune response that results in the activation of immune responses mediated via eosinophils and mast cells.64 Mast cells and eosinophils act as antigen-presenting cells to TH2 lymphocytes, which leads to eosinophil and mast cell degranulation close to nerve fibers, which is responsible for muscle contraction and abdominal pain.80 TH2 cells also trigger immunoglobulin (Ig) class switching in B cells and elevate IgE antibody expression, suggesting an increased number of IgE+ B cells in conditions associated with leaky gut, including FGIDs.81 A recent study showed an increased number of IgE+ mast cells in colonic tissue positively correlated with visceral hypersensitivity and intestinal barrier dysfunction in IBS patients.82 Thus, a healthy gut barrier function may play a fundamental role in gut homeostasis, microbial colonization resistance, community resilience, host defense, drug modifications, and food digestion.

**Gut microbiota alterations modulating visceral sensation.** The “taste” and “smell” of food particles, the gut bacteria, and their derived molecules could be sensed and transduced through the enteroendocrine cells and intrinsic nerves via specific receptors.82 Abnormal activation of a mucosal immune response affects visceral sensitivity in relation to the degree of inflammatory response accompanied by the proximity of immune cells and sensory neurons.83 Visceral hypersensitivity is a critical contributor to the occurrence and severity of symptoms in FGIDs.3 It manifests as an amplified perception of mechanochemical stimuli applied to the gut, which results in the exaggerated sensation of pain and burning.7 The modulators of visceral sensation include transient receptor potential vanilloid subtype 1 (TRPV1) and the transient receptor potential ankyrin 1 channel (TRPA1), serotonin, histamine, tachykinin, cannabinoid, acid-sensing ion channel, protease activated receptors, and voltage-gated calcium and sodium channels.84,85 Activation of TRPV1 by capsaicin, acidic pH, thermal stimulus, nerve growth factor (NGF), prostaglandins, inflammatory mediators, and microbes triggers the release of neuropeptides, such as substance P and calcitonin gene-related peptide-1.84 Several studies showed that the upregulated expression of TRPV1 in colonic biopsies was positively correlated with abdominal pain in IBS-D patients.86,87 One recent study showed a positive correlation between an increase in *Escherichia coli* abundance and visceral hypersensitivity followed by induction of hypersensitivity in response to *Escherichia coli* gavage in Paneth cell-defect mice.88 One state-of-the-art study showed that EC cells were activated via the stimuli through TRPA1 and led to voltage-gated calcium channel-dependent serotonin release, which might be a potential target for the management of FGIDs.89 The gut epithelial cells are observed to provide a barrier between the nerve fibers and lumen, suggesting that TRPA1 serves as the primary detector of luminal irritants in the EC cells prior to direct submucosal damage. Further studies explored a mechanistic link contributing to visceral hypersensitivity by demonstrating the induction of epithelial cannabinoid and μ-opioid receptors after administration of *Lactobacillus* strains; regulation of peripheral and central neuronal pathways and antiinocceptive effects through the inhibition of TRPV1 after treatment with *Lactobacillus reuteri* have been shown.89,90

**Gut microbiota modulates neuroimmunoneuroendocrine interface.** The neuron, immune, and endocrine regulations are anatomically and physiologically interconnected in the gut.91 Microbiota-derived molecules could be detected by TLRs and expressed in host cells, particularly neurons, glial cells, macrophages and dendritic cells, and enteroendocrine cells, which specifically recognize microbe-associated molecular patterns.96 For instance, TLR4 recognizes the lipopolysaccharide of Gram-negative bacteria regulating ENS integrity, as well as neuromuscular function.92 TLR2 detects lipoteichoic acid from Gram-positive bacteria and promoted neurogenesis after antibiotic treatment.

A recent study demonstrated that gut microbiota potentially affects embryonic neuronal development. This study showed that GF mice display an altered prenatal transcriptomic profile and activation of brain microglial cells, which are critical for neuronal development.93 Patients with FGIDs showed increased numbers of mast cells and lamina propria T lymphocytes in close proximity to submucosal neuronal plexus, with decreased responsiveness of the neurons.94 Mast cell infiltration in the colonic mucosa in IBS patients triggers neuronal sprouting, resulting in the release of NGF, which promotes topical neoinnervation and angiogenesis.94,95 Duodenal fine nerve fibers were observed more often in IBS patients compared to healthy controls, and the degree of mast cell activation positively correlated with the grade of nerve fiber sprouting.95

Microfold (M) cells are presented in the epithelial lining of mucosa-associated lymphoid tissues, for instance, Peyer’s patches (PPs) of the gut. Luminal antigens are actively transported through M cells to the underlying lymphoid follicles to propagate an immune response.96 This study demonstrated that TRPV1+ neurons promoted gut immune regulation against the pathogenic bacteria *Salmonella typhimurium*. Another study suggested that the TRPV1+ nociceptors regulated the density of M cells in ileum PP follicle-associated epithelia to limit entry points for pathogenic bacteria.96

Enteroendocrine cells represent less than 1% of the gut epithelium, and they are critical to maintain gut hemostasis.97 Accumulating evidence shows that interplay between enteroendocrine cells and enteric neurons modulate gut physiology via an extended axon-like structure termed “neuropod” that directly crosstalks with neurons through modified synapses.98 EC cells secrete serotonin in response to a wide variety of microbiota-derived molecules in the lumen.85 EC cells were found to be significantly increased in rectal biopsies from patients with IBS.99 One recent study showed that TLR9 colocalized with the satiety hormone cholecystokinin (CCK) in the enteroendocrine cells of the small intestine.100 The authors found that pathogenic bacteria, such as *Shigella flexneri*, *Escherichia coli*, and *Salmonella typhimurium*, evoke CCK secretion in TLR9-expressing enteroendocrine cells.100

**Gut microbiota modulates gut–brain axis.** Dysregulation of the gut–brain axis is one of the primary pathophysiological mechanisms in a subset of patients with FGIDs.4,101 Gut microbiota-regulated alterations in epithelial barrier function triggered by immune system activation resulting in abnormal gut–brain communications.102 Stress-related corticotropin-releasing hormones have an important role in intestinal barrier function.103 In addition, eosinophils release
corticotropin-releasing hormone and substance P, which might result in the activation of mast cells and, subsequently, dysfunction of the intestinal epithelial barrier.\textsuperscript{64} Magnetic resonance imaging studies on FGIDs patients demonstrated that abnormal structural and functional connectivity in the brain were responsible for processing visceral afferent transduction.\textsuperscript{8,104} Gut microbiota alterations could modulate the function of neurotransmitters, such as dopamine, serotonin, \( \gamma \)-aminobutyric acid, and acetylcholine, either by synthesis or consumption, resulting in changes in emotional state and behavior.\textsuperscript{105} Furthermore, BAs and their receptors have been identified in the brain, reinforcing a potential mechanistic role in the gut–brain axis.\textsuperscript{106}

Psychological comorbid conditions, including stress, anxiety, or depression, are associated with FGIDs and contribute to the pathophysiology as part of an integrated biopsychosocial model.\textsuperscript{107} Within this construct, the bidirectional communication of the gut–brain axis is critical in FGIDs. The brain alters gut physiology, such as visceral sensitivity and motility, mediating symptoms of FGIDs.\textsuperscript{108} Meanwhile, changes in the gut provide feedback to the brain, influencing psychological well-being.\textsuperscript{108} A meta-analysis showed high prevalence of both depressive disorders and anxiety (23%), anxiety (39%), and depressive (29%) symptoms in IBS patients.\textsuperscript{109} Psychological comorbidity could be a consequence of chronic GI disease burden and reduced quality of life, and its role in the pathophysiology of FGIDs is still unequivocally broader.

**Microbiota-directed therapeutic interventions for FGIDs**

The current treatment strategies to manipulate gut microbiota dysbiosis include probiotics, antibiotics, fecal microbiota transplantation (FMT), and dietary interventions.

**Probiotics and antibiotics.** The efficacy of probiotic treatment for FGIDs symptoms has been documented but lacks consistency in symptom improvement. Numerous studies suggested the benefit of specific probiotic strains, such as *Bifidobacterium animalis* DN-173010154, *Bifidobacterium lactis* DN-173, and multispecies probiotics in patients with IBS.\textsuperscript{110} One study showed that treatment with *Lactobacillus gasseri* OLL2716 shifted the microbial community composition in gastric fluid as found in healthy subjects.\textsuperscript{111} Other studies demonstrated that treatment with a probiotic containing *Bifidobacterium lactis* accelerated gut transit and improved symptoms in IBS-C patients.\textsuperscript{110}

Interventional studies reported a decrease in breath CH\(_4\) through treatment with either rifaximin and neomycin or rifaximin alone, which led to improvement in constipation in IBS-C and functional constipation patients.\textsuperscript{112,113} Randomized controlled trials on FD patients showed that rifaximin treatment was associated with improvements in dyspeptic symptoms, including abdominal bloating/fullness and belching.\textsuperscript{114} Furthermore, systematic reviews and meta-analysis of rifaximin and other antibiotics showed improvement in SIBO.\textsuperscript{20,115} These studies reinforce the fundamental role of gut microbiota in FGIDs.

**Fecal microbiota transplantation.** FMT is considered to be beneficial in managing microbial dysbiosis due to its ability to restore “healthy” microbes in patients.\textsuperscript{116} However, whether FMT as a treatment for FGIDs is a panacea or placebo is still controversial and warrants a precise definition of gut microbiota dysbiosis and the constitution of healthy microbiota.\textsuperscript{117,118} A recent meta-analysis of randomized controlled trials showed no significant symptom improvement in IBS patients after FMT compared to placebo.\textsuperscript{119} In contrast, a double-blind controlled trial in IBS patients showed improvement in symptom severity after FMT compared to placebo.\textsuperscript{120} However, more rigorous and multicentric larger trials are warranted on this issue.

**Dietary intervention.** The safest treatment intended to restore the healthy gut microbiota would be dietary intervention.\textsuperscript{121,122} A few randomized controlled trials showed that diets low in fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAP) are helpful to treat IBS.\textsuperscript{123,124} A few studies showed that vegetarian diets rich in fiber would increase SCFA production, which further inhibits pathogenic bacterial colonization in the gut.\textsuperscript{125,126}

**Future treatment strategies.** As the field moves toward modern science utilizing a rigorous, robust design, large and multicentric longitudinal human studies might be exploited to integrate multiomic data including physiology and pathophysiological mechanisms to explore unique features of symptom response for individual treatment. Such insight would lead to better stratification of therapeutic approaches, leading to the targeted restoration of altered physiological functions in a subgroup of patients with FGIDs. Gut microbiota and its derived molecules might be key drivers of the FGID pathogenesis. Through the elucidation of the steps involved in the synthesis, the release and consumption of these molecules might explore novel therapeutics avenues. The therapeutic intervention of the gut microbiome might include (i) interventions of replacing an entire gut microbial community with an optimized healthy community\textsuperscript{127}; (ii) introducing single specific microbes that might restore normal gut functions by producing critical gut microbiota-derived molecules for specific pathophysiology, such as genetically engineered bacteria *Bacteroides thetaiotaomicron* that produce tryptamine, which results in increased intestinal secretion and accelerate gut transit\textsuperscript{127}; and (iii) inhibiting and/or removing specific microbes or microbial processes through inhibitors of enzymatic pathways that are specific to the gut microbiota.\textsuperscript{128} Furthermore, the prior screening for SIBO/gut microbiota dysbiosis in FGID patients before antibiotic treatment may lead to better therapeutic response. Unselected patients with IBS treated with antibiotics showed a response rate of 40%.\textsuperscript{129} In contrast, according to a novel Indian study, IBS patients with SIBO on upper gut aspirate culture selected for antibiotics treatment showed a response rate as high as 87%.\textsuperscript{130} These improved antibiotics responses were able to be reproduced in subsequent studies from China and the United States using the lactulose H\(_2\) breath test, although studies with a larger sample size are needed on this issue.\textsuperscript{131,132}

**Conclusion and further directions**

State-of-the-art studies showed the importance of gut microbiota and its derived molecules in the modulation of major
pathophysiological mechanisms in FGIDs. There is substantial progress in characterizing the gut microbiota species in healthy and patients with FGIDs. However, the translational approach of gut microbiome research warrants robust and multicentric longitudinal studies in animals and humans to elucidate the precise role of gut microbes that can be therapeutically targeted to underpin each pathophysiology in FGIDs.\textsuperscript{15} Restoring healthy gut microbiota would maintain the microbiota–gut–brain axis in homeostasis from motility to mood, but it is important to keep in mind that clinical outcomes are more important compared to mechanistic parameters alone.\textsuperscript{117}

Furthermore, the biggest challenge for gut microbiome-based treatment is the use of broad-spectrum antibiotics, leading to the scorching of the gut microbiome, which has faced challenges due to skepticism related to antibiotic resistance, though, hitherto unproven.\textsuperscript{133} Therefore, alternative interventions for antibiotics might be considered, such as optimized healthy gut microbiota and/or its metabolites, which may benefit the patients by hampering the pathogenic bacteria. Traditional approaches to treat patients with FGIDs have beneficial effects in general. Due to the enormous heterogeneous nature of FGIDs, without consideration of the focus on the underlying pathophysiological mechanisms meditated through gut microbiota alterations, it would likely dilute the impact of current therapeutic strategies. The current knowledge gaps warrant a modern approach for a holistic view to characterize patients based on the multomics data from the gut microbiome, metabolome, transcriptome, host epigenome, and dietary profiles based on longitudinal studies. The integration of these factors with physiological changes will enable us to develop targeted approaches to treat the patients, not only relieving symptoms but also restoring gut homeostasis.

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