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REVIEW

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Role of the duodenal microbiota in functional dyspepsia

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Abstract

Background: Functional dyspepsia (FD) is a common and debilitating gastrointestinal disorder attributed to altered gut-brain interactions. While the etiology of FD remains unknown, emerging research suggests the mechanisms are likely multifactorial and heterogenous among patient subgroups. Small bowel motor disturbances, visceral hypersensitivity, chronic microinflammation, and increased intestinal tract permeability have all been linked to the pathogenesis of FD. Recently, alterations to the gut microbiome have also been implicated to play an important role in the disease. Changes to the duodenal microbiota may either trigger or be a consequence of immune and neuronal disturbances observed in the disease, but the mechanisms of influence of small intestinal flora on gastrointestinal function and symptomatology are unknown.

Purpose: This review summarizes and synthesizes the literature on the link between the microbiota, low-grade inflammatory changes in the duodenum and FD. This review is not intended to provide a complete overview of FD or the small intestinal microbiota, but instead outline some of the key conceptual advances in understanding the interactions between altered gastrointestinal bacterial communities; dietary factors; host immune activation; and stimulation of the gut–brain axes in patients with FD versus controls. Current and emerging treatment approaches such as dietary interventions and antibiotic or probiotic use that have demonstrated symptom benefits for patients are reviewed, and their role in modulating the host–microbiota is discussed. Finally, suggested opportunities for diagnostic and therapeutic improvements for patients with this condition are presented.

KEYWORDS
disorders of gut–brain interaction, dysbiosis, functional dyspepsia, functional gastrointestinal disorders, intestinal mucosa, microbiota, small intestinal bacterial overgrowth

1 | INTRODUCTION

Functional dyspepsia (FD) is a complex and heterogenous disorder characterized by chronic or recurrent upper abdominal pain or discomfort including postprandial fullness and early satiety not explained by structural or biochemical abnormalities identified in the routine clinical setting.[1] Similar to the irritable bowel syndrome (IBS), FD is a disorder of gut–brain interaction (DGBI).[2] These disorders were previously known as functional gastrointestinal disorders (FGID)[3] and likely involve several disease mechanisms including

disrupted normal sensorimotor function of the gastrointestinal tract, but with essentially normal endoscopic findings.[3–5]

FD is common with a pooled worldwide prevalence of approximately 5%–7%,[6] with variation across geographical regions[6,7]; however, treatment options are currently limited to chronic symptom relief,[8,9] contributing to a high disease burden with an impaired quality of life,[6,10] and financial hardship.[11–13] Thus, there remains a significant need for further research on the pathophysiology of FD to guide improved diagnostics and therapy for patients.

Based upon Rome IV criteria, FD is stratified into two subtypes: epigastric pain syndrome (EPS), with epigastric pain and/or epigastric burning; and postprandial distress syndrome (PDS), with postprandial fullness and/or early satiety.[3] While different risk factors have been identified for each FD subtype,[14,15] overlap is seen in approximately 30–60% of cases.[16–18] Individuals' symptoms may also change over time.[19] Moreover, many patients with FD have concomitant IBS[20,21] or gastroesophageal reflux disease (GERD).[22,23] These diseases overlap more than expected by chance and are part of a "spectrum" of gastrointestinal diseases with most likely a similar underlying pathogenesis.[3,8,22]

The pathophysiology of FD is likely multifactorial.[24] Increased intestinal permeability,[25,26] gastric hypersensitivity,[27,28] gastric motor disturbances such as delayed gastric emptying and fundic dysaccommodation,[27,28] *H. pylori* infection,[29,30] abnormalities of bile acid metabolism,[31] dietary, environmental, psychosocial, microbiota, and atopic, allergic, and autoimmune changes have all been implicated in the disease process.[32] Emerging evidence shows that microscopic upper gut alterations may exist in some patient subsets[33] and that the pathophysiology of FD is likely due to alterations in duodenal microbiota, which interact with the immune system to trigger symptoms[8] (Figure 1). Here, we review the recent literature on the duodenal microbiota changes in FD and host immunological, dietary and psychological factors, and outline future directions for mechanistic research in this complex condition.

1.1 | The small intestinal microbiome in FD

There is growing interest in the microbiome of the small intestine in FD. The gastrointestinal tract has a large surface and represents one of the major interfaces between the human body and the external environment, with a microbiota made up of over 1000 commensal bacterial species.[34,35] Many factors influence microbial composition including diet, environmental exposures, and medications, leading to high interindividual variation.[36] The microbiome plays a role in gut homeostasis and in particular the integrity of the epithelial barrier or the modulation of the mucosal immune system pathways, including tolerance to commensal microorganisms and digested food antigens in the lumen.[37–39] Furthermore, metabolites of the microbiota, most notably, tryptophan, short-chain fatty acids, and bile salts, interact with a number of pathways in the host.[40] The main two phyla of the gut microbiota are gram-negative Bacteroidetes and gram-positive Firmicutes.[41] While extensive physiological microbiota variation and compartmentalization exists along the length of the gastrointestinal tract,[42–44] this may be disturbed in disease states.[45–47] The small bowel is characterized by higher acidic, oxygen, and antimicrobial peptide levels, and these factors and a singular tightly packed mucosal layer and phasic propulsion at the ileum, limit bacterial density compared with the colon.[44]

The small intestine is dominated by the phyla Firmicutes, Proteobacteria, and Actinobacteria, with fewer Bacteroidetes,[48] since fast-growing facultative anaerobes tolerate this environment and can metabolize the simple carbohydrates available.[44] The taxonomy of these is visualized in Figure 2. Moreover, the microbial compositions of the luminal microbiota and the mucosa-associated microbiota (MAM) are taxonomically and functionally distinct from each other.[44,49] The MAM is posited to have a more direct role in the pathogenesis of gastrointestinal diseases due to its proximity to the epithelium.[42,50] The mucosal layer acts as a barrier against pathogenic microbes, preventing translocation into host tissue[51]; hence, the bacteria that can penetrate this layer and in the MAM may have a high potential to induce pro-inflammatory gene expression in the epithelium.[44,52] Furthermore, the duodenal MAM is again taxonomically distinct from other areas of the gastrointestinal tract,[48] dominated by *Streptococcus*, and lower levels of *Prevotella*, *Veillonella*, and *Neisseria* species.[53]

Microbial "dysbiosis" is defined as alterations in the composition, density, and function of the intestinal microbes that regulate immune and metabolic homeostasis.[54] Gastrointestinal dysbiosis, especially a decrease in bacterial diversity, is an increasingly recognized feature of multiple chronic noncommunicable diseases such as obesity, liver disease, cardiometabolic conditions, type 2 diabetes mellitus, and malnutrition.[55] It is suggested that microbial dysregulation may enable the expansion of opportunistic "pathobionts"—commensals with pathological potential,[54] activating a disease process[56] such as what is increasingly hypothesized to be a feature of IBS and FD.[54,57,58]

1.2 | Small intestinal bacterial overgrowth and FD

DGBIs have been linked to small intestinal bacterial overgrowth (SIBO),[59–64] a clinical condition that is characterized by the presence of excessive and/or abnormal type of microbes in the small intestine.[65] It is currently understood that SIBO involves bacteria that produce hydrogen, while patients that are also colonized with methanogenic archaea, anaerobic organisms producing methane, have an overgrowth termed "intestinal methanogen overgrowth" (IMO), as distinct from SIBO.[65] SIBO is traditionally diagnosed by $>10^5$ organisms per mL of jejunal aspirate,[66] or by a positive hydrogen breath test after peroral glucose or lactulose challenge.[65] However, methane breath tests are now used for IMO diagnosis, and the organism cutoff value is debatable, and a lower threshold of $\geq 10^3$ cfu ml^{-1} cutoff has been proposed.[67] It should be noted that jejunal aspirates are a limited sampling tool for the small intestinal microbiota due to risk of contamination and difficulties sampling hard-to-access areas

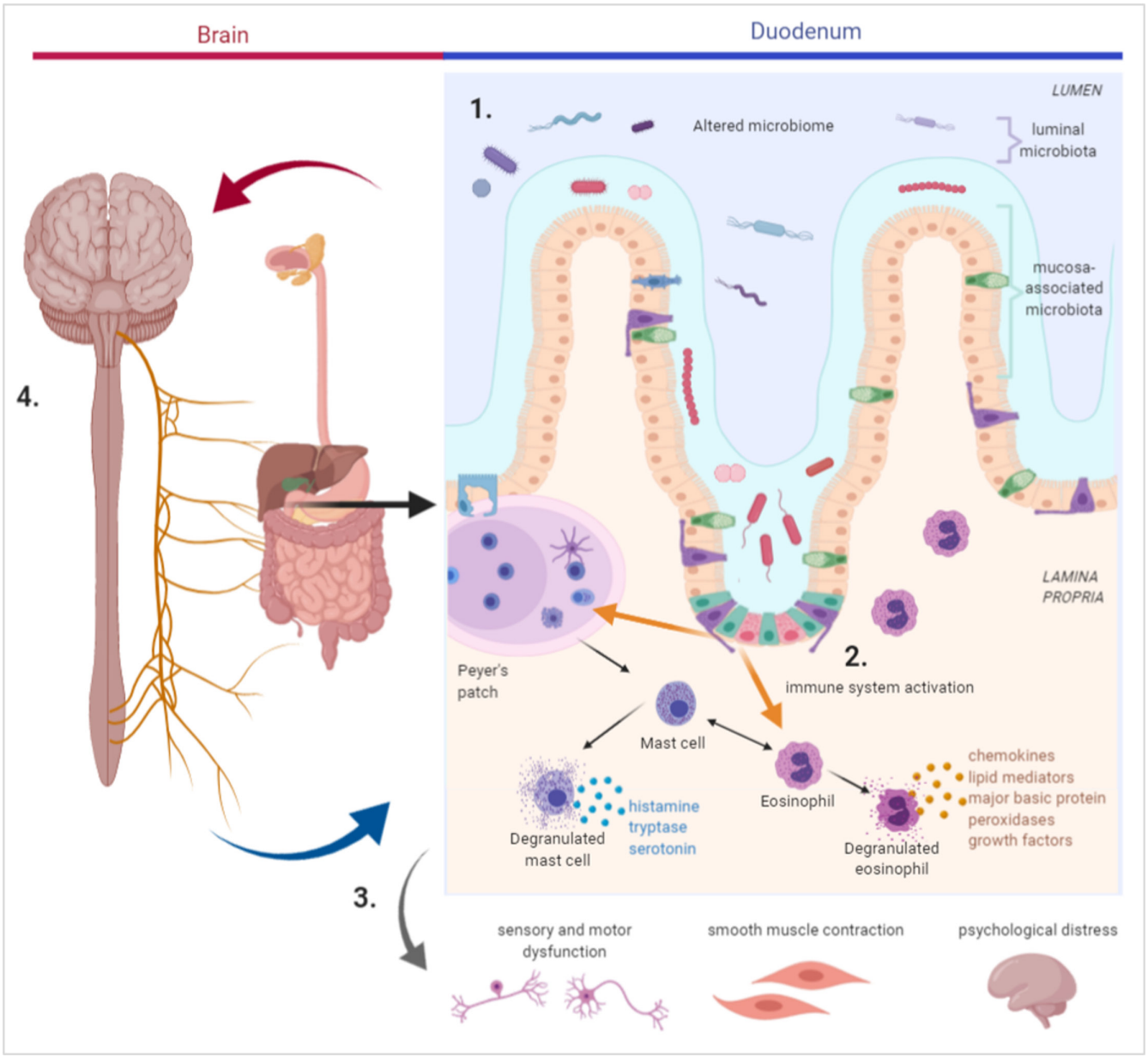

FIGURE 1 Proposed disease model for the pathogenesis of functional dyspepsia. 1. Antigen presentation to the small intestinal mucosa, including food macromolecules or microbial antigens. 2. Activation of eosinophils and mast cells through an immune cascade. 3. Local nerve sensitization and systemic immune activation leading to symptomatology. 4. Maintenance of a low-grade state of inflammation through bidirectional gut–brain and brain–gut pathways [Image created in BioRender]

like blind loops[48,66–68] and because the sampled luminal fluid may not represent the mucosal microbial composition.[69] Breath tests lack sensitivity and specificity confounded by intestinal transit,[66,70,71] and heterogenous methodology.[67,71]

Sequencing of the duodenum has identified microbial alterations in SIBO, including a lower relative abundance of Firmicutes and a higher abundance of Proteobacteria, both positively associated with decreased alpha diversity.[72] These changes also correlated with symptom severity such as bloating,[72] likely as a result of increased microbial fermentation by the imbalanced over-abundant community.[73,74] SIBO has been shown to be increased in chronic gastrointestinal diseases irrespective of the diagnostic method used.[62,75–77] Specifically, the prevalence and risk of SIBO are both increased significantly in chronic uninvestigated dyspepsia and FD compared with controls.[78] A recent study demonstrated elevated duodenal biopsy bacterial loads in DGBI patients compared with controls and increases in breath positivity in some patients, but no association between breath test positivity and duodenal microbial load.[79] Patients diagnosed with SIBO may receive antibiotic treatment such as with the nonabsorbable antibiotic rifaximin,[80] which also provides symptom relief in FD patients.[81] Interestingly, a recent systematic review demonstrated that treatment with rifaximin in combination with fiber,[82,83] *Lactobacilli* and *Bifidobacteria* probiotics[84] or mesalazine,[85,86] augmented the eradication rate of SIBO, further indicating

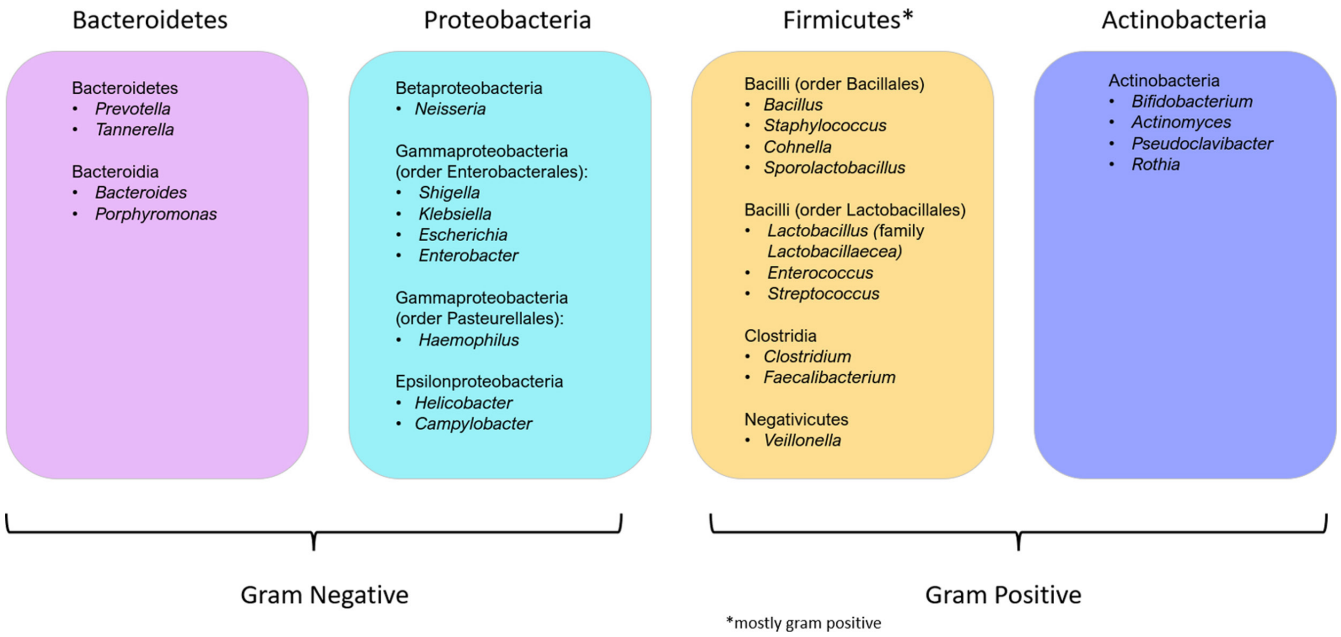

FIGURE 2 Taxonomy of the small intestinal microbiota across the four main bacterial phyla. Pictured are key intestinal microbial species from phylum–class–genus

that the condition is likely not underpinned simply by an increased microbial load alone, but also by an element of dysbiosis and possibly inflammation.[80] It has been postulated that SIBO may not be a distinct disease but a pathogenic mechanism underlying the development of FD.[60] The link between SIBO and DGBIs remains controversial, as the small intestinal microbial alterations in symptomatic patients do not always correspond with a positive SIBO diagnosis.[87]

1.3 | Small intestinal microbial signatures in FD

Recent studies have characterized the duodenal MAM of FD patients to a bacterial genus level. An Australian study first reported the increased relative abundance of *Streptococcus* in patients with FD compared with matched controls, albeit nonsignificant (which may have been because of the small sample size), and a notable inverse relationship between *Streptococcus* abundance and that of the anaerobic genera *Prevotella*, *Veillonella*, and *Actinomyces*, which were significantly reduced in the FD patients. Moreover, a higher bacterial load observed in FD patients negatively correlated with bacterial diversity and reported quality of life scores using the Nepean Dyspepsia Index and positively correlated with the severity of upper gastrointestinal meal symptoms.[88] A Japanese study identified an increase in *Streptococcus* in all sites of the upper gut in an FD cohort that again correlated with patient symptoms scores.[89] Furthermore, the reported beta diversity of the duodenal MAM was significantly different between patients and controls, while the alpha diversity remained unchanged, indicating a more complex microbiota structural change may be involved in the disease rather than changes in relative abundance of particular genera alone.[89] Dyspeptic patients also demonstrated increased anaerobic metabolism in the gastric microbial community in conjunction with increased *Pseudoclavibacter* and *Tannerella*, increased *Veillonella*, *Cohnella*, *Sporolactobacillus*, *Propionigenium* in saliva, and a higher duodenal prevalence of *Rothia*, *Clostridium*, *Haemophilus*, and *Actinobacillus* species.[90] A recent study demonstrated decreased duodenal mucosal *Neisseria* and *Porphyromonas* abundance in FD patients and controls prior to treatment with PPIs, but no differences in microbial load.[91] These findings are presented in Table 1. These bacterial shifts reported in FD patients are typical of the oral microbiota, and one study has confirmed that the healthy duodenum is taxonomically similar to the oral cavity.[42] Importantly, a recent Australian publication describes a new *Streptococcus salivarius* strain isolated from an FD duodenal biopsy.[92] Overall, this literature confirms that distinct duodenal microbial changes in both microbial load and diversity do occur in patients with FD.

1.4 | Effects of antibiotics and probiotics on the microbiome in FD

A pathogenic role of the microbiota in FD is supported by responses to drugs that alter the microbiome (Table 1). Rifaximin is a broad-spectrum antibiotic with gram-positive, gram-negative, aerobic and anaerobic coverage, a high intraluminal bioavailability in the gastrointestinal tract, and minimal systemic adverse effects.[93] It was superior to placebo FD treatment in one randomized trial, wherein relief of dyspeptic symptoms was reported in 79% of patients after taking rifaximin, compared to 47% in the placebo group at 8 weeks.[81] Moreover, the clinical benefits of rifaximin in FD do not appear to be explained by the overlap with IBS.[20] There is some evidence that its efficacy in IBS is due to rifaximin altering the

TABLE 1 Microbial changes in functional dyspepsia patients compared with controls as reported in published literature

Microbial change	Location	Number of FD patients	Number of controls	Citation
Increased relative abundance of *Streptococcus*	Duodenal biopsies	9 FD patients	9 matched endoscopy-negative controls	Zhong et al.[88]
	Mucosal brush sample of all sites in upper gut	11 FD patients	7 healthy controls	Fukui et al.[89]
Streptococcus abundance positively correlated with severe upper gastrointestinal symptoms	Mucosal brush samples of all sites in upper gut	11 FD patients	7 healthy controls	Fukui et al.[89]
Lower abundance of *Prevotella*, *Veillonella* and *Actinomyces* Inverse relationship between *Streptococcus* and *Prevotella*, *Veillonella* and *Actinomyces* Higher bacterial load negatively correlated with bacterial diversity Higher bacterial load and lower bacterial diversity correlated with quality of life scores	Duodenal biopsies	9 FD patients	9 matched endoscopy-negative controls	Zhong et al.[88]
Reduced beta diversity Unchanged alpha diversity Increased Firmicutes	Mucosal brush samples of all sites in upper gut	11 FD patients	7 healthy controls	Fukui et al.[89]
Increased *Pseudoclavibacter* and *Tannerella*, increased *Veillonella*, *Cohnella*, *Sporolactobacillus*, *Propionigenium* in saliva Increased duodenal *Rothia*, *Clostridium*, *Haemophilus*, and *Actinobacillus* Increased gastric *Pseudoclavibacter* and *Tannerella*	Gastric and duodenal mucosal brush samples and saliva samples	25 symptomatic patients with dyspepsia, dysphagia and reflux	11 patients with achalasia	Cervantes et al.[90]
Lower abundance of *Prevotella* Higher abundance of *Bifidobacterium* and *Clostridium* *Prevotella* inversely associated with PDS symptom severity Lower interindividual bacterial diversity	Gastric fluid (via nasogastric tube)	44 FD patients	44 healthy controls	Nakae et al.[101]
Predominance of Bacteroidetes over Proteobacteria Absence of *Acidobacteria* Higher *Escherichia*, *Shigella* and *Bifidobacterium longum*	Gastric fluid (via nasogastric tube)	24 FD patients	21 matched healthy controls	Igarashi et al.[102]
Lower *Neisseria* and *Porphyromonas* abundance	Mucosal brush samples of duodenum	28 PPI-naive FD patients	30 healthy controls	Wauters et al.[103]

Abbreviation: FD, functional dyspepsia.

intestinal gut microbiota,[94] and in other gastrointestinal diseases, it has been shown to preserve colonic flora, increasing the abundance of *Lactobacilli* and *Bifidobacteria*[95] in a "eubiotic" effect of positively modulating the gut microbiota, favoring the growth of beneficial bacteria altering the overall composition.[96] However, these microbial changes with treatment need to be studied in the duodenum in FD cohorts.

Conversely, previous antibiotic treatments are a risk factor for developing FD,[97,98] and it is well known that broad-spectrum antibiotic use can result in long-term reductions in commensal species.[55] Probiotics may have a therapeutic role in FD due to their modulation of gastrointestinal flora, and three studies have shown postprandial fullness and bloating symptom improvements after administration of probiotic *Lactobacillus gasseri* OLL2716 for twelve weeks in *H. pylori*-positive and negative dyspeptic patients.[99–101] Symptom resolution was demonstrated in 35.5% of *H. pylori*-negative FD patients compared to 17% with placebo.[100] A lower abundance of *Prevotella* and a higher abundance of *Bifidobacterium* and *Clostridium* were seen in the gastric fluid of the FD cohort prior to treatment, but post-treatment, FD patients showed decreased gastric fluid volume possibly signifying more efficient gastric emptying, and a gastric fluid microbiota resembling the healthy controls pre-treatment. Interestingly, *Prevotella* abundance specifically was inversely associated with postprandial distress levels, wherein an increase in this genus post-treatment

was related to symptom alleviation.[101] *Lactobacillus gasseri* OLL271 was also shown to "restore" the abnormal gastric fluid microbiota of patients with FD. The gastric fluid of FD patients prior to twelve weeks of probiotics showed a predominance of Bacteroidetes over Proteobacteria (24.2/4.9% compared to 13.5/29.5% for controls) and an absence of Acidobacteria (0.2% compared to 3.8% in controls) and post-probiotic treatment, these proportions shifted to reflect a microbiota composition similar to healthy volunteers.[102] Additionally, while small intestinal bacterial data were not collected, 8 weeks of treatment with *Bacillus coagulans* MY01 and *Bacillus subtilis* MY02 demonstrated a 28% response rate improvement over placebo in FD patients.[103] These findings offer preliminary evidence that patient symptoms are related to abundances of certain genera in the upper gut microbiota, which can be modified by therapeutic antibiotic and probiotic agents. Despite these noted specific changes, further research on larger patient cohorts across more diverse geographical regions is needed to characterize the microbiome alterations in the disease, especially in the duodenum.

Proton pump inhibitors (PPIs) also modulate the microbiome, and patients with DGBIs using PPIs have been shown to have a higher bacterial load in the duodenum compared with non-PPI users,[79] and there an increased risk of SIBO in PPI users has been observed.[104] Studies of the fecal microbiota suggest that PPIs are associated with decreased bacterial diversity,[105,106] and a shift toward a community more closely resembling that of the oral microbiota, such as increased *Streptococci*,[105,106] *Enterococcus* and potentially pathogenic species of *Escherichia coli*,[105] and decreased *Faecalibacterium*.[107] Some duodenal microbiota changes at a family level have been observed between PPI users and non-PPI users,[108] but little is known about how PPIs impact the duodenal MAM in FD. A recent preliminary study using duodenal brushings found significant MAM variation and decreased diversity from baseline after PPI use in FD patients and controls.[91] While the healthy duodenum is taxonomically similar to the oral microbiota,[42] it is of interest to understand whether PPIs reduce the abundance of species specific to the duodenum, resulting in increased similarity to oral bacterial communities. Furthermore, PPIs are a current treatment for FD and gastroesophageal reflux disease (GERD),[109] and oral microbial species have been linked to other gastrointestinal inflammatory diseases, including GERD.[110,111] Thus, there is likely to be a complex interrelationship between microbial load, diversity, and PPI use in the small intestine of patients with FD.

1.5 | Immune activation and the gastrointestinal microbiome in FD

1.5.1 | The microbial antigen or "postinfectious" hypothesis of FD

The microbiota's significance in gastrointestinal inflammation and FD symptomatology is highlighted in cases of postinfectious FD (PI-FD). The prevalence of PI-FD is approximately 10%, with ~2.5 times increased likelihood of development at 6-month post-acute gastroenteritis exposure (OR = 2.54, 95% CI 1.76–3.65, $p < 0.05$).[112] PI-FD is currently understood to be a product of residual dysfunctional immune activation[113,114]; or from permanent gastrointestinal damage from transient inflammation[27] driven by either microbial displacement or antibiotic treatment.[32] Previously, persistent microscopic duodenitis[114] and higher proportions of eosinophils and mast cells activating in close range (<5 nM) of nerve fibers in the gut have been noted in PI-FD patients.[115] Postinfectious gastrointestinal symptoms have been reported up to 10 years after an intestinal infection,[116] and those exposed to gastroenteritis were more likely to develop new-onset dyspepsia[117] and IBS, with relative risks of 5.2 (95% CI 2.7–9.8, $p < 0.05$) and 7.8 times greater (95% CI 3.1–19.7, $p < 0.05$), respectively, at 1-year follow-up.[118]

Moreover, while *H. pylori* infection is now classified as a separate entity,[3,119] its role in the pathogenesis of FD is under scrutiny.[30,32,70] Higher oesophageal and gastric eosinophils[29,120–122] and altered gastric microbiotas including lower bacterial richness have been found in *H. pylori*-positive FD patients compared to those not infected.[123] Some *H. pylori*-positive patients have decreased Firmicutes, Bacteroidetes, Actinobacteria,[123,124] and Fusobacteria,[123] and an increase in Proteobacteria.[124] Moreover, the benefit of long-term symptom relief after *H. pylori* eradication therapy in FD has been postulated to come not from the resolution of the infection, but possibly from the antibiotics' effect on the upper gut microbiota.[125–127] Nonetheless, this supports the role of therapeutics targeting the microbiota in symptom alleviation for FD patients.

1.5.2 | Mucosal barrier disruption, inflammation, and dysbiosis

From infancy, an individual's microbiome matures in conjunction with their immune system.[128] A disruption in the triad of epithelial gut barrier integrity, the gut microbiome, and the immune system, such as intestinal dysbiosis, may lead to pathology and an inflammatory process. Impaired intestinal mucosal barrier integrity has been reported in FD and may relate to microbiome disruption, leading to a conceptualization of the disorder involving a "leaky gut."[129] A relationship between the extent of increased duodenal mucosal permeability and the severity of low-grade duodenal inflammation has been reported in FD patients; however, the mechanism by which the microbiome influences this process is still unknown.

Increased immune mediators and cell populations and abnormally decreased protein expression at epithelial intercellular have been seen in FD patients.[25,26,130] Immune mediator interleukin-1β disrupts the function of epithelial cells,[131] and in FD patients, increased expression was correlated inversely with duodenal epithelial integrity.[26] Inflammasomes have a crucial role in intestinal mucosa homeostasis as NOD-like receptor 6 (NLRP6) deficiency leads to defective intestinal goblet cells and a compromised mucosal barrier,

which abrogates effective clearance of enteric pathogens.[132] This leaves patients vulnerable to infection and intestinal dysbiosis[133,134] and consequential inflammation.[135] In FD patients, duodenal NLRP6 was significantly reduced and correlated with decreased duodenal corticotropin-releasing hormone (CRH) receptor-2 expression compared with controls.[136] This indicates that the upregulation of NLRP6 may lead to mucosal disruption and immune activation in FD.

Eosinophils and mast cells in the proximal small intestine are increased in FD and associated with eosinophil degranulation.[137] These cells may be stimulated after barrier disruption, leading to mucosal nerve activation and visceral hypersensitivity, or alternatively, eosinophil degranulation may induce barrier disruption.[138,139] The involvement of these cells in FD is supported by patients' responses to therapeutics such as montelukast,[140,141] histamine H1/H2 antagonists,[142] and budesonide.[143] Moreover, emerging evidence indicates that PPIs may have therapeutic value through anti-inflammatory actions[144,145] such as reducing duodenal eosinophils and improve mucosal barrier dysfunction.[146,147]

Other documented immune changes in FD include increased duodenal intraepithelial lymphocytes in a subset[148] and circulating TNF-α, IL-1β, and IL-10 cytokines in patients compared with controls.[149] Moreover, higher proportions of gut-homing (positive to CD4, α4 β7 integrins, CCR9-positive) T cells have also been observed and correlated with gastrointestinal symptoms in patients compared with controls, potentially due to localizing immune recruitment in the small intestine in FD.[149] Notably, increased duodenal populations of CD68- and CCR2-positive cells in PI-FD patients compared with non-PI-FD patients and controls have been reported, suggesting a potential cellular response is triggered by bacterial antigens in this subtype.[114] Moreover, upregulated antimicrobial pathways involving toll-like receptors[150,151] and β-defensin 2[152] have been observed in other DGBI patients and controls, but these have not yet been studied in FD.

Interestingly, animal studies have demonstrated a relationship between microbial communities including *Clostridium* and *Bacteroides* and increased regulatory T cell populations.[153–155] While there is emerging evidence for links between barrier integrity, specific microbial changes, and immune activation, associations between specific microbial communities and immune signatures in FD patients are areas for further study.

1.5.3 | Gastrointestinal microbial and immunological relationships with dietary factors in FD

Over 80% of patients with FD report meal-related gastrointestinal symptoms and alleviation with dietary modification.[14,156] Dietary factors are known to play a role in gut symptomatology and may represent a link between food antigens, immune cell activation, and microbiome changes. The potential mechanisms of dietary antigens in this condition have recently been reviewed.[157,158] The relationship between digested food, mucosal barrier dysregulation, and hypersensitivity responses is complex. Digested nutrients activate neurons in the small intestine, such as submucosal sensory neurons, and myenteric motor neurons, and the specific site of neuronal activation is nutrient-specific.[159] FD patients' increased sensory responses to glucose challenge,[159] lipids,[160] and capsaicin[161] are suggested to be linked to barrier dysfunction and gastric acid hypersensitivity, but the role of the intestinal microbiota in this process is unclear. Notably, patient symptoms after a standardized nutrient challenge were significantly correlated with an increased bacterial load dominated by *Streptococcus* and *Prevotella* and reduced *Actinomyces*.[88] Moreover, this increased bacterial load was negatively correlated with bacterial diversity.[88] This suggests an association between meal-related symptoms in FD and both microbial load and diversity changes. Interestingly, avoidance of highly processed, "inflammatory" foods minimized the risk of dyspepsia onset,[162] prevented colonic inflammation,[163,164] and is associated with beneficial fecal microbial changes.[165] Furthermore, fecal microbiota transplant in IBS patients exhibited gastrointestinal and psychological symptom relief benefits,[166] and associations with increased short-chain fatty acid butyrate, a fiber metabolite produced by gut microbes,[167,168] which has been linked to nociception.[169,170] However, these associations are still conjectural and have not yet been investigated in the small intestine of patients with FD.

Fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs) have been speculated to influence FD symptom onset. FODMAPs are carbohydrates that are poorly absorbed by the small intestine, and their incomplete digestion by the small intestinal microbiota may lead to increased fatty acid, gas production, and increased water transport in the colon leading to bloating, pain, and diarrhea in FD and IBS.[171] Patients with IBS have higher reported levels of known microbial fermentation by-products compared with controls,[172] and changes of the microbiota may at least partly explain the clinical effects of low-FODMAP diets in patients with DGBI symptoms.[173–176] Interestingly, high FODMAPs lead to increased gastrointestinal symptoms, higher levels of anger, and a decreased positive affect in IBS patients compared with controls,[177] indicating gastrointestinal dysfunction is more complex than just macromolecule metabolism and is intertwined with psychological processes. However, a randomized trial of a low-FODMAP diet in FD was negative in terms of symptom improvement versus a standard diet, although PDS symptoms improved more on a low-FODMAP diet on a post hoc analysis.[178]

FD symptoms may be directly or indirectly influenced by lipid intake interacting with the intestinal microbiota, bile acid signaling, or both. Fatty foods have been associated with FD symptoms, but the mechanism of this is unclear.[156] Dietary fat intake is an independent risk factor for increased gastrointestinal permeability, and most lipids are absorbed in the first 20 cm of the small intestine.[179,180] Lipids are a microbial substrate, and the most potent nutrient modulators of gastrointestinal motility and distention through hormones such as cholecystokinin (CCK), released from enteroendocrine cells in a vago-vagal loop with the brain to regulate satiety.[181] While yet to be studied in FD, a high-fat diet is associated with a pro-inflammatory immune cascade involving a transition to barrier-disrupting hydrophobic bile acids and changes to the intestinal microbiota.[182,183] An overall reduced bacterial

load and diversity in the gut has been seen[182] and increased Firmicutes and reduced Bacteroidetes[182] (with a predominance of *Bacteriodes* and lower *Prevotella*[183]). This abnormal, higher Firmicutes/Bacteroidetes ratio is associated with disturbances in the intestinal mucosa[184] and may be a hallmark of metabolic pathology[185]; however, the latter remains controversial. Interestingly, these bacterial changes are consistent with those that have been observed independently of dietary fat in FD patients (Table 1).

The relationship between microbial translocation, intestinal mucosal permeability, and bile salts has been investigated in FD. The microbiota alter the bile acid pool composition through biochemical transformation, such as partial dihydroxylation.[186] Bile salt composition, such as lower concentrations of hydroxylated bile salts, has been associated with increased duodenal permeability[31] and slowed gastric emptying in FD.[187] While remediation by administration of an anti-inflammatory, hydrophilic bile salt was observed,[31] the influence of bacterial translocation across the lumen on bile salt composition is unclear in FD.[31] Further analysis of the gut microbiota in FD patients and how it modulates nutrient and bile metabolism, gastrointestinal symptom onset, and intestinal function is needed to understand the pathogenesis of this condition.

1.5.4 | Gut–brain and brain–gut axes in FD

The immune activation and dysbiosis in the pathogenesis of FD may be explained by a relationship with psychological symptoms through the bidirectional brain–gut and gut–brain axes. The brain can influence the microbiota indirectly via gut motility, secretions, and intestinal permeability, or directly through the release of signaling molecules into the lumen from lamina propria cells. Microbes can conversely communicate with the nervous system by direct stimulation of cells in the lamina propria by microbial metabolites, signaling molecules, and hormones to stimulate vagal pathways and contribute to reward processing, pain, sleep, mood, and cognition.[188]

FD was originally conceptualized as psychosomatic,[189] and patients often have extraintestinal symptoms and comorbidities.[190–194] FD patients have a higher prevalence of psychiatric comorbidities such as depression and anxiety compared with non-dyspeptic controls,[195,196] and patients may be able to be classified based on their gastrointestinal symptoms and concomitant psychological burden.[197] Baseline anxiety is an independent predictor of persistent GI symptoms and DGBI onset,[198,199] but the order of onset of psychiatric conditions and gastrointestinal symptoms may vary between patients. One third of patients have psychological conditions that precede their DGBI diagnosis,[200] while many patients experience psychological disturbances after DGBI onset.[16,200–203] Moreover, those with an FD or IBS diagnosis but no psychological comorbidities at baseline had higher levels of psychological distress at 12-month follow-up.[199] Notably, several brain regions in patients with FD demonstrate both white and gray matter anomalies, including the frontal and somatosensory cortices, hippocampus, amygdala, internal capsule, and corpus callosum, suggesting both abnormal central processing (brain to gut) and overactive visceral signaling and pain modulation (gut to brain).[204–209]

Chronic stress is a risk factor for FD and IBS, posited to be mediated by the gut–brain axis and hypothalamic–pituitary–adrenal (HPA) axis.[210] Stress may increase intestinal permeability and potentiate the uptake of noxious agents,[211] dysregulate motility,[212] and lead to visceral hypersensitivity[213] and an activated inflammatory state.[214] Anxiety and depression may contribute to the systemic immune activation seen in FD,[215] as CRH released by the hypothalamus during stress is also produced by peripheral inflammatory cells and increases gastrointestinal permeability.[216] Moreover, it is suggested that eosinophils and possibly mast cells may alter neural structure and function, and this sensitization of the enteric nervous system may be exacerbated by pre-existing psychological problems.[217] Interestingly, anxiety in IBS patients is linked to increased mast cells in the rectum,[218] while gastric mast cell density has been shown to relate to somatization, depression, and anxiety in pediatric FD cases.[219]

The intestinal microbiota have a defining impact on the nervous, neuroendocrine, and metabolic systems as outlined in several comprehensive reviews.[188,220–222] Microbial factors have now been identified in a number of psychiatric diseases,[40,223,224] and it is known that gut microbes produce their own neuroactive molecules, potentially having a modifiable impact on brain signaling.[55] This is demonstrated by healthy female adults exhibiting reduced reactive midbrain neural activity in response to a negative emotional attention task after ingestion of a fermented probiotic product containing *Bifidobacterium lactis DN-173 010* for four weeks.[225] This same probiotic product led to a reduction in gastrointestinal symptoms in undiagnosed subjects[226] and improved symptom severity, abdominal transit, and distension in IBS patients.[227] Moreover, *Bifidobacterium longum* supplementation reduced limbic reactivity and depressive scores in IBS patients.[228] These results may be a product of vagal nerve signaling, as murine and human studies have demonstrated vagal nerve-mediated anxiolytic effects of *Lactobacillus*[229,230] and *Bifidobacterium*[230,231] and increased neural plasticity gene expression, linked to stress circuitry.[232] Furthermore, other bacterial strains have been linked to changes to serotonin metabolism in the brain stem,[233] inhibited pain sensation from visceral distension[234,235] and expression of endogenous opioid and cannabinoid receptors by gut epithelium.[236] These findings exemplify that the intestinal microbiota have a complex relationship with neuroendocrine pathways in the brain–gut and gut–brain axes and likely contribute to both psychological symptoms and gastrointestinal discomfort in FD.

2 | LIMITATIONS OF MICROBIAL ANALYSIS IN DGBIs

Analyzing the role of the intestinal microbiome in FD is complicated by difficulties in studying the gut microbes themselves. This is partly due to wide human microbiota heterogeneity between

individuals,[237,238] and the hostile and dynamic environment of the proximal small intestine wherein microbes have fastidious growth requirements.[36,239–241] Culture-independent methods of analysis remain the mainstay of gastrointestinal microbiological research such as metagenomics and 16S rRNA sequencing.[45,242,243] However, few metagenomic studies exist due to the emerging technology and high cost,[45,243] while the distinct variable rRNA gene regions targeted in different 16S sequencing protocols may influence microbial diversity results and exclude viral and fungal analysis.[244,245] Furthermore, the small bowel is difficult to study due to limitations with current sampling and storage methods.[245] The assessment of MAM in the duodenum requires the analysis of endoscopic biopsies as they adequately capture bacteria deep in the mucosa more effectively than other techniques.[44,246] However, current biopsy techniques pose a risk of cross-contamination of biopsy samples,[247] so the more widespread use of novel techniques such as the Brisbane Aseptic Biopsy Device (BABD) that minimizes cross-contamination from luminal contents, is key for representative analysis of the MAM.[53] Moreover, the heterogenous aggregation of microbial communities along the gastrointestinal tract means may require considered mucosal biopsy technique to obtain samples representative of FD's disease process and accurately capture any subtle microbial changes.[248–250] Multifarious factors influencing mucosal microbial diversity contribute to extensive variation between individuals, revealing a need for further study to better understand the normal microbiome across a variety of populations.[36,251]

3 | CONCLUSION

There is growing evidence that the microbial colonization of the small intestine plays a role in the pathophysiology of disorders of gut–brain interaction. FD has been shown to have component of gastrointestinal dysbiosis and altered mucosal barrier dysfunction. While low-grade inflammation might be the consequence of microbial dysbiosis, the inflammation may play a role in sensory dysfunction. This disease process resulting in the manifestation of dyspeptic symptoms is likely modified by environmental factors such as dietary factors or medications. The recent observations require further research to appropriately delineate cause and consequence and explore interventions that allow individualized treatments targeting the causes for symptoms. While the understanding of FD continues to improve, further analysis of the small intestinal microbiome in conjunction with immune cell activation levels and function is required to clarify the pathophysiological mechanisms of this debilitating condition to guide further diagnostic and curative therapeutic innovations.

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AUTHOR CONTRIBUTIONS

GB formulated the idea, concept and design, contributed to data extraction, data interpretation, and drafting of the manuscript. ECH reviewed the final manuscript. SK contributed to concept and design, drafting of the manuscript, and review of final manuscript. AS reviewed the final manuscript. MMW contributed to concept and design, drafting of the manuscript, and review of final manuscript. GH reviewed the final manuscript. NJT contributed to concept and design, drafting of the manuscript, and review of final manuscript.

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