Chapter 3
Emerging Role of Gut Microbiota in Functional Gastrointestinal Disorders

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1 Introduction

The brain–gut interrelation plays a central role in linking psychological factors and gut dysfunction that clinically present with gastrointestinal (GI) symptoms and disease. The major clinical domains that involve brain–gut axis function include organic (structural pathology at macro-and micro-level), motility (measurable organ dysfunction), and functional GI disorders (FIGD). The latter is specifically defined in the presence of "illness experiences," symptoms rather than signs, strongly linked to psychosocial impact and diagnosed by specific subjective (Rome) criteria. The biopsychosocial concept of disorders of the GI system linked genetics, culture, and environmental factors to stress, personality traits, psychology, coping, cognition, and social functions further to central (CNS) and enteric (ENS) nervous system influences that formed the pathophysiological basis of FGID. In this regard, current research has demonstrated that the motility, sensation, immune function, and mucosal physiology of the gut influenced by food and dietary habits have been linked to alterations of the intestinal microbiota and its functional metabolism. This liaison between the gut microbiota, the local (enteric), and central nervous systems have been shown to influence symptoms, severity, and behavior among patients with

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FGID. Thus, FGID is a syndrome of "clustered" GI symptoms, related to GI functioning, associated with perturbed gut–brain interaction and gut microbiota, associated with visceral hypersensitivity, motility disturbance, and altered mucosal and immune function in the presence of disturbed CNS processing, diagnosed by the Rome Criteria. The Rome IV Criteria classified FGIDs into 33 adult and 20 pediatric variants, primarily based on symptoms into anatomic regions (esophageal, gastroduodenal, bowel, biliary, and anorectal and centrally mediated disorders of GI pain) for easy utilization in clinical practice (Drossman 2016; Schmulson and Drossman 2017).

The role of the luminal microenvironment, especially the microbiota and associated functional metabolism and its relationship with the enteric neuromuscular apparatus and its central connections through the gut–brain axis, was initially noticed in patients who developed and sustained FGID-type symptoms after enteric infections. Gut–brain axis communications are dependent on several complex signaling pathways that involve the sympathetic (splanchnic) and parasympathetic (vagal) nerves, the ENS, hypothalamus–pituitary axis, and CNS that are in turn affected by intestinal microbiota (microbiota–gut–brain axis) and psychosocial factors—a bidirectional interaction. Our knowledge on gut microbiota-associated changes and strong links to FGIDs stems from translational research studies encompassing direct and indirect intestinal microbiota modulation predominantly in patients with functional dyspepsia (FD) and irritable bowel syndrome (IBS) and its subtypes. The interaction between psychosocial and dietary factors (food/food components) trigger morphological changes to the gut epithelium and alters the mucosal endocrine signaling, leading to perturbation in local and systemic immune and inflammatory responses that culminate in FGID—whether the role of intestinal microbiota is a cause or an effect to this ultimate event is a matter of further research (Fig. 3.1). Nonetheless, specific gut microbiota changes have been shown to directly and indirectly (through functional metabolism) promote symptoms, affect the severity, and engage treatment responses in patients with FGIDs (Barbara et al. 2016).

2 Gut Microbiota Associations in FGID

The microbiota–gut–brain axis activity in FGID was demonstrated initially in small animal experiments. When male rat pups were stressed by separating them from their mothers in the immediate postnatal period, increases in plasma cortisol and alterations in fecal microbiota were noticeable compared with an unseparated control group. Similarly, when germ-free mice underwent fecal microbiota transplantation (FMT) with stool derived from severe depressive patients, they demonstrated anxiety-depression behavior compared to a control group undergoing FMT from "normal" human controls. These studies represent the bidirectionality of the role of gut microbiota in FGIDs (Luo et al. 2018). The introduction of pathogenic bacteria or short or repeated antibiotic feeding courses in healthy mice was associated with

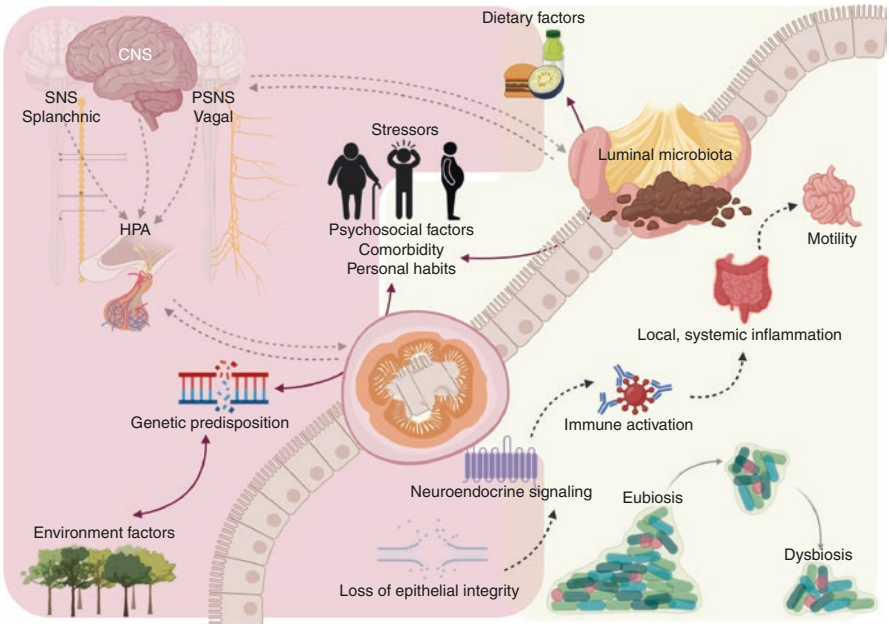

Fig. 3.1 The bidirectional, multifactorial pathophysiology of functional gastrointestinal disorders development. *CNS*, central nervous system; *SNS*, sympathetic nervous system; *PSNS*, parasympathetic nervous system; *HPA*, hypothalamus–pituitary axis

mood changes, anxiety-like behavior, and cognitive decline in the short and long term that occurred in tandem with a modulation of the intestinal microbiota. Germ-free mice demonstrated developmental changes and perturbed gut mucosal immunity, which was partially reversible through recolonization using stool transfer from healthy mice. Correspondingly, mice receiving feces from diarrhea-predominant irritable bowel syndrome (IBS) patients exhibited faster gastrointestinal transit, gut barrier dysfunction, innate immune activation, and anxiety-like responses, demonstrating a strong association between intestinal dysbiosis and intestinal and behavioral manifestations in FGID (De Palma et al. 2017; Ceylani et al. 2018; Kwon et al. 2020). Much of our understanding of microbiota (bacterial taxa) in FGID emanates from quantitative polymerase chain reaction (qPCR), PCR-denaturing gradient gel electrophoresis (DGGE), fluorescent in situ hybridization, pyrosequencing, and the recent, next-generation sequencing (NGS) studies conducted in patients with functional dyspepsia (FD) and IBS. Multiple studies have looked at gut microbial diversities within the adult and pediatric groups, between genders, and at various sites of the GI tract resulting in heterogeneous findings across populations and regions.

Furthermore, striking differences between the luminal microbiota and the mucosa-associated microbiota (MAM), the latter, considered more stable and reflective of host–disease interaction, also portend differences across studies in

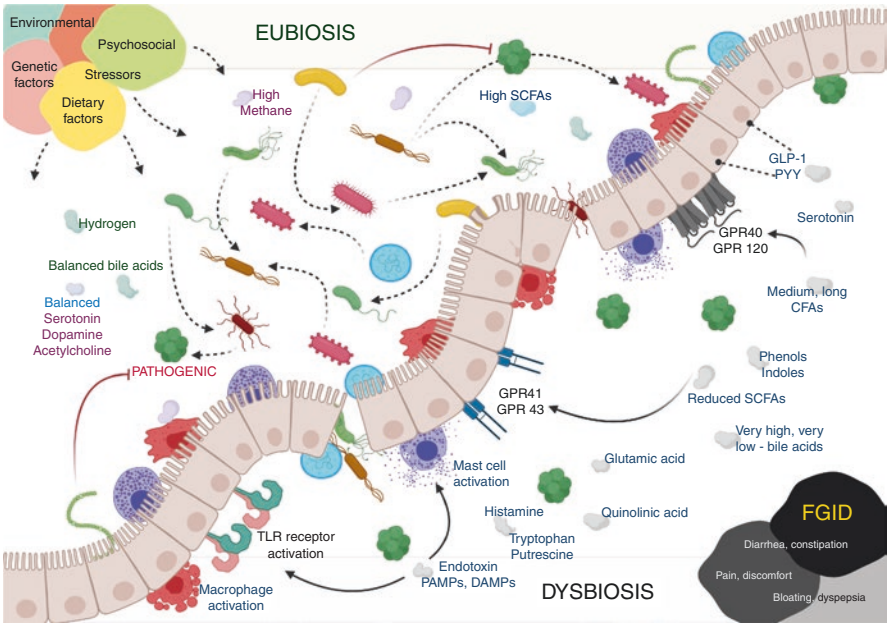

Fig. 3.2 Schematic representation of gut dysbiosis and microbial and related functional changes in patients with functional gastrointestinal disorders (FGIDs). The unbroken red lines with perpendicular dashed ends represent inhibition. *SCFA*, short-chain fatty acid; *TLR*, toll-like receptor; *PAMP*, pathogen-associated molecular pattern; *DAMP*, damage-associated molecular pattern; *GPR*, G-protein coupled receptor; *GLP*, glucagon-like peptide; *PYY*, peptide YY

FGID. Nonetheless, taken together, from a bird's eye view, all these studies provide insights into common associations between specific bacterial taxa and the type of FGID considered (Mottawea et al. 2019) (Fig. 3.2).

2.1 Gut Microbiota in Functional Dyspepsia

In patients with FD [post-prandial distress syndrome (PDS), epigastric pain syndrome (EPS), or PDS–EPS overlap], higher levels of *Prevotella* were notable in gastric fluid aspirate, and those with PDS, an inverse correlation between *Prevotella* abundance and disease severity was noted (Nakae et al. 2016). Similarly, in the gastric fluid, at the phylum level, higher *Bacteroides* compared to *Proteobacteria* and absence of *Acidobacteria* were remarkable in FD patients compared to healthy controls (Igarashi et al. 2017). In studies that looked at MAM (gastric and small intestinal mucosa biopsies), an inverse relationship between *Streptococcus* and *Prevotella* (Zhong et al. 2017), negative correlation between the abundance of *Veillonella* and gastric emptying time (Shanahan et al. 2018), and higher levels of *Firmicutes*, especially *Streptococcus*, positively correlated with symptoms (Fukui

et al. 2020) in patients with FD. Studies have also demonstrated important interactions between *Helicobacter pylori* and gut microbiota in patients with nonulcer and ulcer dyspepsia. *H. pylori*-negative biopsy-proven gastritis was found to be associated with greater enrichment of *Firmicutes*, *Fusobacteria*, *Bacteroidetes*, and *Actinobacteria*. At the same time, in those patients positive for *H. pylori*, the fecal samples were enriched with *Proteobacteria*. Interestingly, it was seen that bacterial species and richness diversity were higher among persons living in less industrialized nonmodern regions in whom *H. pylori* incidence was also very low. Patients with nonulcerative dyspepsia had a greater abundance of *Cutibacterium acnes* at the species level (Gantuya et al. 2019; Chua et al. 2019).

2.2 Gut Microbiota in Irritable Bowel Syndrome

In patients with IBS (diarrhea or constipation-predominant or mixed type and unclassified), deep molecular analysis of microbiota has revealed specific bacterial taxa changes associated with symptoms and severity compared to healthy controls. The first such study to utilize state-of-the-art techniques was performed in 2007 in which authors identified changes in *Coprococcus*, *Collinsella*, and *Coprobacillus* abundances in patients with IBS (Kassinen et al. 2007). In general, a "healthy" microbiota is characterized by a higher prevalence of *Firmicutes* and *Bacteroidetes* and a lack of *Proteobacteria*. The most crucial aspect of gut microbiota related to IBS stems from observations and studies in small animal models and patients with post-infection (following acute gastroenteritis) IBS. It was noted that approximately 10% to 14% of patients within 3 to 12 months after an acute GI infection developed IBS symptoms driven by bacterial and host factors, local and systemic immune activation, and enteric neuronal changes that ultimately led to changes in intestinal motility and development of symptoms. In small animals infected with *Campylobacter jejuni*, it was shown that post-infection, alteration in stool form, increase in rectal lymphocytes, reduction in interstitial cells of Cajal, and bacterial growth predominated along with the production of cytolethal distending toxin resulting in subsequent autoimmunity to enterocyte adhesion protein vinculin impressing the fact that IBS development and progression had strong links to bacteria-driven local as well as systemic immune-related and neuroendocrine changes (Pimentel et al. 2015).

The parasite *Giardia duodenalis* has been shown to reduce thickness and disrupt extracellular matrix compositions and structural integrity of the mucosal microbiota biofilms leading to over-representation of *Clostridiales* and a decreased amount of *Phascolarctobacterium* species in experimental models of post-infection IBS, which was also clearly demonstrated among IBS patients from Italy and Norway (Beatty et al. 2017). From a dysbiosis point of view, at the phylum level, a twofold increase in *Firmicutes* to *Bacteroidetes* ratio, increase in *Actinobacteria*, and reduction in *Bifidobacterium* correlated with symptoms and severity in patients with IBS (Jeffery et al. 2012; Pimentel and Lembo 2020). A large body of evidence from gut

microbiota studies in IBS emphasizes the relative richness of pro-inflammatory bacterial species (Enterobacteriaceae) associated with a parallel decline in beneficial species *Bifidobacterium* and *Lactobacillus*. The differential dysbiosis in IBS has been demonstrated between patients with and without abdominal bloating, further classified into different subtypes based on bowel habits and between patients from various regions. *Subdoligranulum* and *Anaerovorax* (belonging to the families Ruminococcaceae and Eubacteriaceae, respectively) were found to increase in those without bloating. In patients with constipation-predominant IBS, *Collinsella* was increased, while among those with predominantly diarrhea, members of the *Firmicutes* phyla *(Oscillibacter*, *Anaerovorax*, *Streptococcus*, and *Eubacteriaceae)* were significantly decreased (Ringel-Kulka et al. 2016; Zhuang et al. 2017; Ringel et al. 2018). Similarly, in a study on fecal and mucosal microbiota, researchers found that IBS symptom severity was associated negatively with microbial diversity or richness, exhaled methane levels, presence of methanogens, and reduced prevalence of Methanobacteriales or *Prevotella* species. Only two previous studies have shown the predominant role of *Pseudomonas aeruginosa* among patients with IBS (Kerckhoffs et al. 2011; Shukla et al. 2015; Ghoshal et al. 2018a, b).

Contrary to study findings on bacterial communities, in a study published from Korea, authors found that halophilic archaea such as *Halorubrum* and *Halococcus* species predominated. This was possibly due to high-salt food intake notable among Korean populations, implying the role of dietary factors on qualitative and quantitative gut microbial aspects, which is not yet fully weighed into studies on IBS and its subtypes (Nam et al. 2008). Metagenomic analysis on fecal samples from patients with constipation-predominant IBS revealed predominant microbiota directed anti-inflammatory activity when transferred to conventional mice due to increased *Akkermansia muciniphila* even with a decrease in the relative abundance of *Bacteroides*, *Roseburia*, and *Eubacterium rectale* and an increase in pathogens belonging to *Enterobacteriaceae* and *Desulfovibrio* species, demonstrating the importance of cross-talk between microbial taxa and host intestinal homeostasis (Gobert et al. 2016). A recent study analyzed the fecal and mucosa-associated bacterial composition along the GI tract in patients with IBS. The authors found that feces' bacterial profiles and the sigmoid colon mucosa, but not duodenum, differed between IBS patients. The IBS-specific bacterial profiles were linked to the colonic antibacterial gene expression. Furthermore, the fecal bacterial profile differed between IBS subtypes, while the mucosa-associated bacterial profile was significantly associated with IBS symptom severity (Sundin et al. 2020). Large-scale studies on specific microbial changes and interactions in patients with IBS subsets seem incomplete in current literature. Nonetheless, a recent study showed that diversity richness was reduced, and levels of *Faecalibacterium* and *Dorea* were lower and higher, respectively, in patients with diarrhea-predominant IBS (Maharshak et al. 2018). Another study demonstrated an increased abundance of *Prevotella* and association with a high risk of diarrhea-predominant IBS in the Chinese population (Su et al. 2018). In a systematic review and meta-analysis that included differential expression of intestinal microbiota in patients with IBS versus healthy controls and subgroup analysis, authors found lower levels of *Lactobacillus*, *Bifidobacterium*,

and *Faecalibacterium prausnitzii* in patients with diarrhea-predominant IBS (Liu et al. 2017). A recent systematic review involving 777 patients and 461 healthy controls demonstrated that, for most studies, those with IBS had lower α-diversity in both fecal and mucosal samples. Relatively consistent findings on intestinal microbiota analyses included increased *Firmicutes*, decreased *Bacteroidetes*, and increased *Firmicutes*:*Bacteroidetes* ratio at the phylum level and increased *Clostridia* as well as decreased *Bacteroides* (Duan et al. 2019). A more recent meta-analysis showed that the family Enterobacteriaceae (phylum Proteobacteria), the family Lactobacillaceae, and the genus *Bacteroides* were increased. In contrast, uncultured Clostridiales I, *Faecalibacterium*, and *Bifidobacterium* were decreased in patients with IBS (Pittayanon et al. 2019). A study comparing fecal and mucosal gut microbial signatures among patients with inflammatory bowel disease (IBD), IBS, and healthy controls showed that *Erysipelotrichi* was a potential biomarker of IBS. In contrast, *Enterococcus* was significantly identified in patients with IBD (Lo Presti et al. 2019). Several authors have described changes associated with intestinal bacterial communities at the luminal and mucosal level in patients with IBS since the original description more than a decade ago. A summary of gut microbial (bacterial) interactions in patients with the FGIDs, FD, and IBS and its subsets are illustrated in Fig. 3.3.

3 Gut Microbiota and Functional Metabolites in FGID

Metabolomics, the exhaustive study and profile generation of small-molecule metabolic products of cells, tissues, and organisms at a specific point in time, is a novel approach to analyzing complex interactions between gut microbiota functions. Mass spectrometry and magnetic resonance spectroscopy are powerful tools to identify, quantify, and apply biostatics and mathematical models and discern biologically significant metabolites from large data sets (Liu and Locasale 2017). Apart from this, analysis and identification of functional metabolism based on metagenomic data and biomarker discovery, in the absence of quantification, can be achieved through bioinformatic pipelines such as Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt), which allows inference of the functional profile (significant pathways of metabolism) of a microbial community using operational taxonomic units-based marker gene sequence and survey (Douglas et al. 2020). Fermentation of polysaccharides and generation of short-chain fatty acids (SCFAs; acetate, propionate, butyrate) by intestinal bacteria lead to hydrogen, methane, and other by-products that affect gut mucosal barrier, gut permeability, and bowel motility. Intestinal bacteria also play a central role in gut–brain interactions through the production or degradation of various locally acting neurenteric and systemic neuroactive substances such as the anti-inflammatory S-adenosylmethionine, neurotoxin quinolinic acid, glutamic acid, hydroxybutyric acid, dopamine, acetylcholine, kynurenine, histamine, and serotonin (Valles-Colomer et al. 2019). The bacteria also harbor hormonal receptors that mediate

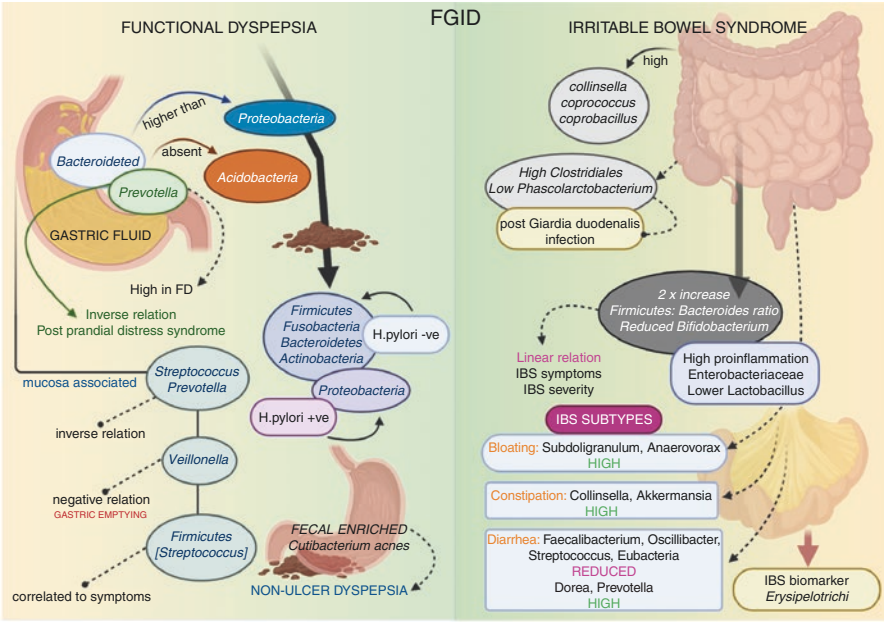

Fig. 3.3 Summary of bacterial interactions and associated relationships with symptoms in patients with functional dyspepsia and irritable bowel syndrome (IBS). FGID, functional gastrointestinal disorder; *H. pylori*, *Helicobacter pylori*

cross-talk between the host and microbiota. SCFAs are an important energy source for colonocytes but are also chemical messengers or signaling molecules for various host cells. The G-protein coupled receptors (GPR41, GPR43), also known as the free fatty acid receptor types 3 and 2 (FFAR), have been identified as receptors for SCFAs and expressed in a variety of cells, including colonic endocrine L cells, adipose tissue, neutrophils, monocytes, and mucosal mast cells. SCFAs upregulate the secretion of glucagon-like peptide 1 (GLP-1) and peptide tyrosine-tyrosine (PYY) that take part in "ileal-brake," a primary inhibitory feedback mechanism to control the transit of a meal through the GI tract to optimize nutrient digestion and absorption. Similarly, medium—and long-chain fatty acids produced by bacterial activity on dietary substrates within the host act on GPR40 and GPR120 receptors on the enterocytes and promote cholecystokinin's secretion and regulatory activity and glucose-dependent insulinotropic polypeptide/gastric inhibitory polypeptide. Similarly, reducing certain gut microbiota metabolites and subsequent increase in certain others (due to host, environment, or associated comorbidity) lead to mucosal barrier dysfunction leading to leakage of pathogens into the lamina propria of intestinal mucosa triggering the mucosal immune system. This results in pro-inflammatory cytokine production and stimulation through direct bacterial or indirect bacterial products-related activity on the toll-like receptors on the enterocytes, mast cell, and macrophage activation. This local and subsequent systemic immune activation and pro-inflammatory profile have been considered to play a central role in

neurovisceral sensitivity and associated symptoms in patients with FGID, especially IBS (Fukui et al. 2018). Thus, it is evident that secondary bacterial metabolites play a central role in intestinal function and systemic neuroendocrine and immune regulation in humans. In patients with FGID, especially those with IBS, it was found that perturbed metabolite profiles in fecal samples, such as an increase in gaseous hydrogen, phenols, and indoles, were associated with symptoms and disease severity. Colonic spore-forming bacteria belonging to the *Clostridiales* order, enriched in *Ruminococcaceae* and *Lachnospiraceae*, were associated with biosynthesis and release serotonin from intestinal enterochromaffin cells and modulate intestinal motility, a serotonergic dysfunction notable in patients with IBS (Yano et al. 2015; Labus et al. 2019). Metabolism of polysaccharides in the gut lumen leads to the production of hydrogen and methane by-products. Colonic bacteria also produce short-chain fatty acids such as acetate, propionate, and butyrate that affect intestinal permeability and motility. Methane gas production, specifically by methanogens in the colon, slows intestinal transit and augments small intestinal contractility. It was shown that reduction in butyrate production and butyrate-producing taxa was found among patients with diarrhea and mixed (diarrhea, constipation)-type IBS. Similarly, lower methane production in the intestinal lumen was notable in patients with diarrhea-predominant IBS, while higher levels were noted in patients with constipation-predominant IBS. The symptoms of flatulence in FGID patients have been linked to reduced hydrogen gas removal from the colon due to decreased sulfate-reducing bacterial taxa (Pozuelo et al. 2015; Tap et al. 2016; Chong et al. 2019). The role of metabolite production and its effects on gut motility, mucosal immunity, local immune regulation, and symptom development in IBS and FD patients have been identified through prebiotic interventional studies and subsequent observations. It was shown that inulin-type fructans and arabinoxylan oligosaccharides fermentation capacity by Bifidobacteria strains depended on bacterial cooperation, and the metabolites rich in short-chain fatty acids thus produced acted on metabolite-sensing G-protein-coupled receptors to regulate inflammatory responses and motility. An essential metabolite of the human colon, butyrate, the central energy source for the colon epithelial cells, maintains gut mucosal integrity and promotes immunomodulatory and anti-inflammatory properties within the intestinal milieu. *Bifidobacteria* and other butyrate-producing bacteria, such as *Faecalibacterium prausnitzii*, *Anaerostipes*, *Eubacterium*, and *Roseburia,* interact with each other through cross-feeding pathways that generate metabolites beneficial for the host (Rivière et al. 2016, 2018; Chong et al. 2019). Bile acid metabolites are produced from cholesterol in hepatocytes with cholic and chenodeoxycholic acids. Intestinal bacteria deconjugate bile acids to form secondary bile acids such as lithocholic acid and deoxycholic acid. These stimulate enterocyte secretion through their actions on sodium and chloride channels. Excessive bile acid secretion is negatively controlled by fibroblast growth factor 19 (FGF-19). This inhibitory molecule and its regulation are affected by gut microbiota functions and metabolite production. The levels of bile acids within the intestinal lumen decide motility functions—high levels lead to diarrhea-like symptoms. In contrast, very low levels result in constipation in the host. Thus, gut microbial metabolite generation affects the host, which

depends on the quality and diversity of intestinal microbiota (Raskov et al. 2016). In a China study, authors identified gut microbiota and metabolite signature in patients with IBS using gas chromatography coupled to time-of-flight mass spectrometry (GC-TOFMS) and 16S rDNA amplicon sequencing. They found that metabolites ornithine, putrescine, N-acetyl tryptophan, and L-tryptophan were associated with abdominal pain and discomfort and stool characteristics. In contrast, eicosatrienoic acid, oxoadipic acid, L-phenylalanine, L-valine, and gamma-aminobutyric acid were associated with the duration of symptoms in patients with IBS (Zhu et al. 2019). Thus, it is clear that the quality and type of bacterial taxa, their interactions, and beneficial cooperation lead to favorable metabolite generation that acts at the local and systemic levels to promote or improve intestinal function. These findings led to the use of healthy donor FMT or prebiotic use, which promotes advantageous metabolite generation for the treatment of FGID, especially in patients with IBS.

4 Fecal Microbiota Transplantation in FGID

Fecal microbiota transplantation or FMT is the infusion of screened fresh or stored (frozen or encapsulated) feces from a healthy donor into the GI tract of a patient with a specific disease addressable to intestinal dysbiosis, intending to restore microbial homeostasis and advantageous functionality toward the host. The FMT procedure gained interest with its extremely beneficial therapeutic role in patients with a mild and severe recurrent form of *Clostridium difficle* infection, a condition well known to be associated with intestinal dysbiosis (Cammarota et al. 2017; Cheng et al. 2020). The use of FMT includes stepwise, scrutinized, protocol-based donor screening followed by different methods for feces infusion that is dependent on the treating unit's expertise. In brief, donor screening must include a thorough clinical history, including history of chronic as well as recent drug and medications such as antibiotics and proton pump inhibitors; GI symptoms, history of travel within 3 months; neuropsychiatric disorders, the latter, in the donor as well as first degree relatives; and physical examination and blood investigations to rule out acute as well as chronic infections, metabolic disorders, and possible transmissible diseases. A minimum of 30 g of freshly donated or frozen stool material (stored at -80 °C with added glycerol to a final concentration of 10%) homogenized with normal saline (three to five times larger volume of solvent) through blending and gauze filtering or manual/device straining can be infused into the recipient through a colonoscope into the lower GI tract or through a fluoroscopy-guided, nasally placed tube or gastroduodenoscopy-directed introduction into the upper GI tract (Wang et al. 2019; Kim and Gluck 2019; Cammarota et al. 2019). Researchers from China extracted and analyzed microbiota in feces from constipated donors who had undergone effective therapy with FMT and transplanted the extracted microbiota into pseudo-germ-free mice while measuring parameters of intestinal motility. They found that the treated mice developed lower pellet frequency and stool water percentage, smaller pellet size, delayed gastrointestinal transit time, and weaker

spontaneous contractions of colonic smooth muscle. To identify the mechanism underlying delayed gut motility in detail, the authors evaluated microbial metabolites. They found that SCFAs and secondary bile acids were decreased in mice receiving microbiota from constipated donors. They also demonstrated that the compositional changes of gut microbiota in constipated patients (taxa and the species richness and alpha diversity) were greater than healthy volunteers (Ge et al. 2017). The effect of allogenic and autologous FMT on IBS symptoms, visceral sensitivity, and compositional changes in fecal and mucosa-adherent microbiota was studied by researchers from Finland and Sweden in a randomized controlled study. They showed that single FMT via colonoscopy might have beneficial effects in patients with IBS. Still, allogenic fecal material was not superior to autologous feces, suggesting that prior bowel cleansing may contribute to symptoms and gut microbiota changes in IBS. This study sheds light on discovering standardized practices that minimize inadvertent microbiota modulation in patients treated with FMT (Holster et al. 2019). A single-arm open-labeled study included patients with IBS who underwent colonoscopy-directed FMT with a change in Bristol stool form scale (to types 3 or 4) at 4 weeks post-treatment as the primary endpoint. The authors noted that among responders to FMT, stool bacterial diversity increased with improved psychological status (measured using the Hamilton Rating Scale), especially in donor feces enriched in *Bifidobacterium* (Mizuno et al. 2017). Improvement in depression and anxiety symptoms after FMT in a group of patients with IBS, functional diarrhea, or functional constipation associated with microbial alpha diversity improvement was demonstrated in an open-label observational study from Japan (Kurokawa et al. 2018). A randomized placebo-controlled, double-blind study from Denmark on FMT in patients with moderate to severe IBS demonstrated significant improvement in symptoms gauged by amelioration in the IBS-Severity Scoring System (IBS-SSS) in patients receiving placebo compared to those on fecal capsules. Even though FMT improved bacterial richness and diversity compared to placebo, clinical improvements per predefined primary endpoints were notably absent in the FMT group (Halkjær et al. 2018).

In another double-blind, randomized, placebo-controlled, parallel-group, single-center trial from Norway, 90 participants with moderate to severe IBS were randomly assigned to receive either freshly processed feces (50 to 80 g stool in 200 mL saline and 50 mL of 85% glycerol) or patients' own feces as placebo along with loperamide for retention benefit. On modified intention-to-treat analysis (55 in the active treatment group and 28 in the placebo group), 65% of participants receiving active treatment versus 43% receiving placebo showed a response in the form of graded symptom improvements at 3 months (Johnsen et al. 2018). The previous Danish and the current Norwegian studies were contrasting, probably because of the higher dosing and better route of FMT utilized in the latter, which beckons standardization of the FMT procedure in specific subsets of patients with FGID. This was also confirmed in a recent study in IBS patients wherein authors repeated the FMT procedure by infusing 60 g of freshly prepared faces into the duodenum through a gastroscope in patients not responding to the initial 30 g volume FMT. It was shown that repeated and higher dosed FMT improved responses and alleviated

symptoms in patients who did not initially respond to the treatment (El-Salhy et al. 2019).

A more recent study showed that in patients with diarrhea-predominant IBS, several intestinal microbiota taxa and SCFAs, which were significantly different in the patients at baseline compared to their donors, normalized by the third week following FMT in parallel with significant improvement in symptoms and quality of life that was maintained up to 28 weeks post-treatment (Mazzawi et al. 2019). In a meta-analysis of eight single arm (SATs) and five randomized controlled trials ($N = 105$ patients on FMT and 105 controls), the authors found that 59.5% of IBS patients had significant improvement in the former of symptoms. In contrast, there were no differences between FMT and control treatment in IBS symptom, severity, or quality of life in the latter. This meant that randomized controlled trial results were dependent on and affected by the placebo effect; dosing, route, and FMT source were confounding factors. The effectiveness of FMT was dependent on the IBS subtype (Myneedu et al. 2019).

In a meta-analysis, the authors examined the efficacy of FMT in 267 IBS patients. They found that, for all individuals, there was no improvement in IBS symptoms as compared to placebo and concluded that the dose and method of delivery might have influenced response, and that fresh or frozen donor stool delivered by colonoscopy or nasojejunal tube may be associated with the better response, which needs further validation through larger more rigorously conducted trials (Ianiro et al. 2019). In a more recent systematic review and meta-analysis on 742 citations with ultimately 254 eligible participants with IBS undergoing FMT, the authors noted no significant difference in the global improvement of IBS symptoms at 12 weeks in those receiving FMT to placebo. The heterogeneity among studies was significant, and subgroup analyses revealed benefits of single-dose FMT using colonoscopy and nasojejunal tubes in comparison with autologous FMT for placebo treatment (number needed to treat = 5, RR = 1.59) and a reduction in the likelihood of improvement of multiple-dose capsule FMT RCTs (number needed to harm = 3, RR = 0.54). The authors also found that the placebo response was 33.7% in non-oral FMT RCTs and 67.8% in capsule FMT RCTs. Thus, current evidence from RCTs does not suggest a benefit of FMT for global IBS symptoms (Xu et al. 2019) (Fig. 3.4).

5 Conclusions and Future Directions

Current evidence sheds light on the influential role of intestinal dysbiosis in patients with FGIDs. However, our knowledge regarding specific taxa and their functions in different subsets of FGID remains limited to patients with IBS and, to some extent, FD. Even so, there remain wide variations in observed changes in the species and genera of these patients dependent on the region (Asian versus European), methodology (16 s RNA sequencing versus shotgun sequencing), and sites (fecal versus mucosal; duodenum versus colon) studied. With improved standardization of study methods, our comprehension of the precise role of qualitative and quantitative gut

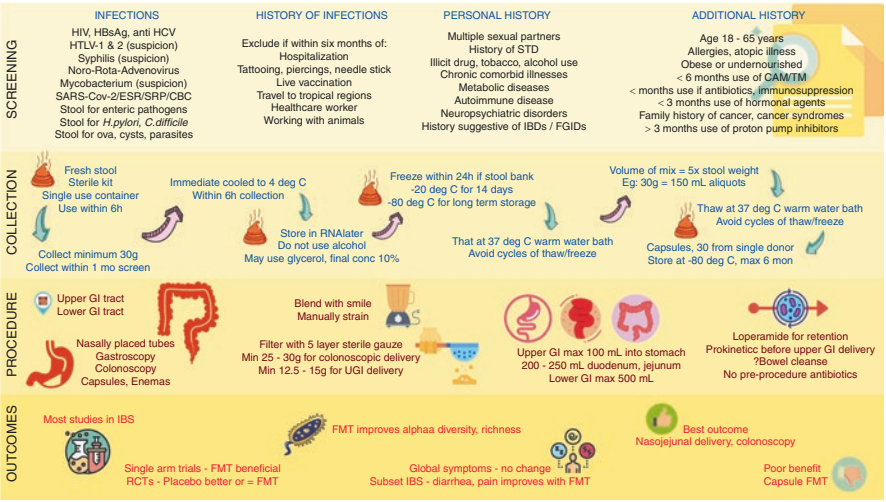

Fig. 3.4 Brief summary of FMT in IBS patients. *HIV*, human immunodeficiency virus; *HTLV*, human T-lymphotropic virus; *SARS-Cov-2*, novel coronavirus; *ESR*, erythrocyte sedimentation rate; *CRP*, C-reactive protein; *CBC*, omplete blood counts; *STD*, sexually transmitted disease; *IBD*, inflammatory bowel disease; *FGID*, functional gastrointestinal disorder; *CAM*, complementary and alternative medicine; *TM*, traditional medicine; *mo*, month(s); deg. *C*, degree centigrade; *GI*, gastrointestinal; *RCT*, randomized controlled trial

microbial functions in patients with FGID and subgroups has been steadily improving. In similar lines, FMT's use as a therapeutic option in patients with FGID, especially those with IBS, has not yielded favorable results due to differences in dosing, route, and duration of therapy utilized across studies. In the future, understanding and identifying specific groups of patients with FGID in whom intestinal dysbiosis plays a central role in the pathogenesis of the disease, independent of other factors, who would benefit from gut microbial modulation, through large population-based observational and randomized controlled interventions needs effectuation.

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