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TOPIC HIGHLIGHT

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Unraveling the ties between irritable bowel syndrome and intestinal microbiota

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Abstract

Irritable bowel syndrome (IBS) is the most prevalent functional gastrointestinal disorder. It is a multifactorial disorder. Intestinal microbiota may cause the pathogenesis of IBS by contributing to abnormal gastrointestinal motility, low-grade inflammation, visceral hypersensitivity, communication in the gut-brain axis, and so on. Previous attempts to identify the intestinal microbiota composition in IBS patients have yielded inconsistent and occasionally contradictory results. This inconsistency may be due to the differences in the molecular techniques employed, the sample collection and handling methods, use of single samples that are not linked to fluctuating symptoms, or other factors such as patients' diets and phenotypic characterizations. Despite these difficulties, previous studies found that the intestinal microbiota in some IBS patients was completely different from that in healthy controls, and there does appear to be a consistent theme of *Firmicutes* enrichment and reduced abundance of *Bacteroides*. Based on the differences in intestinal microbiota composition, many studies have addressed the roles of microbiota-targeted treatments, such as antibiotics and probiotics, in alleviating certain symptoms of IBS. This review summarizes the current knowledge of the associations between intestinal microbiota and IBS as well as the possible modes of action of intestinal microbiota in the pathogenesis of IBS. Improving the current level of understanding of host-microbiota interactions in IBS is important not only for determining the role of intestinal microbiota in IBS pathogenesis but also for therapeutic modulation of the microbiota.

Key words: Irritable bowel syndrome; Intestinal microbiota; Dysbiosis; Antibiotics; Probiotics

Core tip: The intestinal microbiota is altered in some Irritable bowel syndrome (IBS) patients, and the symptoms of IBS can be alleviated by treatments that target the microbiota. Over the past several years, many studies have attempted to identify the intestinal microbiota composition in IBS patients and intestinal dysbiosis in IBS is characterized by *Firmicutes* enrichment and reduced abundance of *Bacteroides*. Based on the differences in intestinal microbiota composition, the roles of microbiota-targeted treatments, such as antibiotics and probiotics, were investigated in alleviating certain symptoms of IBS.

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INTRODUCTION

Irritable bowel syndrome (IBS) is characterized by abdominal discomfort, bloating, and disturbed defecation in the absence of any identifiable abnormalities indicative of organic gastrointestinal disease[1]. IBS is the most commonly diagnosed gastrointestinal disorder, and it accounts for about 30% of all referrals to gastroenterologists[2]. In the general population worldwide, its prevalence has been reported to range from 5% to 25%[1,3-6]. IBS worsens patients' quality of life significantly, and both patients and healthcare systems incur huge costs toward its treatment[6]. Several treatments and therapies help alleviate the symptoms of IBS; however, they do not cure this condition. Thus, the chronic nature of IBS and the challenge of controlling its symptoms can be frustrating for both patients and healthcare providers[1,2].

IBS is a multifactorial disorder, and its underlying pathophysiology is unclear[1]. Therapeutic strategies have traditionally focused on alterations in gastrointestinal motility and visceral hypersensitivity influenced heavily by stress[7]. However, some drugs that target gastrointestinal motility and visceral hypersensitivity, such as antidepressants, alosetron, and tegaserods, have only a narrow therapeutic window, limiting their clinical application, especially in mild cases of IBS[8]. Therefore, studying the pathophysiology of IBS is important, especially in light of the possibility of developing targeted therapies. More recent studies have focused on the role of altered intestinal microbiota[7,9,10].

Since prospective studies have demonstrated that 3%-36% of enteric infections lead to new, persistent IBS symptoms[10], the concept that gut microbes play an important role in the pathogenesis of IBS was confirmed. Recent studies have demonstrated an unimagined level of complexity in human intestinal microbiota, with thousands of phylotypes, 80% of which remain uncultured[11]. The introduction of culture-independent techniques for studying intestinal microbiota has increased our understanding of the role of intestinal microbiota in human diseases, and emerging studies have demonstrated changes in intestinal microbiota in patients with IBS[12-14]. The restoration of altered intestinal microbiota may be a new therapeutic option for treating IBS[15]. Previous randomized controlled trials (RCTs) have documented that the symptoms of IBS can be improved by treatments that target the microbiota, such as antibiotics and probiotics[7]. Herein, the evidence of associations between the intestinal microbiota composition and IBS is reviewed, and the possible roles of specific microbial groups in IBS management are discussed in light of the most recent findings.

HUMAN INTESTINAL MICROBIOTA

The human body is inhabited by a complex community of microbes that are collectively referred to as human microbiota. The human intestinal microbiota constitutes a complex and metabolically active ecosystem that is now well recognized for its impact on human health and disease[16]. It is estimated that the human microbiota number more than 10^{14} cells, which exceeds the number of human cells in our bodies[7]. The microbiota is taxonomically classified according to the traditional biological nomenclature (phylum-class-order-family-genus-species), and currently, more than 50 bacterial phyla have been described, of which 10 inhabit the colon and three bacterial phyla, *Firmicutes*, *Bacteroidetes* and *Actinobacteria* predominate[17]. Genotypic sequencing studies based on the 16S ribosomal RNA (16S rRNA)-encoding gene have been used for demonstrating that the human gastrointestinal tract can be populated by any of 1000-1150 different species[18]. Despite this diversity, a core of 18 species was found in all individuals, and 57 were found in 90% of individuals, indicating considerable dominance and inter-individual stability of these species across humans[18]. Faith *et al*[19] analyzed the fecal microbiota of 37 individuals and found that, on average, 60% of the bacterial strains present remained stable for up to 5 years; many were estimated to remain stable for decades.

Recent analyses of human-associated bacterial diversity have tried to categorize individuals into "enterotypes" based on the abundances of key bacterial genera in the intestinal microbiota[20]. Arumugam *et al*[21] reported that a set of 22 Sanger-sequenced European fecal metagenomes from Danish, French, Italian, and Spanish individuals was shown to fit into three distinct clusters (enterotypes), each characterized by variations in the numbers of *Bacteroides* (enterotype 1), *Prevotella* (enterotype 2), and *Ruminococcus* (enterotype 3). Recent meta-analysis including the 16S rRNA sequences and whole genome shotgun sequences from the Human Microbiome Project, Metagenomics of the Human Intestinal Tract consortium, and additional studies yielded only bimodal distributions of *Bacteroides* abundances in gut samples[20]. Enterotype identification depends not only on the structure of the data but also on the methods used for identifying clustering strength[20].

The diversity of intestinal microbiota within and among individuals is strongly influenced by factors such as age, diet, and diseases[9]. In a large cross-sectional study of an elderly population using pyrosequencing, the intestinal microbiota of the elderly subjects was found to be different from that of younger adults, with higher *Bacteroides* and *Clostridia cluster IV*, as well as some signature sequences that were present only in older people[22]. The impact of food intake on the microbiota is being explored. Habitual long-term diet has been shown to be strongly associated with enterotype, with protein/animal fat being associated with *Bacteroides* abundances and carbohydrate being associated with *Prevotella* abundances[23]. In a comparative study in children from urban Europe and rural Africa, rural African children showed significant enrichment in *Bacteroidetes* and depletion in *Firmicutes*, with a unique abundance of bacteria from the genus *Prevotella* and *Xylanibacter*, which are known to contain a set of bacterial genes for cellulose and xylan hydrolysis and were completely lacking in the urban European children[24]. In addition, obese individuals show an increase in

Firmicutes and a decrease in *Bacteroidetes*, probably owing partly to differences in diets[25]. Furthermore, manipulation of dietary macronutrients in gnotobiotic mice was shown to account for the majority of the change in their microbiota[26]. Moreover, many dietary prebiotics including oligo-fructose[27], lactulose[28], lupin kernel[29], inulin-containing juices[30], and arabinoxylan-oligosaccharides[31] significantly alter human fecal microbiota.

Characterization of intestinal microbiota, however, has been limited to Western people. A recent study investigated the overall intestinal microbiota composition of 20 Koreans using pyrosequencing[32]. Microbial communities were dominated by five previously identified phyla: *Actinobacteria*, *Firmicutes*, *Bacteroidetes*, *Fusobacteria*, and *Proteobacteria*. Cluster analysis showed that the species composition of intestinal microbiota was host-specific and stable over the duration of the test period, but the relative abundance of each species varied among individuals. The results were compared with those of individuals from the United States, China, and Japan, and it was found that human intestinal microbiota differed among countries, but tended to vary less among individual Koreans. The gut microbial composition may be related to the internal and external characteristics of each country member, such as host genetics and dietary patterns[32].

INTESTINAL MICROBIOTA COMPOSITION OF IBS PATIENTS

Numerous diseases have been associated with alterations in the microbiota, which are referred to as dysbiosis, ranging from systemic disorders such as obesity and diabetes to gastrointestinal disorders such as IBS[9,33]. The major physiological and immunological functions of the gut cannot be carried out in the absence of the intestinal microbiota[34,35]. The differences in the intestinal microbiota of IBS patients and those of healthy controls have been studied. A previous study that used cultures of fecal material obtained from patients with IBS reported decreased fecal *Lactobacilli* and *Bifidobacteria*, increased facultative bacteria dominated by *Streptococci* and *Escherichia coli*, as well as higher counts of anaerobic organisms such as *Clostridium*[36,37]. Traditional microbiology studies and microbial genome sequencing relied upon cultivated clonal cultures. Such culture-based assessment of fecal microbiota is cheap, widely available, and easy to use, but it grossly underestimates fecal populations because more than 80% of the bacteria in the human intestinal tract cannot be cultured using currently available methods[38].

A revolution in DNA sequencing technologies would be to define genetic material recovered directly from environmental samples. Metagenomics refers to culture-independent and sequencing-based studies of the collective set of genomes of mixed microbial communities (metagenomes) with the aim of exploring their compositional and functional characteristics[39]. In 1977, Woese *et al*[40] identified 16S rRNA, which is a component of the 30S small subunit of prokaryotic ribosomes, having relatively short gene sequences and highly conserved primer binding sites and containing hypervariable regions that can provide species-specific signature sequences useful for bacterial identification. Since then, the molecular profiling of bacterial communities *via* 16S rRNA-gene based approaches such as terminal restriction fragment length polymorphism, PCR temperature/denaturing gradient gel electrophoresis, and fluorescent *in situ* hybridization, has been performed[41]. In the last decade, Sanger sequencing was used for generating data in most microbial genomics and metagenomics sequencing projects; however, recent advances in molecular biology have resulted in the application of DNA microarrays and next-generation sequencing (NGS) technologies for studying complex intestinal microbiota. DNA microarrays comprising hundreds or thousands of DNA fragments arrayed on small glass slides were originally developed for gene expression profiling. These were subsequently applied to the study of different aspects of microbial ecology, including total microbial diversity and a range of biogeochemical functions[42]. Alternatively, NGS approaches, including pyrosequencing (introduced by 454 Life Sciences, Inc.) as well as other platforms such as Solexa (Illumina, Inc.) and SOLiD (ABI, Inc.), offer rapid and highly parallel sequencing of many DNA fragments from complex samples or transcriptomes[39]. Pyrosequencing is particularly suited to microbial ecology studies because of its relatively long read length compared with other NGS technologies platforms, and it has therefore been widely adopted by microbial ecology researchers; other platforms have also been recently adopted in this field[42]. Table 1 lists the advantages and disadvantages of the principal techniques used for characterizing intestinal microbiota.

Studies using culture-independent molecular-based techniques revealed changes in the intestinal microbiota composition in IBS patients compared with those of healthy controls. Thus far, the results of studies on the intestinal microbiota of IBS patients are inconsistent and occasionally, contradictory (Table 1). This inconsistency in results may be ascribed to several reasons, including differences among the various molecular techniques employed, sample collection and handling methods, , as well as definitions of IBS and IBS subtypes[16]. Table 2 lists the advantages and disadvantages of the principal techniques used for characterizing intestinal microbiota. In studying human intestinal microbiota, classical approaches suffer from individual advantages and limitations[7,16]. NSG and phylogenic metagenomics update the bacterial community profiles of patients with IBS. The sample collection method can influence the intestinal microbiota composition. Namely, fecal samples show distal colonic luminal microbiota, whereas biopsy samples show only mucosa-attached microbiota. Although feces or fecal swabs are the most convenient samples, they do not accurately reflect the microbiota composition or activities in the proximal colon. Colon biopsies also do not represent the microbiota in its physiologic state because extensive colon preparation for cleaning intestinal contents removes

Table 1 Summary of molecular studies of intestinal microbiota in irritable bowel syndrome

Ref.	Ethnicity	IBS patients, *n*	Mean age (range), yr	Male gender, *n*	IBS subtype			Controls, *n*	Sample	Method	Changes in intestinal microbiota composition in IBS
					IBS-C	IBS-D	IBS-M				
Malinen *et al*[83] 2005	Finland	27	46.5 (20-65)	7	9	12	6	22 (age, gender matching)	Feces	qPCR covering bacteria 300 bacterial species	IBS-D: ↓ lactobacillus spp. IBS-C: ↑ veillonella spp. Overall IBS: ↓ clostridium coccoides subgroup, Bifidobacterium catenulatum group
Mättö *et al*[46] 2005	Finland	26	46 (20-65)	7	9	12	5	25 (age, gender matching)	Feces	Culture, PCR-DGGE	Temporal instability in the bacterial population ↑ coliform bacteria ↑ aerob:anaerob ratio
Maukonen *et al*[84] 2006	Finland	24	45 (24–64)	5	6	7	3	16	Feces	PCR-DGGE, Transcript analysis with the aid of affinity capture for Clostridial groups	Temporal instability in the bacterial population IBS-C: ↓ clostridium coccoides-Eubacterium rectale group
Kassinen *et al*[43] 2007	Finland	24	47.3 (21–65)	5	8	10	6	23 (age, gender matching)	Feces	GC-profiling + high-throughput *16S rRNA* gene sequencing of 3753 clones	Coverage of the clone libraries of IBS subtypes and control subjects differed
Kerckhoffs *et al*[85] 2009	The Netherlands	41	42 ± 2.12	12	11	11	16	26	Feces, Duodenal mucosa	FISH, qPCR	↓ bifidobacterium catenulatum
Krogius-Kurikka *et al*[86] 2009	Finland	10	46.5	4	0	10	0	22	Feces	G + C (%G + C) -based profiling and fractioning combined with *16S rRNA* gene clone library sequencing of 3267 clones	↑ proteobacteria ↑ firmicutes ↓ actinobacteria ↓ bacteroidetes
Lyra *et al*[87] 2009	Finland	20	IBS-D: 43.6 (26-60), IBS-C: 48.6 (24-64), IBS-M: 50.8 (31-62)	6	8	8	4	15	Feces	qPCR	IBS-D: ↑ ruminococcus torques, ↓ clostridium thermosuccinogenes IBS-C: ↑ ruminococcus bromii-like IBS-M: ↓ ruminococcus torques, ↑ clostridium *thermosuccinogenes*
Tana *et al*[88] 2010	Japan	26	21.7 ± 2.0	13	11	8	7	26 (age, gender matching)	Feces	Culture, qPCR	↑ veillonella spp. ↑ lactobacillus spp.
Codling *et al*[89] 2010	Ireland	47	43.6 (24–66)	0	-	-	-	33	Feces, Colonic mucosa	PCR-DGGE	Significantly more variation in the gut microbiota of healthy volunteers than that of IBS patients
Ponnusamy *et al*[90] 2011	South Korea	11	47.5 (18-74)	6	-	-		8	Feces	DGGE + qPCR of *16S rRNA* genes	↑ diversity of Bacteroidetes and Lactobacillus groups
Rinttilä *et al*[91] 2011	Finland	96	47 (20-73)	27	15		81	23	Feces	qPCR	17% of IBS samples (n = 15) tested positive for staphylococcus aureus
Rajilić-Stojanović *et al*[45] 2011	Finland	62	49 (22−66)	5	18	25	19	42	Feces	Phylogenetic 16S rRNA microarray and qPCR	2-fold ↑ firmicutes:Bacteroidetes ratio↓ bacteroidetes, ↑ dorea, ruminococcus, clostridium sppBifidobacterium faecalibacterium spp
Carroll *et al*[51] 2011	United States	16	35.6 (23–52)	5	0	16	0	21	Feces, Colonic biopsies	T-RFLP fingerprinting of 16S rRNA-PCR	↓ microbial biodiversity in D-IBS fecal samples
Parkes *et al*[52] 2012	United Kingdom	53	IBS-D: 36.2 (32.1–40.3), IBS-C: 32.4 (28.1–36.7)	28	26	27	0	26	Colonic mucosa	FISH, confocal microscopy	Expansion of mucosa-associated microbiota; mainly bacteroides and clostridia; association with IBS subgroups and symptoms

Jeffery *et al*[92] 2012	Sweden	37	37 ± 12	11	10	15	12	20	Feces	Pyrosequencing 16SrRNA	Clustering of IBS patients: normal-like *vs* abnormal microbiota composition (increase of firmicutes-associated taxa and a depletion of bacteroidetes-related taxa)

IBS: Irritable bowel syndrome; IBS-D: Diarrhea-predominant irritable bowel syndrome; IBS-C: Constipation-dominant irritable bowel syndrome; IBS-M: Alternating type or mixed irritable bowel syndrome; PCR-DGGE: PCR denaturing gradient gel electrophoresis; FISH: Fluorescent *in situ* hybridization; qPCR: Quantitative PCR; 16S rRNA: 16S ribosomal RNA.

some of the outer mucus layers and, in turn, the mucosa-attached microbes as well as their normal attachment sites[16]. In addition, different studies used different sample handing methods; some studies used frozen samples, whereas others used fresh samples. The use of single samples cannot be linked to fluctuating symptoms and probably to other factors such as diet and patients' phenotypic characterization[7]. Although most studies used the Rome criteria for IBS, the proportions of the enrolled numbers of IBS subtypes differed among the studies. There is suggestive evidence of an association of intestinal microbiota in certain IBS subtypes. Kassinen *et al*[43] pooled fecal samples by an IBS subgroup diarrhea-predominant IBS (IBS-D), constipation-dominant irritable bowel syndrome (IBS-C), and IBS mixed type (IBS-M) and controls, extracted the bacterial DNA, and analyzed it using high-throughput 16S rRNA sequencing. Population analysis found significant differences between each IBS subgroup and controls[43].

It is difficult to determine whether alterations in microbiota are the primary events that lead to the development of IBS or merely the secondary effects of the syndrome. Despite these difficulties, previous studies found that the intestinal microbiota of some IBS patients was different from that of healthy controls, and there does appear to be a consistent theme of *Firmicutes* enrichment and reduced abundance of *Bacteroides*.

PATHOGENIC ROLE OF INTESTINAL DYSBIOSIS IN IBS

Intestinal microbiota can be divided into two distinct ecosystems: luminal bacteria, which are either dispersed in liquid feces or bound to food particles, and mucosa-associated bacteria, which are bound to a mucus layer adjacent to the intestinal epithelium[16]. Although microbial trafficking will occur between the two ecosystems with a distinct microenvironment, each ecosystem has the potential to play a different role in IBS symptomatology (Figure 1). Luminal microbiota constitutes the majority of the gastrointestinal tract microbiota and plays a crucial role in gut homeostasis. In IBS, luminal microbiota may play a key role in bloating and flatulence through carbohydrate fermentation and gas production. Bacterial fermentation of undigested carbohydrate leads to short-chain fatty acid production, with gaseous byproducts such as carbon dioxide, hydrogen, and methane. The metabolites and toxins of luminal microbiota can modulate the host immune system[44]. Rajilić-Stojanović *et al*[45] prepared a phylogenetic 16S rRNA microarray and performed qPCR using fecal samples from 62 IBS patients and 46 healthy adults. Adult patients with IBS had a two-fold greater ratio of *Firmicutes* to *Bacteroidetes* than controls, resulting from an approximately one-and-a-half-fold increase in the numbers of *Dorea*, *Ruminococcus*, and *Clostridium spp*. In addition, they observed a two-fold decrease in the number of *Bacteroidetes* and a one-and-a-half-fold decrease in the numbers of *Bifidobacterium* and *Faecalibacterium spp*[45]. Furthermore, the instability and temporal variation in the intestinal microbiota of IBS subjects was addressed, and a trend was noted wherein some *Clostridium spp.* increased and *Eubacterium spp.* decreased in IBS patients[46].

Meanwhile, the mucosal microbiota, although fewer in number, may influence the host *via* immune-microbial interactions[35]. Recently, mucosal microbiota has attracted increased research interest. Mucosal microbiota is bound to a mucus layer consisting of glycosylated polysaccharides and glycocalyx. The mucus layer contains binding sites for commensal and pathogenic bacteria that help minimize adherence to the intestinal epithelium below. The vast majority of the microbiota is trapped in a complex biofilm containing a diverse population, and only those bacteria that are able to penetrate the mucus and that possess suitable adhesion proteins can directly interface with the apical surface[47]. Luminal interaction occurs *via* pattern recognition receptors such as toll-like receptors (TLRs) and NOD2. TLRs are expressed on the apical and basolateral membranes of enterocytes and on the processes of dendritic cells that pass from the lamina propria into the lumen through tight enterocyte junctions. Differential expression of TLRs was observed in patients with IBS, with increased TLR-4 and TLR-5 expression and decreased TLR-7 and TLR-8 expression compared with controls[48]. In addition, bacteria can pass through the epithelial layer and are presented to dendritic cells. The pathogenicity of the bacteria determines whether the dendritic cells either auto-induce tolerance *via* the secretion of anti-inflammatory cytokines such as IL-10 and TGF-β or respond aggressively. Studies have also shown that bacteria such as *Bifidobacteria* and *Lactobacilli* stimulate IL-10 and TGF-β production by dendritic cells and inhibit the release of proinflammatory cytokines from dendritic cells[49]. A recent study revealed that some *Bifidobacterium* strains showed the highest production of IL-17 as well as poor secretion of interferon γ and tumor necrosis factor α, suggesting stimulation of the Th17 pathway[50]. The plasticity of

Table 2 Advantages and limitations of the principal techniques used in the characterization of the intestinal microbiota[16,39]

	Advantages	Limitations
Culture	Cheap, easy to use	Limited estimate intestinal microbiota
PCR-T/DGGE	High sensitivity in detecting difference in bacterial populations, semi-quantitative	Does not identify bacteria unless bands on the gel are cut out and sequenced
FISH	Microbial *in situ* identification, high sensitivity, quantitative	Few species can be simultaneously detected, only known species are detected
T-RFLP	Low cost	Low biodiversity resolution, no species-level identification, not quantitative
Quantitative PCR	Can detect small number of bacteria and quantify them	Laborious
Phylogenetic microarray	High biodiversity resolution, quantitative	Only known species are detected
NGS phylogenetic analysis (*e.g.*, pyrosequencing)	Enormous quantities of data at individual Species level	Very costly, need bioinformatics analysis

16S rRNA: 16S ribosomal RNA; PCR-T/DGGE: PCR temperature/denaturing gradient gel electrophoresis; FISH: Fluorescent *in situ* hybridization; T-RFLP: Terminal restriction fragment length polymorphism; qPCR: Quantitative PCR; NGS: Next-generation sequencing.

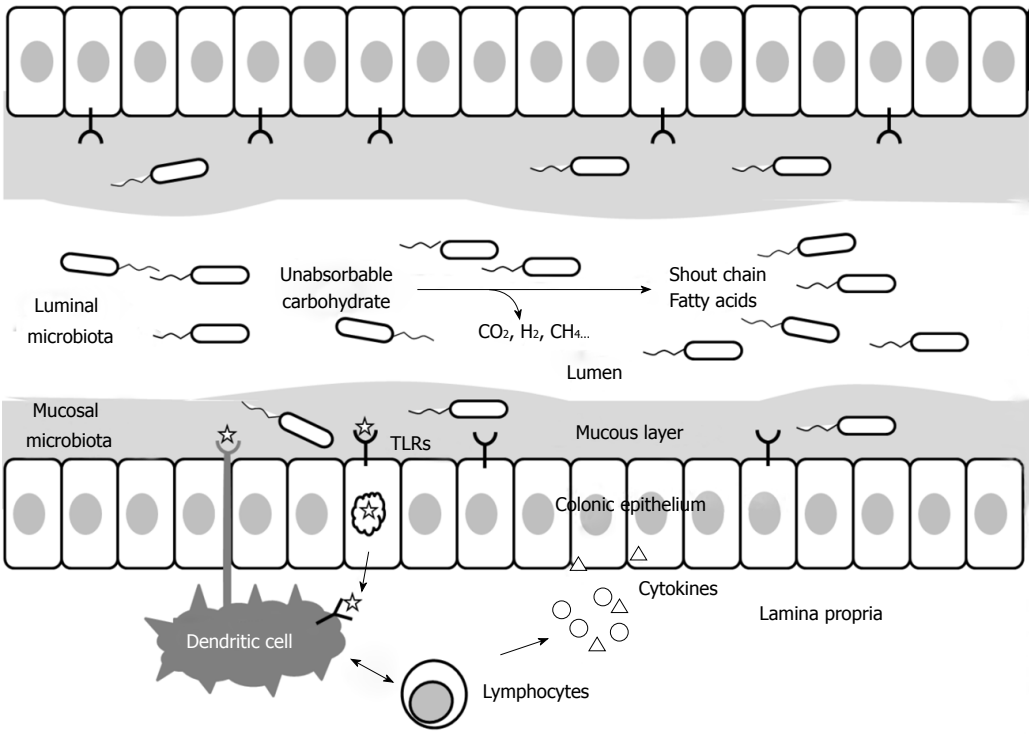

Figure 1 Luminal and mucosal intestinal microbiota and roles in gut homeostasis.

Treg/Th17 populations and the commensal bacteria play a key role in mucosal tolerance and T cell reprogramming[50]. It is, therefore, readily apparent that a disturbance in the mucosal microbiota could lead to an upregulation of the immune system. However, recent studies that examined the mucosal microbiota of IBS patients reported different results. Carroll *et al*[51] performed microbial community composition analyses on fecal and mucosal samples from patients with IBS-D and healthy controls using terminal-restriction fragment length polymorphism fingerprinting of the bacterial 16S rRNA gene. There were compositional differences in the luminal- and mucosal-associated microbiota of IBS-D patients and those of healthy controls as well as diminished microbial biodiversity in the IBS-D fecal samples. There were no differences in the biodiversities of the mucosal samples of IBS-D patients and healthy controls[51]. In contrast, Parkes *et al*[52] performed an analysis of frozen rectal biopsies taken at colonoscopy and bacterial quantification by hybridizing frozen sections with bacterial-group-specific oligonucleotide probes. They found expansion of mucosa-associated microbiota in IBS patients, mainly *Bacteroides* and *Clostridia*, and association with IBS subgroups and symptoms. In addition, they found that the mucosal *Bifidobacteria* were lower in IBS-D patients than in controls, together with a negative correlation between mucosal *Bifidobacteria* and the number of days patients experienced pain or discomfort. However, the studies on the mucosal microbiota of IBS patients are limited because doing so requires endoscopic examination of subjects' gastrointestinal tracts and carrying out biopsy, unlike the luminal microbiota, which can be readily examined in feces.

Intestinal microbiota may be involved in the pathogenesis of IBS by contributing to abnormal gastrointestinal motility, low-grade inflammation, visceral hypersensitivity, communication in the gut-brain axis, and so on. *Lactobacillus paracasei* NCC2461 significantly attenuated muscle dysfunction in a murine model of postinfective IBS[53]. The probiotic yeast *Saccharomyces boulardii* modulated the expression of neuronal markers in the submucous plexus of pigs[54]. There also seems to be an inflammatory component and dysregulation of pro- and anti-inflammatory cytokines in IBS patients[55]. Most interestingly, *Bifidobacterium infantis* (*B. infantis*) 35624 was shown to restore the balance of pro- and anti-inflammatory cytokines in patients[56]. *Lactobacillus farciminis* treatment prevented stress-induced hypersensitivity, increase in colonic paracellular permeability, and colonocyte myosin light chain phosphorylation in rats[57,58]. Modulation of the microbiota induces visceral hypersensitivity in mice, which is reduced by *L. paracasei* NCC 2461-secreted products[53]. Recently, Rousseaux *et al*[59] demonstrated that *Lactobacillus acidophilus* (*L. acidophilus*) contributes to the modulation and restoration of the normal perception of visceral pain through the NF-κB pathway and by inducing mu-opioid receptor 1 (MOR1) and cannabinoid receptor 2 (CB2) expression. Only the *L. acidophilus* NCFM strain was able to induce a significant *in vitro* expression of MOR1 and CB2 messenger in RNA and protein, respectively. To confirm these results *in vivo*, the researchers administered *L. acidophilus* NCFM orally to rats and mice at a clinically relevant concentration (10^9 CFU) and compared colonic samples from these rodents with those from untreated control rodents. MOR1 and CB2 expression was induced in 25%-60% of the intestinal epithelial cells from treated animals compared with only 0%-20% of those from the control group. In addition, visceral perception was assessed in rats using colorectal distension. Oral administration of the *L. acidophilus* NCFM strain for 15 d decreased normal visceral perception in the rats and increased their pain threshold by 20%. In further experiments of chronic colonic hypersensitivity on a rat model, treatment with *L. acidophilus* NCFM resulted in an analgesic effect similar to that of 1 mg morphine administered subcutaneously, thus increasing the colorectal distension threshold by 44% compared with that in untreated rats[59]. Transient perturbation of the microbiota with antimicrobials alters brain-derived neurotrophic factor expression, exploratory behavior, and colonization of germ-free mice, suggesting that the impact of the intestinal microbiota is not limited to the gut and the immune system[60].

SMALL INTESTINAL BACTERIAL OVERGROWTH AND ANTIBIOTICS

Since Pimentel *et al*[61] reported that 84% of IBS patients had small intestinal bacterial overgrowth (SIBO) and that patients with IBS were over 26 times more likely to harbor SIBO than controls, the potential role of SIBO in IBS pathogenesis has gained considerable research attention[62]. In addition, bacterial fermentation in IBS has been highlighted in recent studies on SIBO[16]. Bacterial overgrowth in stagnant sections of the small intestine leads to malabsorption, diarrhea, bloating, and pain, and it can be treated with antibiotics. However, a subsequent study on the SIBO-IBS link showed similar results, whereas other studies were unable to establish an association[62].

A SIBO diagnosis test includes jejuna aspirate and culture, ^{14}C-xylose breath test, and hydrogen (H_2) breath tests (HBT) using either glucose (GHBT) or lactulose (LHBT) as the substrate. Jejunal aspirate and culture is considered as the gold standard (> 10^5 CFU after 48 h of culture); however, it is invasive and time consuming. In contrast, HBT is noninvasive and cheap, but prone to error. Following the ingestion of glucose or lactulose, serial breath H_2 measurements are performed. SIBO is defined by either a rise in H_2 > 20 ppm in < 90 min or a "double peak" demonstrating distinct small intestinal and colonic bacterial populations[63]. Meta-analysis of 12 studies containing 1921 subjects meeting the Rome criteria for IBS revealed that the pooled prevalence of a positive LHBT or GHBT was 54% (95%CI: 32%-76%) and 31% (95%CI: 14%-50%), respectively, but showed marked statistical heterogeneity between study results[64]. In addition, the prevalence of a positive jejunal aspirate and culture was only 4% (95%CI: 2%-9%). These results suggested that it is premature to accept a firm etiologic link between SIBO and IBS. Moreover, despite a decade of investigation on the relationship between SIBO and IBS, it remains unclear whether SIBO causes IBS or is a bystander of something else altogether[62].

However, the idea of treating IBS patients with an antibiotic was developed as a consequence of the SIBO concept[65]. Neomycin therapy eradicated SIBO and reduced symptoms of IBS[61,66]. Considering the chronic, relapsing nature of IBS and the undesirability of long-term systemic antibiotic therapy, the efficacy of rifaximin, a nonabsorbable antibiotic, began to be explored in IBS[67]. In a RCT, rifaximin treatment for 10 d resulted in symptom improvement that lasted for up to 10 wk in some IBS patients who did not document bacterial overgrowth[68]. Subsequently, a double-blind, placebo-controlled trial phase Ⅲ study reported that rifaximin treatment for 2 wk provided significant relief from IBS symptoms such as bloating, abdominal pain, and loose or watery stools[69]. A recent meta-analysis of 5 studies found rifaximin to be efficacious for global IBS symptom improvement (OR = 1.57, 95%CI: 1.22-2.01) and more likely to improve bloating (OR = 1.55, 95%CI: 1.23-1.96) compared with a placebo[70].

EVIDENCE OF THE ROLE OF POTENTIALLY PROBIOTIC BACTERIA IN IBS

An improved understanding of host-microbiota interac-

Table 3 Systemic reviews for randomized controlled trials of probiotics in irritable bowel syndrome

Ref.	Selection criteria	*n* of identified studies	Results
McFarland *et al*[73] 2008	RCTs in humans published as full articles or meeting abstracts in peer-reviewed journals	20 RCTs	Global IBS symptoms: RR = 0.77 (95%CI: 0.62-0.94)/ abdominal pain: RR = 0.78 (95%CI: 0.69-0.88)
Brenner *et al*[76] 2009	RCTs; adults with IBS defined by Manning or Rome Ⅱ criteria; single or combination probiotic *vs* placebo; improvement in IBS symptoms and/ or decrease in frequency of adverse events reported	16 RCTs → 11 studies showed suboptimal study design	*Bifidobacterium infantis* 35624 has shown efficacy for improvement of IBS symptoms. Most RCTs about the utility of probiotics in IBS have not used an appropriate study design
Hoveyda *et al*[74] 2009	RCTs compared the effects of any probiotic therapy with placebo in patients with IBS	14 RCTs → 7 RCTs providing outcomes as dichotomous variable and 6 RCTs providing outcomes as continuous variable	Overall symptoms: dichtomous data - OR = 0.63 (95%CI: 0.45-0.83)/continuous data - mean ± SD, 0.23 (95%CI: 0.07-0.38) Trials varied in relation to the length of treatment (4-26 wk), dose, organisms and strengths of probiotics used
Moayyedi *et al*[75] 2010	RCTs comparing the effect of probiotics with placebo or no treatment in adult patients with IBS (over the age of 16 yr)	19 RCTs → 10 RCTs providing outcomes as a dichotomous variable	Probiotics appear to be efficacious in IBS (Probiotics were statistically significantly better than placebo, but there was statistically significant heterogeneity). The magnitude of benefit and the most effective species and strain are uncertain
Ortiz-Lucas *et al*[77] 2013	RCTs comparing probiotics with placebo in treating IBS symptoms	24 RCTs → 10 RCTs providing continuous data performed with continuous data summarized using mean ± SD and 95%CIs	Pain scores: improved by probiotics containing *Bifidobacterium breve*, *Bifidobacterium longum*, or *Lactobacillus acidophilus* species Distension scores: improved by probiotics containing *B. breve*, *B. infantis*, *Lactobacillus casei*, or *Lactobacillus plantarum* species Flatulence: improved by probiotics containing *B. breve*, *B. infantis*, *L. casei*, *L. plantarum*, *B. longum*, *L. acidophilus*, *Lactobacillus bulgaricus*, and *Streptococcus salivarius ssp. thermophilus*

IBS: Irritable bowel syndrome; RCT: Randomized controlled trial.

tions in IBS is not only important for its pathogenesis but also for assessing the possible benefits of potential probiotic strains in IBS management. Probiotics are defined as live organisms that when ingested in adequate amounts yield a health benefit to the host[9]. Clinically acceptable probiotics should be species-specific; should be of human origin; should survive passage from the oral cavity through the gastric acid barrier, digestive enzymes, and bile acids; should travel down the small bowel into the colon; nidate; and should proliferate therein[54]. Probiotics offer protection against potential pathogens through enhancement of mucosal barrier function by secreting mucins; providing colonization resistance; producing bacteriocins; increasing production of secretory immunoglobulin A; producing a balanced T-helper cell response; and increasing production of IL-10 and TGF-β, both of which play a role in the development of immunologic tolerance to antigens. For example, a specific strain of *B. infantis* 35624 has been shown to prevent NF-κB and IL-8 activation as well as to inhibit the secretion of chemokine ligand 20 in response to *Salmonella typhimurium*, *Clostridium difficile*, and *Mycobacterium paratuberculosis*[71]. Current evidence suggests that probiotic effects are strain-specific[72].

Probiotics should be administered at an adequate dose, preferably greater than 10 billion CFU/g in adults; their viability and concentration should be maintained; and they should have a dependably measurable shelf life at the time of purchase and administration. When these criteria are fulfilled, randomized, placebo-controlled, double-blind trials should be performed on an appropriate population. Five systematic reviews with RCTs of adult IBS patients were published[73-77]. Most of the meta-analyses indicated a beneficial effect of probiotics on global symptoms, abdominal pain, and flatulence, whereas the influence on bloating was equivocal (Table 3). However, aggregation of the effects of different probiotics into a meta-analysis should be undertaken with caution. Different probiotics have different microbiological characteristics, which inevitably influence their efficacy. The most commonly studied probiotic species are *Lactobacilli* and *Bifidobacteria*. Products range in delivery systems (*e.g.,* yogurts, fermented milk drinks, powders, and capsules) and dose (10^6-10^{10} CFU). *Lactobacillus plantarum*, *B. infantis*, and VSL 3 (*Lactobacillus casei*, *L. plantarum*, *L. acidophilus*, *Lactobacillus delbrueckii*, *Bifidobacterium longum*, *Bifidobacterium breve*, *B. infantis*, and *Streptococcus salivarius*) have demonstrated efficacy in patients with IBS[56,78,79].

Recently, we isolated have been isolated new strains, *i.e.*, *L. acidophilus*-SDC 2012, 2013, from Korean infants'

feces[8]. In Korea, the prevalence of IBS is reported to be around 2.2%-6.6%[1], while that in Western countries is around 10%-20%[2]. Based on the relatively lower prevalence of IBS in Korea and previous reports on the efficacy of probiotics for treating IBS symptoms, we hypothesized that the newly isolated *L. acidophilus*-SDC 2012, 2013 may help control the symptoms of IBS patients. The result of our RCT showed that *L. acidophilus*-SDC 2012, 2013 were effective in alleviating IBS symptoms, irrespective of the bowel habit subtype[8]. Although *Lactobacilli* or *Bifidobacteria* have demonstrated efficacy in IBS patients, the benefits of one given species or organism have not been found to be better than that of other species or organisms. In an RCT of composite probiotics, Kim *et al*[80] reported that VSL3 reduced flatulence and retarded colonic transit without altering bowel function in patients with IBS and bloating.

Recent guidelines published by the British Dietetic Association have therefore made strain-specific recommendations considering the limited weak evidence for *B. lactis* DN 173010 in improving overall symptoms, abdominal pain, and urgency in constipation-predominant IBS and the limited weak evidence for VSL3 in reducingg flatulence in IBS patients[32]. People with IBS who choose to try probiotics should be advised to consume a given product for at least 4 wk while monitoring the effect. Probiotics should be consumed at the dose recommended by the manufacturer[75,76,81].

A number of RCTs have been performed for investigating the effectiveness of probiotics in IBS. However, most RCTs of probiotics had a suboptimal study design with inadequate blinding, trial length, sample size, and/or lack of intention-to-treat analysis. Despite these limitations, there is a possibility of greater efficacy of probiotics in patients whose IBS pathogenesis is known to be related to the intestinal microbiota. In addition, the probiotics include strains present in normal intestinal microbiota, and probiotic-associated adverse events are very rare. Thus, probiotics are good candidates for controlling the symptoms of IBS, especially when treatment safety is paramount in a nonlethal disorder such as IBS[82]. The evidence from clinical trials and systematic reviews are largely supportive of the use of specific probiotics strains in IBS[9].

CONCLUSION

Multiple recent studies have consistently proven that intestinal dysbiosis is associated with this IBS. An improved understanding of host-microbiota interactions in IBS is important not only for determining its pathogenesis but also for enabling therapeutic modulation of the microbiota. In addition, such evidence has encouraged investigations of the potential roles of antibiotics and probiotics in this disorder. Although the interactions of microbiota-targeted treatments with the host immune and visceral nervous systems are yet to be fully understood, they have the potential to play a key role in the management of IBS.

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