

## 2016 Irritable Bowel Syndrome: Global view

## Gut microbiota role in irritable bowel syndrome: New therapeutic strategies

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### Abstract

In the last decade the impressive expansion of our

knowledge of the vast microbial community that resides in the human intestine, the gut microbiota, has provided support to the concept that a disturbed intestinal ecology might promote development and maintenance of symptoms in irritable bowel syndrome (IBS). As a correlate, manipulation of gut microbiota represents a new strategy for the treatment of this multifactorial disease. A number of attempts have been made to modulate the gut bacterial composition, following the idea that expansion of bacterial species considered as beneficial (*Lactobacilli* and *Bifidobacteria*) associated with the reduction of those considered harmful (*Clostridium*, *Escherichia coli*, *Salmonella*, *Shigella* and *Pseudomonas*) should attenuate IBS symptoms. In this conceptual framework, probiotics appear an attractive option in terms of both efficacy and safety, while prebiotics, synbiotics and antibiotics still need confirmation. Fecal transplant is an old treatment translated from the cure of intestinal infective pathologies that has recently gained a new life as therapeutic option for those patients with a disturbed gut ecosystem, but data on IBS are scanty and randomized, placebo-controlled studies are required.

**Key words:** Irritable bowel syndrome; Gut microbiota; Probiotics; Prebiotics; Synbiotics; Antibiotics; Fecal transplantation

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**Core tip:** In the last decade, the gut microbiota has provided support to the concept that a disturbed intestinal ecology could promote development and maintenance of symptoms in irritable bowel syndrome (IBS). As a correlate, manipulation of gut microbiota represents a new strategy for the treatment of this multifactorial disease. Probiotics appear an attractive option in terms of both efficacy and safety, while prebiotics, synbiotics and antibiotics still need for-

mation. Fecal transplant has recently gained a new life as therapeutic option for those patients with an altered gut ecosystem, but data on IBS are scanty and randomized, placebo-controlled studies are required.

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## INTRODUCTION

Irritable bowel syndrome (IBS) is a disorder characterized by chronic abdominal pain and discomfort associated with alterations of bowel habits in the absence of a demonstrable pathology<sup>[1,2]</sup>. Other common symptoms are abdominal distension, bloating and flatulence, straining and urgency. IBS is a common gastrointestinal (GI) disorders in the industrialized world with a 10%-15% prevalence in the general population<sup>[3]</sup>. This high prevalence together with the associated co-morbidities has a significant impact on both patients and society, especially in terms of quality of life and medical costs<sup>[4]</sup>.

IBS is a heterogeneous functional disorder that, depending on the prevailing bowel habit, has been subtyped into IBS with constipation (C-IBS), IBS with diarrhea (D-IBS), mixed, alternate IBS (A-IBS) with both constipation and diarrhea plus unsubtyped IBS with neither constipation nor diarrhea<sup>[1,2]</sup>. Alterations of bowel habits are likely related to dysregulation of the autonomic system in the gut, whereas symptoms of abdominal pain and discomfort are thought to involve additional changes in the bidirectional communication between the gut and the brain, known as "gut-brain axis", that cause a modified perception of visceral events in the form of hyperalgesia or allodynia<sup>[5,6]</sup>. The etiology of IBS is incompletely understood and evidence is growing that IBS might be a post-inflammatory and stress-correlated condition<sup>[7,8]</sup>. Both host and environmental factors, including diet, play a key role in triggering symptoms. Among the host factors, central alterations (*i.e.*, aberrant stress responses, psychiatric co-morbidity and cognitive dysfunctions) and peripheral alterations (*i.e.*, intestinal dysmotility, visceral hypersensitivity, low-grade immune activation and altered intestinal barrier function) are both involved<sup>[9]</sup>. Despite considerable research efforts, the treatment of IBS remains a significant challenge mainly due to its poorly defined pathophysiology.

## HUMAN MICROBIOTA

Human microbiota is a complex living ecosystem consisting of unicellular microbes, mainly bacterial,

but also metagenomic archaeal (*i.e.*, *Methanobrevibacter*), viral (*i.e.*, bacteriophages) and eukaryotic (*i.e.*, yeast), that occupies almost every mucosal and cutaneous surfaces of our body. It has been estimated that microbes that stably live in human body amount to 100 trillion cells, ten-fold the number of human cells<sup>[10]</sup> and the majority inhabits the gut where the intestinal microbiota is widely regarded as a virtual organ that actively influences and mediates several physiological functions. These living microorganisms encode for over three millions of genes, the so-called "microbiome"<sup>[11]</sup>, outfitting the human genome by approximately 100-fold<sup>[12]</sup>. The intestinal microbiota is composed by 17 families, 50 genera and more than 1000 species of bacteria: its composition varies among individuals, changes during life and depends on environmental factors, mainly lifestyle, diet, drugs, stress and invasive medical procedures. The intestinal microbiota is dominated by four main phyla: *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria*. In the adult life *Bacteroidetes* and *Firmicutes* are usually prevalent, whereas *Actinobacteria* and *Proteobacteria* are less represented. In the healthy status, the gut microbiota interacts with the human host in a mutualistic relationship, the host intestine provides the bacteria with an environment to grow and the bacterial ecosystem contributes to maintain homeostasis within the host by modulating several physiological functions such as gut development<sup>[13]</sup>, nutrient processing and digestion<sup>[14,15]</sup>, immune cell development and immune responses<sup>[16-18]</sup>, resistance to pathogens<sup>[19,20]</sup>, control of host energy and lipid metabolism<sup>[14,15]</sup> and brain development and function. Changes in bacterial number and composition, the so-called dysbiosis, may induce a dysregulation of this deep relationship and cause the appearance of a spectrum of diseases including metabolic syndrome, diabetes, cancer, inflammatory conditions, neurological pathologies and psychiatric disorders.

Throughout its communication with gastrointestinal epithelial, immune and nerve cells, the gut microbiota generates and releases molecules that can signal to distant organs. It is now recognized that a significant portion of the metabolites circulating in mammalian blood derives from the intestinal microbial community<sup>[21-25]</sup> and the presence or absence of the gut microbiota influences the metabolic profile in regions distant from the gut such as the brain<sup>[26]</sup>. Moreover, it releases factors that target specific neuronal systems involved in the gut-brain axis, generating neurotransmitters and neuromodulators as dopamine, noradrenaline, acetylcholine and gamma-aminobutyric acid (GABA)<sup>[27-31]</sup>. Direct contact of certain probiotics (*i.e.*, *Lactobacillus acidophilus*) with epithelial cells induce the expression of opioid and cannabinoid receptors in the gut and contribute to the modulation and restoration of the normal perception of visceral pain<sup>[32]</sup>. Finally, as the result

of intestinal microbial colonization, metabolism, and subsequent fermentation, the human microbiota produces a significant proportion of the gases present in the gut, including carbon dioxide (CO<sub>2</sub>), hydrogen (H<sub>2</sub>), methane (CH<sub>4</sub>), and hydrogen sulfide (H<sub>2</sub>S). Since H<sub>2</sub>S has been recently recognized as a gaseous neuromodulator/neurotransmitter capable of modulating intestinal inflammation and sensitivity<sup>[33-37]</sup>, it may be hypothesized that the intestinal microbiota plays a significant role in modulating visceral pain also by producing this gaseous mediator. The term microbiota-gut-brain axis is now currently used to indicate the deep correlation among these three functional "organs".

## MICROBIOTA AND IBS

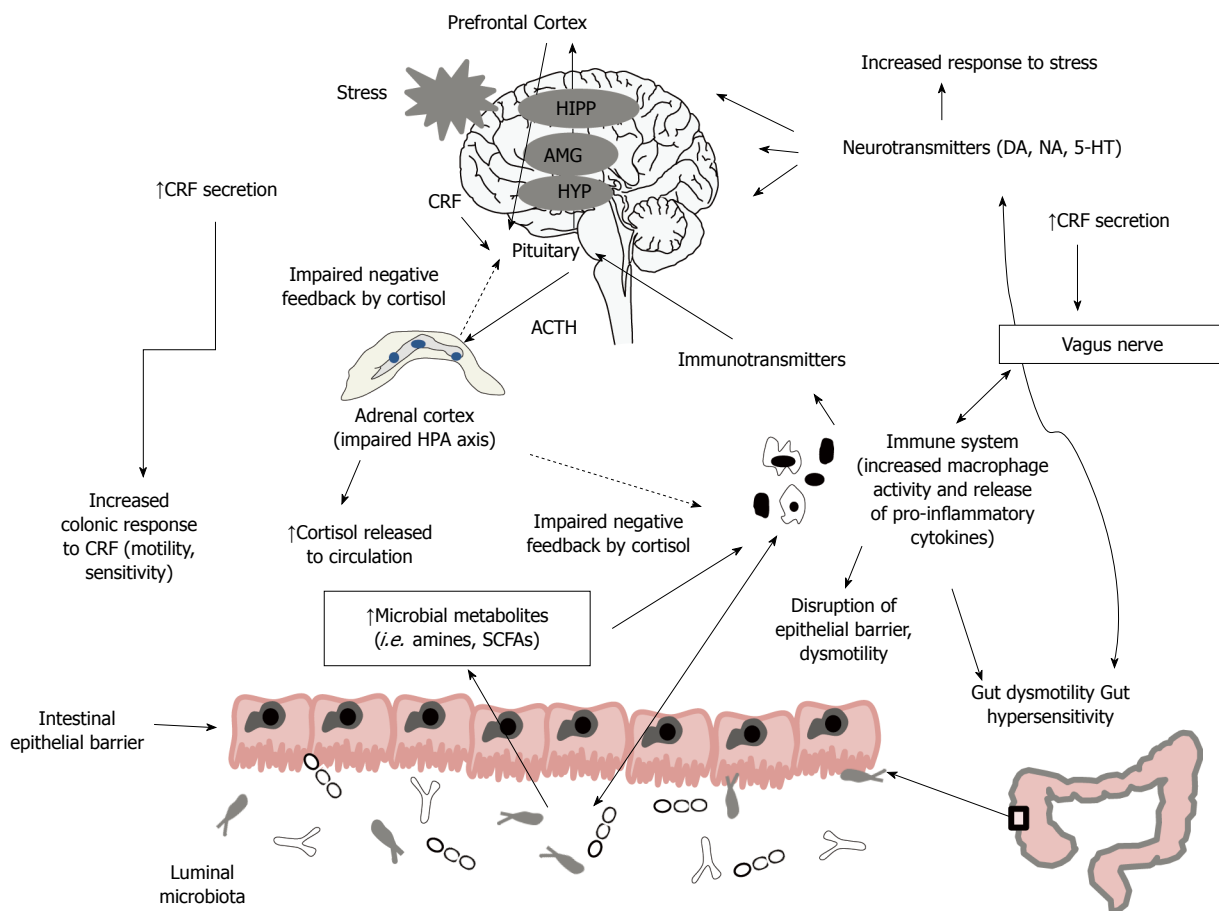
IBS can be considered a multifactorial syndrome in which several pathogenic mechanisms are involved. Gut microbiota interferes with normal intestinal functions at diverse levels, acting as both cause and target of abnormalities of intestinal motility, sensitivity and neuroimmune signaling, including alterations of mucosal barrier and pattern recognition receptors expression, as well as dysfunctions of hypothalamus-pituitary-adrenal (HPA) axis.

### *Perturbation in microbiota composition*

In recent years, perturbations in the intestinal microbiota have been linked to the pathophysiology of IBS (Figure 1), thought that studies investigating the composition of intestinal microbiota in IBS have produced non univocal results<sup>[38]</sup>. Nevertheless the majority of data support the notion that the composition of luminal and mucosal microbiota differs among specific subgroups of IBS patients and healthy individuals<sup>[39-63]</sup> (Table 1). Using culture-based techniques and a 16S rRNA gene-based phylogenetic microarray analysis, it has been demonstrated that the diversity of microbial population is reduced, the proportion of specific bacterial groups is altered and the degree of variability in the microbiota composition is different in IBS patients when compared with healthy subjects. Furthermore, a higher degree of temporal instability of the microbiota among IBS patients has been detected. Examples of these modifications are a decreased amount of *Lactobacilli* and *Bifidobacteria* along with an increased amount of aerobes relatively to anaerobes in IBS patients. Finally mucosal bacteria have also been found to be more abundant in IBS patients than in healthy controls (Table 1). Consistent with this view, the clinical guidance regarding the modulation of intestinal microbiota in IBS provided by the Rome Team Working Group has recently concluded that there is good evidence supporting the concept that the intestinal microbiota is perturbed in patients with IBS<sup>[64]</sup>.

A part of the abnormal composition of gut

microbiota in IBS, others factors support the notion that intestinal flora plays a key role in determining IBS. First, there is increasing evidence of an activation of the intestinal immune system in IBS leading in a micro-inflammation, with studies demonstrating increased concentrations of mucosal intra-epithelial lymphocytes<sup>[65,66]</sup>, mast cells<sup>[66-69]</sup> and 5-hydroxytryptamine-secreting enterochromaffin cells<sup>[65]</sup>. Gut microbiota influences mucosal inflammation in inflammatory bowel disease (IBD) patients, *i.e.*, ulcerative colitis (UC) and Crohn's disease. Animal-based studies emphasize the critical role of gut microbiota in the balance between immunosuppression and inflammation in the GI tract, involving Toll-Like Receptor (TLRs) signaling pathways<sup>[70]</sup>. Indeed, helminthic based treatment with *Heligmosomoides polygyrus* could ameliorate colonic inflammation in murine model of IBDs, shifting the composition of intestinal bacteria<sup>[71]</sup>. Furthermore, Kuehbachner *et al.*<sup>[72]</sup> analyzed the gut microbiota of 73 patients with IBD demonstrating that alteration of TM7 bacteria and the genetically determined antibiotic resistance of TM7 species in these patients, could be a relevant part of a more general alteration of bacterial microbiota in IBD patients, *i.e.*, as a promoter of inflammation at early stages<sup>[72]</sup>. Recent studies demonstrate that reductions in protective bacteria and increases in inflammatory bacteria are associated with pouch inflammation in patients with UC who underwent pouch surgery<sup>[73]</sup>. Moreover, the presence of *Ruminococcus gnavus*, *Bacteroides vulgatus* and *Clostridium perfringens* and the absence of *Blautia* and *Roseburia* in faecal samples of patients with UC before surgery is associated with a higher risk of pouchitis after ileal pouch-anal anastomosis<sup>[74]</sup>. Given the evidence for the role of intestinal microbiota in the profound inflammatory state in IBD, it might be speculated that luminal antigens should play a similar role in development of subclinical inflammation in IBS. Second, it has been reported that approximately 10% of IBS patients refer that their symptoms began following an episode of infectious diarrhea<sup>[75-77]</sup>, the so-called post infectious IBS (PI-IBS), a condition with a clear infective trigger that may alter the normal intestinal microbiota. Third, there is a strong association between IBS and prior use of antibiotics<sup>[78]</sup>. Fourth, the intestinal microbiota strongly interacts with exogenous factors, in particular diet, which may directly or indirectly cause IBS symptoms<sup>[79]</sup>. Fifth, it is well known that alteration of gut microbiota could interfere with behavior and mood in humans<sup>[80,81]</sup>; on the other hand, psychiatric disorders such as anxiety and depression are highly present as co-morbidities in individuals with IBS<sup>[82]</sup>. An high *Firmicutes: Bacteroides* ratio is found in some IBS patients and appears to correlate with depression and anxiety<sup>[60]</sup>, while in another study it has been reported that in IBS patients with clinically significant anxiety, daily treatment with a prebiotic galactooligosaccharide



**Figure 1** Gut microbiota influences the bidirectional communication between the enteric nervous system and the central nervous system, modulating gut development and several physiological functions, including intestinal motility, sensitivity, secretion and immunity. In irritable bowel syndrome (IBS), the altered composition and/or activity of microbiota may induce a disruption of this communication leading to activation of immune system and production of pro-inflammatory cytokines, production of microbial metabolites as short-chain fatty acids (SCFAs) that are toxic at high concentration, activation of hypothalamic-pituitary-adrenal (HPA) axis with increase of cortisol that feeds back to the pituitary, hypothalamus (HYP), amygdala (AMG), hippocampus (HIPP) and prefrontal cortex to shut off the HPA axis and increase of corticotropin releasing factor (CRF). These effects lead to alterations of intestinal motility and sensation, disruption of epithelial barrier and impaired production of neurotransmitters with an increased response to stressful events. On turn, stress may provoke systemic pro-inflammatory cytokines production that activates the HPA axis that signals to both enteric nervous system and the central nervous system and may alter microbiota composition.

mixture for 4 wk reduced anxiety scores and had a significant positive impact on quality of life<sup>[83]</sup>. Taken together, these factors support the notion that an imbalance in the intestinal microbiota composition may directly or indirectly interfere with the normal function of the microbiota-gut-brain axis, leading to the development of central and peripheral abnormalities of either intestinal motility and viscerosensory network.

**Microbiota and colo-intestinal motility**

Although in the past decades the alterations of intestinal and colonic motility have been considered to play a major role in the development of symptoms in IBS patients<sup>[84-86]</sup>, their influence has been reduced over time as intestinal manometry failed to identify any diagnostic abnormality in IBS patients<sup>[87,88]</sup>. The abnormal manometric findings found in IBS patients are heterogeneous and range from minimal changes to severe qualitative abnormalities. For example, the incidence of “clustered” contractions is similar in healthy subjects and IBS patients and greatly varies

overtime<sup>[89,90]</sup>, acute psychologic stress alters duodeno-jejunal motility in both healthy subjects and IBS patients<sup>[91]</sup> and more than half of IBS patients has an entirely normal 24-h manometry<sup>[92]</sup>. Nonetheless gut microbiota modulates gut motor function, which in turn can alter the intestinal microbiota composition. Indeed, not only germ-free animals show profound altered motility patterns that is reversed upon reconstitution with normal flora<sup>[93,94]</sup>, but the influence of the intestinal microbiota on small intestinal myoelectric activity is species-dependent<sup>[95,96]</sup>. The modulatory effects of microbiota on colo-intestinal motility may be dependent on interaction of bacteria with the gastrointestinal tract through receptors on the epithelial cell such as TLRs and nucleotide oligomerization domain (NOD) receptors and, although bacterial translocation, defined as passage of viable bacteria to mesenteric lymph nodes or other organs, is minimal<sup>[97]</sup>, secreted products of bacteria normally gain access to the submucosa to stimulate the mucosal immune system and to induce changes in intestinal

Table 1 Perturbations in the intestinal microbiota in irritable bowel syndrome patients

Ref.	Methods	N° of Pts.	Diagnostic criteria	Results in IBS in comparison with healthy subjects
Dlugosz <i>et al</i> <sup>[38]</sup> Balsari <i>et al</i> <sup>[39]</sup>	qPCR (small bowel)	35	Rome II	No differences ↓ <i>Coliforms</i> ↓ <i>Lactobacilli</i> ↓ <i>Bifidobacteria</i>
Si <i>et al</i> <sup>[40]</sup>	Culture	25	Rome II	↓ <i>Bifidobacteria</i> ↑ <i>Enterobacteriaceae</i>
Malinen <i>et al</i> <sup>[41]</sup>	qPCR	27	Rome II	↑ <i>Veillonellain</i> C-IBS ↓ <i>Lactobacillus</i> in D-IBS
Mättö <i>et al</i> <sup>[42]</sup>	Culture/DGGE	26	Rome II	↑ Aerobes Temporal instability
Swidsinski <i>et al</i> <sup>[43]</sup>	FISH	20	A-IBS C-IBS D-IBS	↑ Mucosal bacteria ↑ <i>Eubacterium rectale</i> ↑ <i>Clostridium coccoides</i>
Maukonen <i>et al</i> <sup>[44]</sup>	PCR-DGGE	16	A-IBS C-IBS D-IBS	Higher instability of the bacterial population ↑ Clostridial groups
Kassinen <i>et al</i> <sup>[45]</sup>	16S ribosomal RNA gene cloning	24	Rome II	Significant differences in several bacterial genera belonging to the genera <i>Coprococcus</i> , <i>Collinsella</i> and <i>Coprobacillus</i>
Lyra <i>et al</i> <sup>[46]</sup>	16S ribosomal RNA gene cloning	20	A-IBS C-IBS D-IBS	↑ <i>Clostridium thermosuccinogenes</i> in D-IBS ↑ <i>Ruminococcus torques</i> in D-IBS ↑ <i>Ruminococcus bromii</i> -like in C-IBS
Krogius-Kurikka <i>et al</i> <sup>[47]</sup>	16S ribosomal RNA gene cloning	10	Rome II	↑ <i>Proteobacteria</i> and <i>Firmicutes</i> in D-IBS ↓ <i>Actinobacteria</i> and <i>Bacteroidetes</i> in D-IBS
Kerckhoffs <i>et al</i> <sup>[48]</sup>	FISH, PCR	41	A-IBS C-IBS D-IBS	↓ <i>Bifidobacteria</i>
Carroll <i>et al</i> <sup>[49]</sup>	16S ribosomal RNA gene cloning	2	Rome III	↑ <i>Bacteroidetes</i> ↑ <i>Proteobacteria</i>
Salonen <i>et al</i> <sup>[50]</sup> Review				
Tana <i>et al</i> <sup>[51]</sup>	Culture	26	Rome II	↑ <i>Lactobacillus</i>
Carroll <i>et al</i> <sup>[52]</sup>	qPCR	10	Rome III D-IBS	↑ <i>Veillonella</i> ↓ Aerobic bacteria ↑ <i>Lactobacillus</i>
Codling <i>et al</i> <sup>[53]</sup>	16S rRNA-DGGE	47	Rome II	↓ Bacterial richness
Ponnusamy <i>et al</i> <sup>[54]</sup>	rRNA-specific 16S rRNA-DGGE	54	Rome II	Same total bacterial quantity Higher diversity of <i>Bacteroidetes</i> and <i>Lactobacilli</i> Lower diversity of <i>Bifidobacter</i> and <i>Clostridium coccoides</i>
Kerckhoffs <i>et al</i> <sup>[55]</sup>	PCR 16S rRNA-DGGE	37	Rome II	↑ <i>Pseudomonas aeruginosa</i>
Rajilić-Stojanović <i>et al</i> <sup>[56]</sup>	qPCR 16S rRNA qPCR	62	Rome II	↑ Ratio of the <i>Firmicutes</i> to <i>Bacteroidetes</i> ↑ <i>Dorea</i> , <i>Ruminococcus</i> , <i>Clostridium spp</i> ↓ <i>Bacteroidetes</i> , <i>Bifidobacterium</i> , <i>Faecalibacterium spp</i>
Carroll <i>et al</i> <sup>[57]</sup>	16S rRNA	16	Rome III D-IBS	Lower biodiversity of microbes
Carroll <i>et al</i> <sup>[58]</sup>	16S rRNA	23	D-IBS	↓ Bacterial richness ↑ <i>Enterobacteriaceae</i> ↑ <i>Proteobacteria</i> ↓ <i>Faecalibacterium</i>
Parkes <i>et al</i> <sup>[59]</sup>	FISH	47	Rome III	More total bacteria numbers ↑ <i>Bacteroides</i> , <i>Clostridia coccoides</i> - <i>Eubacterium rectale</i>
Jeffery <i>et al</i> <sup>[60]</sup>	16S rRNA	37	Rome II	A sub-group of IBS showed normal-like microbiota A sub-group of IBS showed large microbiota-wide changes with ↑ <i>Firmicutes</i> and ↓ <i>Bacteroidetes</i>
Chassard <i>et al</i> <sup>[61]</sup>	FISH/16S rDNA Functional approaches	14	Rome II Rome III C-IBS	↑ <i>Enterobacteriaceae</i> ↑ Sulfate-reducing bacteria ↓ Lactic acid bacteria population ( <i>bifidobacteria</i> and to a lesser extent, <i>lactobacilli</i> )
König <i>et al</i> <sup>[62]</sup> Review				
Sundin <i>et al</i> <sup>[63]</sup>	16S rRNA	19	Rome III	↑ <i>Bacteroidetes</i> in the PI-IBS group (13 patients) ↑ <i>Firmicutes</i> (more specifically <i>Clostridium</i> in IBS)

IBS: Irritable bowel syndrome; IBS-D: Diarrhea predominant IBS; IBS-C: Constipation predominant IBS; A-IBS: Alternate IBS; PI-IBS: Post-infectious-IBS; DGGE: Denaturing gradient gel electrophoresis; PCR: Polymerase chain reaction; FISH: Fluorescence in situ hybridization.



immunity and physiology. In this view, specific bacteria have been reported to induce significant changes in colo-intestinal motility. *Bacteroides thetaiotaomicron*, a common gut commensal in mice and humans, was found to alter expression of genes involved in smooth-muscle function and neurotransmission<sup>[98]</sup>, soluble factors from the probiotic *Escherichia coli* Nissle 1917 enhance colonic contractility by direct stimulation of smooth muscle cells<sup>[99]</sup> and lipopolysaccharide (LPS) from a pathogenic strain of *Escherichia coli* impairs colonic muscle cell contractility<sup>[100]</sup>. Finally, exposure of human colonic muscle cells to *Lactobacillus rhamnosus* GG resulted in a significant dose- and time-dependent impairment of acetylcholine-stimulated contraction<sup>[101]</sup> and in the restoration of the intrinsic myogenic response in a model of LPS-induced alterations of muscle cells<sup>[102]</sup>.

### **Microbiota and visceral sensitivity**

The connections among gut and brain involve several integrated structures that transport the sensorial information from peripheral (gut) to the central (CNS) stations. Each stimulus from splanchnic visceral afferences (*i.e.*, distensive, chemical, thermal, osmotic) pass throughout the gut intrinsic innervation enteric nervous system (ENS), is received in the spinal dorsal horn and is transmitted to supraspinal sites, the final integration of the painful perception occurring in the cortex<sup>[103]</sup>. These complex communications connect to the extrinsic innervation [the autonomic nervous system (ANS)] which interacts with the HPA axis affecting the visceral sensory motor functions<sup>[104]</sup>. Vagal afferences activation plays a modulatory role on the spinal visceral pain pathway<sup>[105]</sup>. Visceral hypersensitivity may develop at several levels of the brain-gut axis, *i.e.*, ENS, spinal cord and supraspinal sites<sup>[106]</sup> and plays a key role in the pathogenesis of IBS, the main physiopathological alteration being represented by a reduced pain threshold to rectal distension<sup>[6,107,108]</sup>. Moreover, an altered rectal compliance<sup>[109,110]</sup> and/or an increased sensorial colonic response to intestinal lipid perfusion may be present<sup>[110-112]</sup>. An abnormal central processing of intestinal stimuli could be the cause of visceral hypersensitivity, as indicated by brain imaging studies that have shown an altered vascularization of certain areas of the CNS in response to intestinal distension in IBS patients, such as the anterior cingulate cortex, the amygdale and the dorso-medial frontal cortex<sup>[113]</sup>. At supraspinal sites, interactions with emotional or stressful stimuli can modulate the visceral sensitivity resulting to increased pain perception<sup>[114]</sup>. Recent data have shown that gut microbiota may directly modulate several systems involved in visceral hypersensitivity. Indeed, antibiotics-induced intestinal dysbiosis modified colonic pain-related and motor responses by upregulation of TLR4 and TLR7 and downregulation of the antinociceptive cannabinoid 1 and mu-opioid

receptor in mice<sup>[115]</sup>. Moreover, manipulations of the commensal microbiota by stressful events was able to enhance the local expression of visceral sensory-related systems within the colon, as cannabinoid receptor type 2 and tryptophan hydroxylase isoform 1 (TPH1), leading to an excitatory modulation of visceral sensitivity<sup>[116]</sup>. Functional dysbiosis caused visceral hypersensitivity in patients affected by IBS (including PI-IBS), SIBO and chronic constipation by acting on local or systemic immune activation and altered intestinal fermentation<sup>[117]</sup>, and gut commensals modulate the activations of intestinal sensory endings<sup>[118]</sup>. Probiotic strains directly modulate visceral perception of nociceptive stimuli. For example, *Lactobacillus reuteri* inhibited the autonomic response to colorectal distension in rats through effects on enteric nerves<sup>[119]</sup>, modulated vagal afferents<sup>[120]</sup> and decreased the *in vitro* and *in vivo* activation of the transient receptor potential vanilloid 1 (TRPV1) channel which activity may mediate nociceptive signals<sup>[121]</sup>.

### **Microbiota and autonomic nervous system, hypothalamic-pituitary-adrenal axis, enteric nervous system, mucosal barrier and neuro-immune signalling**

The bidirectional communication network among central and peripheral regions includes the CNS, the spinal cord, the ANS, the ENS and the HPA axis. The autonomic system, *via* the sympathetic and parasympathetic branches, drives both afferent and efferent signals, while the HPA axis modulates the adaptive responses of the organism to stressors of any kind<sup>[122,123]</sup>. Stressful events, as well as elevated systemic pro-inflammatory cytokines, activate this system that, through secretion of the corticotropin-releasing factor (CRF) from the hypothalamus, stimulates adrenocorticotrophic hormone secretion from pituitary gland that, in turn, leads to cortisol (an hormone that has a predominantly anti-inflammatory role on the systemic and GI immune system) release from the adrenal glands. Both neural and hormonal responses induce activation of several effector cells including immune cells, epithelial cells, enteric neurons, smooth muscle cells, interstitial cells of Cajal and enterochromaffin cells. Once activated, these systems exert a profound influence on gut microbiota composition both indirectly by modulating several GI functions (including motility, secretions, maintenance of intestinal permeability and integrity of immune response) and directly *via* signaling molecules<sup>[124]</sup>. On the other hand, these systems are under the influence of the gut microbiota composition that interacts not only locally with intestinal cells and ENS, but also directly with CNS through neuroendocrine and metabolic pathways<sup>[125-130]</sup>.

### **Microbiota and pattern recognition receptors**

The balance of innate signaling in the intestine is crucial to homeostasis and microbiota integrity is

essential in maintaining the neuro-immune function in the GI tract. The detection of pathogens by the host is obtained through the families of pattern recognition receptors (PRRs) that recognize conserved molecular structures known as pathogen-associated molecular patterns and induce production of innate effector molecules. These signaling receptors can be divided into three families: TLRs, retinoic acid inducible gene I-like receptors, and NOD-like receptors. The TLR family is the best characterized, and 13 receptors have been reported in mice and humans<sup>[131]</sup>. These PRRs play a key role in detecting pathogens and inducing the innate response. In particular, TLRs respond to specific microbial ligands and to harmful signals produced by the host during infection, initiating a downstream cascade that activates both innate and adaptive immunity. This cascade includes epithelial cell proliferation, secretion of IgA into the gut lumen and production of  $\alpha$ -defensins,  $\beta$ -defensins and other bactericidal substances termed antimicrobial peptides (AMPs)<sup>[132-134]</sup>. Gut microbiota, through PRRs, can modulate the expression of genes involved in inflammatory and pain responses and the production of AMPs. In turn, the expression of PRRs affects the structure of gut microbiota in both health and disease. For example, alterations in the composition of the commensal microbiota as seen in dysbiosis may induce profound change in TLR4 and TLR7 expression, leading in alteration of colonic motility and sensitivity<sup>[115]</sup>, while microbiota protects against ischemia/reperfusion-induced intestinal injury through NOD2 signaling<sup>[135]</sup>. In turn, TLR signaling maintains segregation between bacteria and the epithelium through production of AMPs<sup>[136-138]</sup>, but deficiency in PRRs such as NOD2 and TLR5 can alter the gut microbiota composition in mice<sup>[139]</sup>.

## MODULATION OF THE INTESTINAL MICROBIOTA IN IBS

### Probiotics

The term "probiotic" as originally defined by FAO/WHO refers to "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host"<sup>[140]</sup>. However, in order to be beneficial, probiotic bacteria must be able to survive along the gastrointestinal tract, to resist to gastric acid, bile and pancreatic juice action and to demonstrate functional efficacy<sup>[141]</sup>. Several clinical trials that have investigated the therapeutic benefits of probiotics on either overall or specific IBS symptoms, but these studies are highly heterogeneous. Thus, although a number of meta-analysis or systemic reviews indicate that probiotics may be helpful in the treatment of IBS symptoms, their conclusions vary because of inadequate sample size, poor study design and use of various probiotic strains in the reviewed studies. This review has been focused on a number of meta-analysis and extensive

reviews, published in the last 5 years, that have screened randomized and controlled trials (RCTs) conducted on IBS patients by using different probiotic strains.

Moayyedi *et al.*<sup>[142]</sup> have examined 18 RCTs including 1650 patients with IBS and, although there was a significant heterogeneity among studies, a preference toward probiotic treatment was detected. Indeed, probiotic administration was significantly better than placebo at improving overall symptoms. No major difference was apparent between various types of probiotics used, with *Lactobacillus* (three trials, 140 patients)<sup>[143-145]</sup>, *Bifidobacterium* (two trials, 422 patients)<sup>[146,147]</sup>, *Streptococcus* (one trial, 54 patients)<sup>[148]</sup> and combinations of probiotics (four trials, 319 patients)<sup>[149-152]</sup>, all showing a trend towards benefit, with no side effects reported. Moreover probiotics showed a statistically significant effect in improving individual symptoms such as pain, flatulence and bloating, but not urgency.

Lactic acid bacteria (LAB)<sup>[153]</sup>, the most commonly used bacteria in probiotic preparations that include both typical and atypical species, covering *Lactobacilli*, *Bifidobacteria*, *Enterococci* and *Streptococci* have also been widely used in clinical trials. Analysis of 42 RCTs by Clarke *et al.*<sup>[153]</sup> indicates that, despite a significant studies heterogeneity, 34 studies reported beneficial effects on at least one pre-specified endpoints or symptoms. Indeed, 20 of the 34 trials involving LAB reported improvement in abdominal pain/discomfort, 12 of the 24 trials reported improvement on abdominal bloating/distension, and benefits over placebo were reported in 13 of the 24 trials assessed using an index of defecatory function. Both *Bifidobacteria*<sup>[147,154-157]</sup> and *Lactobacilli*<sup>[144,145,154,158-165]</sup> were found effective in ameliorating IBS symptoms, while the beneficial effects of the multispecies LAB preparations, including the multistrain preparation VSL#3, were less evident<sup>[149,150,152,166-171]</sup>.

A more strictly selected list of 16 RCTs was evaluated by Brenner *et al.*<sup>[172]</sup>. These Authors found that 11 trials were inadequately blinded, of too short duration, of too small sample size, and/or lacked intention to treat analysis; they concluded that only two of the studies - those using *Bifidobacterium infantis* 35624<sup>[147-154]</sup> - showed significant improvements in abdominal pain/discomfort, bloating/distension and/or bowel movements, compared to the placebo. However, none of the studies provided quantifiable data about both tolerability and adverse events.

A systematic review by Hungin *et al.*<sup>[173]</sup> has selected 19 studies and included 1807 patients. The majority of these studies tended to include all IBS subtypes, with only two studies focusing on C-IBS<sup>[156,157]</sup>, and three studies focusing on D-IBS<sup>[149,174,175]</sup>. Although reported trials were extremely different for probiotic strains (above all *Lactobacilli* and *Bifidobacteria*, but also *Streptococcus salivaris*, *Saccharomyces boulardii*

and probiotic mixtures such as VSL#3), study design and definition of treatment response with a responder rates of 18%-80% in IBS group and 5%-50% in healthy controls, this review<sup>[149,156,157,173-188]</sup> detected several positive effects of probiotics on IBS symptoms and health-related quality of life. Probiotics had a favorable safety profile with no difference in adverse events among the 23 specific probiotic treatments and placebo<sup>[173]</sup>.

Another systematic review with meta-analysis has been recently published by Didari *et al.*<sup>[189]</sup>. They retrieved 11748 publication on probiotics, but only 15 were used for the meta-analysis, 9 of which were reviewed in deep. Again, the majority of the study was excluded for poor clinical design, lack of inclusion criteria, time limitation, and lack of a control group and use of probiotics in combination with herbal medications or prebiotics. The 15 trials used for the meta-analysis included 882 patients with D-IBS, C-IBS and A-IBS according to Rome II, Rome III, International Classification of Health Problems in Primary Care and World Organization of Family Doctors criteria<sup>[145,152,155,166,169,170,174-177,179,190-193]</sup>. Although the studies differ in term of bacterial strain used, probiotic dosage, duration of either treatment or follow-up and endpoints/outcome, probiotics were more effective than placebo in reducing abdominal pain after 8 and 10 wk of treatment; the effect was higher at week 8, suggesting a reduced effectiveness with long-term use. Furthermore, probiotics administration improved general IBS symptoms and the severity of symptoms was decreased, although not significantly in comparison with placebo. Few adverse events were reported in both probiotics and placebo groups. The same results have been reached by the extensive review of other 9 studies that, according to Rome II or Rome III criteria, included 324 patients with C-IBS, D-IBS and A-IBS<sup>[156,161,167,182,194-198]</sup>.

As several trials have demonstrated the superiority of probiotics over placebo in controlling IBS symptoms (above all, *Lactobacilli* and *Bifidobacteria*, but also other species including bacterial mixtures such as VSL#3), there is now a general agreement on their efficacy as a therapy for this elusive syndrome (extensively discussed in Reference 112 and Table 2). However, given the controversies in IBS pathophysiology, patient heterogeneity, or lack of clear and reproducible evidence for gut microbiota abnormalities in patients with IBS, additional RCTs with appropriate end points and design are needed for determining to which extent (and in which IBS subpopulations) probiotics are a useful therapeutic strategy in the management of IBS symptoms.

#### **Putative mechanisms of action of probiotics**

As the pathogenesis of IBS is multifactorial, probiotics have been shown effective in modulating several mechanisms that might have a mechanistic role in

IBS pathogenesis, including effects on composition of intestinal microbiota, gastrointestinal dysmotility, visceral hypersensitivity, altered gut epithelium and immune function, luminal metabolism, dysfunction of gut-brain axis, psychological distress.

#### **Composition of intestinal microbiota in IBS**

Only few trials on IBS patients have examined the composition of intestinal bacteria before and after the supplementation therapy, therefore the effect (if any) of probiotics administration on resident microbiota is not fully understood. However, it has been suggested that probiotics might reshape the intestinal eco-system generating an ecological milieu that is unfavorable for the growth of harmful species by increasing the number of *Lactobacilli* and *Bifidobacteria*<sup>[199]</sup> that will stabilize the intestinal microbiota<sup>[174,179]</sup>. As bacteria compete for nutrients and produce substances that directly affect the growth of other bacteria, probiotics can provide a two-fold protection against a broad range of pathogens, including certain forms of *Clostridium*, *Escherichia coli*, *Salmonella*, *Shigella* and *Pseudomonas*: aside from competing for the nutrients, probiotics also produce metabolites (*i.e.*, lactic acid, short chain fatty acids and hydrogen peroxide) and soluble factors (*i.e.*, bacteriocins as sakacin, lactocin, amylovorin, acidophilin, bifidin, bifidocin) that are inhibitory for some pathogenic bacteria<sup>[200,201]</sup>. Moreover, probiotics reduce the adherence of pathogenic bacteria by promoting the production of mucins<sup>[202-204]</sup>.

#### **Gastrointestinal dysmotility**

From almost 4 decades, the assumption that IBS is characterized by impaired intestinal motor functions<sup>[205-207]</sup> and by gas retention<sup>[208]</sup> has driven the treatment of IBS to small bowel and colonic dysmotilities. In this conceptual framework probiotics are thought to directly affect the intestinal motility. Indeed, *Bifidobacterium Lactis* HN019 and *Bifidobacterium lactis* DN-173 010 decreased intestinal transit time in adult constipated patients<sup>[209]</sup> and a recent meta-analysis of randomized controlled trials have revealed that *Bifidobacterium lactis*, but not for *Lactobacillus casei* Shirota, reduced whole gut transit time and increased stool frequency in constipated patients<sup>[210]</sup>. Moreover, fermented dairy product containing *Bifidobacterium lactis* DN-173 010 reduced distension in association with acceleration of gastrointestinal transit and improvement of symptoms in IBS with constipation<sup>[156]</sup>, daily *Bifidobacterium lactis* supplementation decreases WGTT and the frequency of functional GI symptoms in a dose-dependent manner in subjects suffering from irregular bowel movements and flatulence<sup>[185,211]</sup> and a combination of probiotics (*Bacillus subtilis* and *Streptococcus faecium*) was effective for relief of symptoms in patients with non-diarrheal-type IBS<sup>[212]</sup>. Probiotics are useful also on D-IBS, as a probiotic mixture containing *Lactobacillus*



Table 2 Efficacy of probiotics in irritable bowel syndrome<sup>[112]</sup>

Authors' statement	Parameter scored	Conclusion	Grade of evidence for effect	Ref.
1	Global symptom assessment	Specific probiotics help relieve overall symptom burden in some patients with IBS	High	[147,149,150,152,156-157,169,174,176-178]
2	Global symptom assessment	Specific probiotics help relieve overall symptom burden in some patients with C-IBS	Low	[147,156-157]
3	Global symptom assessment	Specific probiotics help relieve overall symptom burden in some patients with D-IBS	Moderate	[147,149,174-175]
4	Abdominal Pain	Specific probiotics help reduce abdominal pain in some patients with IBS	High	[145,147,149-150,152,155-157,168-169,174-119]
5	Bloating/distension	Specific probiotics help reduce bloating/distension in some patients with IBS	Moderate	[147,149-150,155-156,168-170,174-177,179-183]
6	Flatus	Probiotics tested to date do not help reduce flatus in patients with IBS	Low	[147,149-150,156,168,174-175,178-179,184]
7	Constipation	Specific probiotics may help reduce constipation in some patients with IBS	Low	[155-156,183,185]
8	Bowel habit	Specific probiotics help improve frequency and/or consistency of bowel movements in some patients with IBS	Moderate	[145,147,149-150,152,155-157,168-170,174-180,182,185-186,188-189]
9	Diarrhoea	Probiotics tested to date do not reduce diarrhea in patients with IBS	Very low	[174,179,181-183,185,187]
12	Health-related quality of life	With specific probiotics, improvement of symptoms has been shown to lead to improvement in some aspects of health-related quality of life	Moderate	[147,150,152,155,169-170,174,176-177,179-180,183-184,186]

IBS: Irritable bowel syndrome; D-IBS: Diarrhea predominant IBS; C-IBS: Constipation predominant IBS.

*acidophilus*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Bifidobacterium breve*, *Bifidobacterium lactis*, *Bifidobacterium longum*, and *Streptococcus thermophilus* has shown to be effective in controlling symptoms<sup>[174]</sup>, but the effect on intestinal motility and transit time in this subtype of patients is less proven<sup>[149]</sup>.

### Visceral hypersensitivity

Mechanistic data provided mainly by animal studies highlight that probiotics exert a direct anti-nociceptive action through the modulation of bacterial metabolites production (*i.e.*, neurotransmitters, neuroactive substances including GABA and serotonin) on sensitive nerve endings in the gut mucosa<sup>[26-31,130]</sup>, or by targeting specific central neurosensitive pathways. For instance, various strains of probiotics have been shown effective in reducing visceral nociceptive reflex responses in several experimental models of IBS<sup>[213,214]</sup> by directly modulating a number of central anti-nociceptive and pro-nociceptive pathways<sup>[32,214-216]</sup>. However, only few studies have investigated the effects of probiotics on visceral sensitivity in humans. Indeed, in healthy subjects a non-fermented milk product contained *Bifidobacterium animalis subsp Lactis*, *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and *Lactococcus lactis subsp Lactis* was able to affect activity of brain regions that control central processing of emotion and sensation<sup>[81]</sup>; on the other hand, in IBS patients the multispecies probiotic Winlove 801 containing six bacterial species (*Bifidobacterium lactis* W52, *Lactobacillus casei* W56, *Lactobacillus salivarius* W57, *Lactococcus lactis* W58, *Lactobacillus acidophilus*

NCFM and *Lactobacillus rhamnosus* W71) failed to ameliorate visceral hypersensitivity in comparison with placebo<sup>[217]</sup>. However, this limited information is insufficient to translate the animals findings to "hypersensitive" disease states involving disturbances in the gut/brain axis such as IBS.

### Epithelial barrier/intestinal inflammation/immune activation

Despite IBS being considered as a "functional", non-organic disease, it is widely accepted that persistence of IBS-like symptoms occurs in a small percentage of patients after a documented episode of intestinal bacterial or viral infection. The fact that IBS could be a state of "low grade inflammation" is gaining acceptance based on the fact that animal and epidemiological studies indicate that IBS is characterized by an increased intestinal permeability, a biomarker of impaired epithelial barrier function. Further on, an increased activity of innate immune (mainly represented by accumulation of mast cells and antigen-presenting cells such as dendritic cells and macrophages) and an activated adaptive immune response in the intestinal mucosa and in blood, including an increased levels of systemic or mucosal cytokines, such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-1 $\beta$ , IL-6, IL-8, IL-12, associated to a decrease of anti-inflammatory cytokines (*i.e.*, IL-10) has been described in IBS<sup>[218-220]</sup>. Probiotics appear effective in reducing the inflammatory components of IBS. Thus, probiotics administered either as a single strain or in combination, maintain the integrity of intestinal epithelial barrier in inflammatory models<sup>[221-225]</sup>

and in humans<sup>[161,175]</sup>, modulate both innate and adaptive immunity<sup>[226,227]</sup>, restore the imbalanced ratio between pro-inflammatory and anti-inflammatory cytokines (*i.e.*, IL-10/IL-12)<sup>[154]</sup> and decrease the levels of pro-inflammatory cytokine as TNF- $\alpha$  and IFN- $\gamma$ <sup>[228-230]</sup>. Human studies have demonstrated that a probiotic combination containing *Lactobacillus gasseri* KS-13, *Bifidobacterium bifidum* G9-1 and *Bifidobacterium longum* MM2 175 induced a less inflammatory cytokine profile in older adults<sup>[231]</sup>, *Saccharomyces boulardii* supplementation induced a significant decrease in blood and tissue levels of proinflammatory cytokines IL-8 and TNF- $\alpha$  and an increase in anti-inflammatory IL-10 levels, as well as an increase in the tissue IL-10/IL-12 ratio ameliorating the quality of life of D-IBS<sup>[232]</sup> and the symptomatic response induced by *Bifidobacterium infantis* 35624 in IBS was associated with normalization of the ratio of an anti-inflammatory to a proinflammatory cytokine<sup>[154]</sup>.

### Luminal metabolism

The gut microbiota produces several metabolites including short-chain fatty acids (SCFAs), neurotransmitters, metabolites of bile acids, and cytokines that target enteric cells *via* specific receptors and signal to the brain *via* afferent vagal or endocrine pathways. SCFAs like acetate, propionate and butyrate derive from the fermentation of undigested and unabsorbed carbohydrates, *i.e.*, resistant starches and dietary fibers and are used as a fuel by the colonic microbiota. While propionate is largely taken up by the liver and acetate enters the systemic circulation to be metabolized by the peripheral tissues, butyrate works as major energy source for colonocytes. Butyrate modulates epithelial proliferation, apoptosis and cell differentiation in the large intestine<sup>[233]</sup>, inhibits nuclear factor kappa B activation<sup>[234]</sup> and stimulates intestinal mucus production<sup>[235]</sup>, thereby supporting the mucosal barrier function. Furthermore, butyrate plays a major role in inflammation-related repairs<sup>[236]</sup>, offers protection against colonic carcinogenesis in rats<sup>[237]</sup> while in humans improves visceral perception<sup>[238]</sup>, suggesting a possible beneficial effect in "hypersensitive" disorders such as IBS.

In contrast acetic and propionic acid-producing bacteria (*i.e.*, *Veionella* and *Lactobacilli* spp) have been reported in IBS patients<sup>[51]</sup> leading to enhanced production of SCFAs that are toxic at high concentrations and stimulate 5-HT release from the intestinal nerve endings<sup>[239]</sup>. As 5-HT initiates high-amplitude colonic contractions, accelerates intestinal transit and increases colonic motility, *i.e.*, all possible features of IBS, it might be speculated that these fermentation products play a role in IBS symptoms. However, fecal concentrations of SCFAs in IBS patients differ only slightly in comparison to healthy subjects<sup>[51,240]</sup> and IBS symptoms show only a slight correlation with SCFAs fecal concentrations.

Moreover, studies that have examined the effect of probiotics supplementation on fecal SCFAs in humans have provided conflicting results. Thus, the probiotic *Lactobacillus paracasei* DG modulated fecal butyrate concentration in healthy subjects<sup>[241]</sup>, while bifidobacteria-fermented milk increased fecal butyrate, propionate and short-chain fatty acid concentrations but ameliorated symptoms in patients affected by UC<sup>[242]</sup>. Finally, the *Bifidobacterium lactis* Bb12 increased the fecal level of acetate and lactate in preterm infants<sup>[243]</sup>.

However studies demonstrating a strong correlation between the assumption of probiotics and the level of fecal SCFAs in IBS patients are lacking.

### Dysfunction of the brain-gut axis and psychological distress

There are evidence that the gut microbiota modulates the CNS *via* the ENS, the ANS, the HPA axis and *vice versa*<sup>[124,213]</sup>: a deep correlation that influences both brain development and responses, intestinal motility, sensitivity, secretions and immunity. An intestinal dysbiosis may lead to alteration of brain-gut axis and probiotics may restore the normal interaction among all components of this pathway. Several beneficial actions of probiotics on brain-gut axis including the maintenance of intestinal barrier that "protects" the ENS, the effects of certain probiotics and their products on intestinal sensory and motor nerves of ENS, ANS and on several receptors (*i.e.*, opioid and cannabinoid receptors) and the modulation of cytokines profile leading to an anti-inflammatory action have been mentioned earlier in this review. Importantly, the HPA axis is a neuroendocrine system essential for the normal stress response to challenges in vertebrates which integrity is important in the pathogenesis of IBS<sup>[244,245]</sup>. There is evidence that certain probiotics directly influence the exaggerated HPA axis response observed in several experimental models of IBS. Indeed, *Bifidobacterium animalis subsp lactis* BB-12<sup>®</sup> and *Propionibacterium jensenii* 702 induced activation of neonatal stress pathways and an imbalance in gut microflora but also improved the immune environment of stressed animals and protected against stress-induced disturbances in adult gut microflora<sup>[246]</sup>, while probiotic preparation containing live *Lactobacillus rhamnosus* strain R0011 and *Lactobacillus helveticus* strain R0052 improved gut dysfunction induced by maternal separation, at least in part by normalisation of HPA axis activity<sup>[247]</sup>. Moreover, probiotics alleviate anxiety- and depression-related behavior that are a typical feature of IBS, as *Lactobacillus rhamnosus* (JB-1) reduced the stress-induced elevation in corticosterone in stressed animals *via* modulation of GABA receptors implicated in anxiety behavior<sup>[216]</sup>, *Bifidobacterium longum* 1714 had a positive impact on cognition in stressed mice<sup>[248]</sup> and the probiotic mixture VSL#3 induced an increase in brain-derived

neurotrophic factor (BDNF) expression and reduced age-related alterations in the hippocampus in a murine model of deterioration in cognitive functions<sup>[249]</sup>.

### **Probiotics and gene expression: a new mechanism of action?**

Finally, probiotics administration might result in a central (*i.e.*, CNS) and peripheral (*i.e.*, intestinal) reprogramming of genes. In the maternal separation (MS) rat model of IBS, *Bifidobacterium breve* 6330 influenced hippocampal BDNF gene expression<sup>[250]</sup>, while the probiotic mixture VSL#3 downregulated the colonic expression of several genes (*i.e.*, TPH1, CCL2, CCR2, NOS3, NTRK1, BDKRB1, IL10, TNFRSF1B and TRPV4) encoding for proteins involved in nociception<sup>[214]</sup>, maternal probiotic intervention also increased the gene expression of ileal mucin-2 (MUC2)<sup>[230]</sup>, indicating that the mechanism of action of probiotics is deeper and more complex than previously thought.

## **PREBIOTICS**

The Food and Agriculture Organization of the United Nations (FAO) defines prebiotic as "a nonviable food component that confers a health benefit on the host associated with modulation of the microbiota"<sup>[251]</sup>. They represent an alternative strategy for reprogramming the gut microbiota by providing regular doses of a specific substrate engineered to be readily metabolized by specific desirable bacteria, thereby encouraging their growth in contrast to the development of harmful microbial species. Also known as "functional" foods<sup>[252]</sup>, they escape absorption in the small bowel and enter the colon where they provide for nutrients for specific bacteria, mainly *bifidobacteria* and *lactobacilli*. Several prebiotics belong to the group of non-digestible carbohydrates: monosaccharides (*i.e.*, fructose), disaccharides (*i.e.*, lactose), oligosaccharides [*i.e.*, fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS)], and polyols (*e.g.*, sorbitol), the so-called Fermentable Oligo- Di- and Mono-saccharides And Polyols or FODMAPs, the prototype of which is inulin, a non-digestible carbohydrate naturally present in a large variety of plants that, when enzymatically hydrolyzed, produces oligofructose<sup>[252]</sup>. All of them occur in many fruits, cereals, vegetables and in human milk, which contains more than 1000 oligosaccharides<sup>[253]</sup>. Furthermore, long-chain polyunsaturated fatty acids have been tested as active prebiotics<sup>[254]</sup>.

Experimental data<sup>[255,256]</sup> and human studies have shown a beneficial effect of prebiotic supplementation in different pathological conditions, including infections<sup>[254]</sup>, allergies<sup>[257,258]</sup>, pregnancy-related disorders<sup>[259-260]</sup>, metabolic disorders<sup>[261,262]</sup>, hepatic and gastrointestinal diseases including cirrhosis<sup>[263]</sup>, IBD<sup>[264]</sup> and chronic constipation<sup>[265]</sup>. However, few studies have evaluated the efficacy of prebiotics in IBS and existing

results are conflicting. Two trials, using oligofructose in one case<sup>[266]</sup> and fructo-oligosaccharides in the other<sup>[267]</sup>, failed to confirm any beneficial effects of prebiotics, while two other studies have demonstrated symptoms improvement, with FOS ameliorating symptoms<sup>[268]</sup> and GOS lowering flatulence and bloating while also improving the anxiety score<sup>[83]</sup>. The clinical response may be dependent on the prebiotic type and dose, taking into account that low doses may be ineffective and high doses may stimulate colonic gas production. Indeed, some reports indicate that prebiotics may exacerbate IBS symptoms. Thus, lactulose and bran, that are successfully used for constipation, tend to produce substantial amounts of gas and abdominal pain and may aggravate symptoms of IBS<sup>[269-271]</sup>. Although fructose and sorbitol mixtures produce a modest increase in stool weight, they often increase flatulence and bloating in both healthy volunteers and IBS patients<sup>[272,273]</sup>. Ong *et al.*<sup>[274]</sup> have recently shown that a diet rich in FODMAPs increases abdominal pain, bloating and flatulence in IBS patients, focusing the attention on the necessity of restricting fermentable carbohydrates in these patients<sup>[275-277]</sup>. Finally, fructose, sorbitol and a range of poorly absorbed polyhydric alcohols enter the gut where they retain a substantial amount of water causing unwanted diarrhea. However, low FODMAP diet recommended for reduction of IBS symptoms will have adverse effects on colonic luminal microenvironment and microbiota in healthy populations, reducing the absolute and relative bacterial abundance and diversity<sup>[278]</sup>.

## **SYNBIOTICS**

An important safety feature of probiotics is that they have a short lifespan within the gut and need repeated doses to keep a constant level. Indeed, an alternative strategy to maintain constant levels of beneficial bacteria within the gut is the contemporary administration of probiotic strains and prebiotics, the so-called synbiotic therapy. Few open label trials<sup>[64,279]</sup> and PCT studies have evaluated the efficacy of synbiotics in both functional pain<sup>[280]</sup> and IBS, all demonstrating a superiority of the probiotic/prebiotic combinations vs placebo<sup>[281-283]</sup>. Although the concept of combining prebiotic with probiotic is theoretically attractive, the limited experience and the poor quality of published studies do not allow any recommendation on their use in IBS.

## **ANTIBIOTICS**

The alteration in the gut microbiota composition is increasingly considered to be a relevant pathogenetic factor in IBS, leading to the use of antibiotics as a treatment for this multifactorial syndrome. In addition, a SIBO may be relevant to at least a subset of IBS patients, thus justifying an antimicrobial approach.

However, it should be considered that lactose hydrogen breath test, routinely used as a surrogate marker for SIBO, has a low sensitivity and specificity<sup>[284-286]</sup>. The first antibiotic investigated in a clinical trial was neomycin, which is not adsorbed in the gut. Pimentel *et al.*<sup>[285]</sup> have demonstrated that neomycin caused a 50% improvement in global IBS symptoms compared to placebo-treated patients, but also induced a rapid clinical resistance. Other broad-spectrum antibiotics reducing bacterial overgrowth have been challenged in the treatment of IBS, including tetracycline, amoxicillin clavulanate, metronidazole and fluoroquinolones such as norfloxacin<sup>[287,288]</sup>; however, these drugs are absorbable causing local and systemic side effects and their use in IBS patients is not recommended. The semisynthetic, antibacterial, rifamycin-derivative rifaximin has virtually no systemic absorption, is effective in improving IBS symptoms with low bacterial resistance profile and has a favorable side-effects profile. Since the first trial published almost a decade ago<sup>[289]</sup>, several trials have shown that rifaximin is an effective treatment for IBS. The strongest evidence comes from two large-scale, multicenter studies: TARGET 1 and TARGET 2<sup>[290]</sup>, which included a total of 1260 patient affected by IBS without constipation. In these phase 3, double-blind, placebo-controlled trials, significantly more patients in the rifaximin group than in the placebo group had adequate relief of global IBS symptoms during the first 4 wk of treatment (40.7% vs 31.7% respectively in the two studies combined). In addition, more patients in the rifaximin group than in the placebo group had adequate relief of bloating, abdominal pain and stool consistency (40.2% vs 30.2% respectively in the two studies combined). The incidence of adverse events was similar. In general, available metanalysis tend to show a beneficial profile of rifaximin vs placebo on global IBS symptoms and bloating<sup>[291]</sup>. Furthermore, a pooled analysis by Schoenfeld *et al.*<sup>[292]</sup> has confirmed the safety and tolerability profile of rifaximin in comparison with placebo, with similar incidence of drug-related adverse events. In conclusion, rifaximin is the only antibiotic which can play a role in the treatment of IBS, but limitations should be considered: (1) it is effective in less than 50% of patients; (2) large phase III study has included only patients without constipation, indeed studies on C-IBS are needed; (3) its long-term effects have not been investigated; and (4) the effect of rifaximin on gut microbiota composition is essentially unknown.

## FECAL TRANSPLANTATION IN IBS: A NEW LIFE FOR AN OLD TREATMENT?

Fecal microbiota transplant (FMT) is an interesting strategy proposed for a large spectrum of diseases<sup>[293]</sup>, including recurrent *Clostridium Difficile* infection (CDI) resistant to conventional antibiotic therapies (that

represents the main gastroenterological indication for FMT), chronic constipation, IBD, recurrent metabolic syndrome, multiple sclerosis, autism and chronic fatigue syndrome. A systematic review of 325 cases of FMT for CDI suggested a lower success rate for upper gut administration (76%), as compared with colonoscopy (89%) and enema (95%) administration<sup>[294]</sup>. Moreover, a large case series of CDI patients has showed rapid response and a cure rate of nearly 90%, without significant adverse event rate<sup>[295]</sup>. Colonic infusion of donor human intestinal flora can reverse UC in a small series of selected patients, determining endoscopic and histological remission<sup>[296]</sup>. This report was followed by a number of small studies of FMT in children and adults with UC, CD, and pouchitis with mixed results<sup>[297-302]</sup>. It has been recently demonstrated that sensitivity to colonic distension of IBS patients can be transferred to rats by the fecal microbiota transplant<sup>[303]</sup>.

From a pure technical point of view, FMT is an easy technique that requires a healthy donor (usually a patient's family member or an anonymous donor), with no risk factors for transmissible diseases or any issues that may alter the cellular composition, particularly prior antibiotic use. The FMT Working Group have recently published guidelines for FMT donor selection criteria and screening tests<sup>[304]</sup>. The steps for an adequate treatment of the fecal material include dilution in saline solutions, homogenization and filtration, while the route of administration can be naso-duodenal, transcolonoscopic or enema based. Naso-duodenal administration is the method patients favour the most. Colonoscopy allows direct assessment of the colonic mucosa for the assessment of disease severity and exclusion of coexistent pathology. Enema administration is effective, cheap and safe and carries less procedural or institutional admission costs.

To date, only anecdotal data have been reported about the efficacy of FMT for IBS treatment, results being far conclusive. The first case series on FMT in IBS has been published in 1989 by Borody *et al.*<sup>[305]</sup> demonstrating approximately a 50% of relief in symptoms of IBS after FMT; however, the study also included IBD and CDI patients and did not show any distinction within the results between these different diseases. Since that publication, the lead Author has treated with FMT more than 300 D-IBS patients whose symptoms had failed to respond to conventional therapies<sup>[306]</sup>. Other preliminary studies indicate a beneficial effect in patients with chronic constipation (*i.e.*, relief in defecation and reduction of bloating)<sup>[307]</sup>, but these data need confirmation in rigorous clinical trials. Pinn *et al.*<sup>[308]</sup> have reported promising results using FMT in IBS patients with refractory disease: 70% of the patients achieving resolution or improvement of the symptoms. Taken together, these data indicate that FMT is a promising treatment option for serious and recurrent CDI<sup>[309,310]</sup>. However, many questions



should be answered before it may be recommended as routine standard treatment of IBS<sup>[31]</sup> and randomized, double-blinded, placebo-controlled trials are required.

## CONCLUSION

Evidence regarding the manipulation of gut microbiota composition as an effective cure for IBS is increasing and, to date, probiotic supplementation and antimicrobial therapy with not absorbable antibiotics are promising treatment. However, all meta-analysis point out to the weakness of the majority of the studies and recommend additional RCT trials to confirm the positive findings reported by small studies. Specifically, additional information on type of probiotic, doses, side effects and time of administration are required, as well as data on patient subtypes. Prebiotics are often burdened by unwanted side effects and synbiotics lack of sufficient numbers of clinical trials on their efficacy and safeness. FMT might be a reasonable option for treating IBS, as it is an inexpensive and easy treatment, but standardized controlled trials are necessary to ascertain which patients are eligible, the most effective regiment as well as the most acceptable method of administration of the donor's microbiota. For these therapeutic options, a careful selection of patients, a close monitoring of clinical data and side effects and a long-term follow-up are necessary, as well as more information on modification of host microbiota composition.

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