Gut Microbiota as Potential Orchestrators of Irritable Bowel Syndrome

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Irritable bowel syndrome (IBS) is a multifactorial functional disorder with no clearly defined etiology or pathophysiology. Modern culture-independent techniques have improved the understanding of the gut microbiota’s composition and demonstrated that an altered gut microbiota profile might be found in at least some subgroups of IBS patients. Research on IBS from a microbial perspective is gaining momentum and advancing. This review will therefore highlight potential links between the gut microbiota and IBS by discussing the current knowledge of the gut microbiota; it will also illustrate bacterial-host interactions and how alterations to these interactions could exacerbate, induce or even help alleviate IBS. (Gut Liver 2015;9:318-331)

Key Words: Irritable bowel syndrome; Microbiota; Immunity; Dysbiosis; Probiotics

INTRODUCTION

Patients with functional bowel disorders (FBDs) have no clear structural or biochemical alterations on routine examinations, making diagnosis and treatment challenging. A number of FBDs affect the lower gastrointestinal (GI) tract with irritable bowel syndrome (IBS) being the most prevalent, affecting approximately 10% to 20% of the population in the Western world.1-3 IBS is characterized by abdominal discomfort or pain associated with disturbed bowel habits,4 but also other GI symptoms such as distension and bloating, with patients often reporting more stress and anxiety than the general population.5 Recent data supports the notion that there is a link between bacterial composition and gut wellbeing, therefore this review article will focus on gut microbiota in relation to IBS.

THE IMPORTANCE OF MICROBIOTA FOR GUT HOMEOSTASIS

Regarded by some as a neglected organ,6 the GI microbiota comprises around 400 species and greatly outnumber the cell count of all other established organs combined.7,8 Commensal bacteria are seen to be necessary in healthy digestion, with roles such as producing enzymes and metabolites which help the body absorb otherwise unavailable essential nutrients and vitamins.9,10 Presence of bacteria is also important for normal development and function of the intestinal immune system which must be both tolerant to food antigens and commensal bacteria, but also able to mount a response to pathogens.11,12 Commensal bacteria also contribute to the maintenance of gut homeostasis by the secretion of bacteriocins,13 proteins that are able to inhibit bacterial toxins,14 and the pH lowering short-chain fatty acids15-17 which withhold an aggressive defence against colonization by noncommensal intruders. Finally, by outcompeting for resources and filling distinct colonization niches,18 commensal microbiota are able to block pathogenic organisms from gaining an all-important foothold in the intestinal microbiota ecosystem.

Generally, the intestinal microbiota composition of healthy individuals is relatively stable; however, changes in the microbiota community may lead to a permanent imbalance known as dysbiosis.19 Several factors, such as antibiotics, diet (including specific probiotic and prebiotic consumption), the host immune system and acidic milieu have been seen to affect the microbiota composition of the gut (Fig. 1). Disturbances to the gut microbiota ecosystem resulting in dysbiosis can lead to maladies of the GI tract20,21 with current research suggesting dysbiosis to have potential significance in IBS, but also other conditions such as obesity,22,23 diabetes,24,25 metabolic syndrome,26 cardiovascular disease,27 and IBD.28

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Fig. 1. Factors that might influence the composition of the gut microbiota. Consumption of antibiotics, prebiotics, and probiotics as well as dietary habits have been shown to affect the species of microbiota residing in the gut. These factors will generally dictate which species will become more prevalent. If an expansion of beneficial species occurs through probiotic or prebiotic use, the local pH level is likely to be altered by the beneficial commensals as a means to hamper pathogenic proliferation. Detrimentally, a bout of gastroenteritis in itself alerts the immune system which employs means to remove the offending species. However, the administration of antibiotics in an attempt to solve the problem has potential side effects by depleting levels of commensal microbiota, thus resulting in an opening for nonbeneficial microbiota to establish themselves.

SMALL INTESTINAL BACTERIAL OVERGROWTH IN IBS

In a healthy individual the small intestine contains a much lower density of bacteria than the large intestine. IBS has been suggested to be associated with small intestinal bacterial overgrowth (SIBO), defined as a bacterial density (colonial bacteria) of ≥10^3 colony-forming units (cfu) per mL of intestinal fluid, measured by the "gold standard" jejunal culture method. SIBO is, however, often diagnosed through culture-independent techniques such as glucose hydrogen and lactulose hydrogen breath tests. There has been much deliberation over these studies and the findings due to the validity of the techniques used. A meta-analysis study by Ford et al. concluded that although SIBO was seen in IBS patients at a higher rate when compared to controls, the prevalence to which this occurred varied substantially between studies and centres. Due to such discrepancy, the true influence SIBO has with regards to IBS symptom generation is still unclear.

POSTINFECTIOUS IBS

Postinfectious IBS (PI-IBS) is likely the strongest evidence in the case of microbiota being important for the development of IBS, and may present after a bout of gastroenteritis caused by viral, parasitic or bacterial infections. Enteric pathogens such as Staphylococcus aureus, Shigella, Clostridium perfringens, Bacillus cereus, and Campylobacter species are potential culprits of PI-IBS, and could increase the risk of developing PI-IBS by at least six-fold. Other variables such as severity and duration of initial infection, as well as age and gender are additional risk factors of developing PI-IBS, with risk of development being higher amongst young females.

An episode of gastroenteritis will cause an inflammatory response of the gut, and may potentially lead to an intestinal dysbiosis. For example, C. jejuni and Shigella infections cause detrimental depletion of intestinal macrophages, which could potentially hamper the clearance of pathogens. Additionally, enterohemorrhagic E. coli have deleterious impacts on the epithelial barrier, which raise the risk for bacterial breach to the epithelial mucosa and subsequent inflammation. Thus, a previous gut infection may temporally cause changes to the immune system as well as the balance of the gut microbiota, resulting in an opening for nonbeneficial microbiota to establish themselves.

MICROBIOTA AND IMMUNITY IN IBS

The interplay of intestinal microbes and host immunity being widely acknowledged in promoting a normally functioning immune system is strengthened with studies suggesting that an altered gut microbiota composition may lead to an altered immune activity, potentially leading to low-grade inflammation in IBS. The putatively increased immune activity in IBS patients may be due to exogenous or endogenous triggers; however, the immune activity pattern of IBS is far from being fully understood and likely involves both the innate and adaptive immunity.

1. The innate immune system

Having a fundamental role in the innate immune system, mast cells are on the front line barrier between the host and the external environment. Numerous studies have reported an increase in number, level of activation and area occupied by mast cells in the intestinal mucosa of IBS patients when compared with healthy controls although other studies are not in agreement. These discrepancies potentially arise through methodological inconsistencies such as from which intestinal region the biopsy was taken from and the techniques used for detection and counting of the cells. Considering how intertwined the gut microbiota and immune system are, and the reports of increase in mast cell numbers in IBS, it could be reasoned that a shift in gut microbiota composition could mediate an immune response leading to a subsequent mast cell increase, potentially contributing to visceral hypersensitivity. However, there is still no evidence supporting an association between an altered microbiota composition and levels of mast cells in IBS, so this putative link remains to be determined.

Another aspect of innate immunity is the phagocytic macrophages. Currently, the number of macrophages is still under discussion with reports of increased and decreased levels of this cell population seen in IBS. Potentially, for a subgroup of IBS patients, it could be proposed that the immune system may
be compromised and therefore less capable to respond to pathogenic microorganisms. This theory is supported by the report of decreased levels of secreted chemotactrants such as CXCL-9 and MCP-1, 74 known to recruit dendritic cells and other immune cells. However, data on the expression of intestinal chemotactrants is also under discussion as an elevation of MCP-1 has also been reported in IBS. 75 Moreover, increased levels of proinflammatory serum cytokines such as interleukin (IL)-6, IL-8, and tumour necrosis factor α, tend to be found in IBS patients 61,75-79 once again evoking the idea of discord in the gut microbiota subsequently provoking an immune response to tackle any pathogenically caused disturbances.

Widely expressed by many cells, including but not limited to epithelial cells and macrophages are Toll-like receptors (TLRs). TLRs are utilized in the recognition of danger signals such as structures found on bacteria and viruses. Studies showing altered TLR expression in IBS patients compared to healthy controls 80 with specific increases in TLR2, TLR4, and TLR5 80-83 and decreases in TLR7 and TLR8, 84 present the notion that IBS is linked to an altered activation of the immune system in response to microorganisms of the gut. Also, defensins such as human β-defensin 2, antimicrobial peptides secreted by colon epithelial cells in response to proinflammatory cytokines or pathogenic microorganisms, have been shown to be increased in IBS patients. 84

2. The adaptive immune system

Following the hypothesis of a low-grade immune activation occurring in IBS, the T cells of the adaptive immune system have been suggested to have an increased presence in IBS patients 87,90-92 and subgroups such as PI-IBS, 93 however, a contradictory study by Braak et al. 94 suggests T cells to be decreased in IBS patients. Similar to an increase in activity of the innate immune system regarding IBS, an increased presence or activation of T cells may contribute to symptom generation. Therefore, the increased frequencies of blood T cells expressing activation markers such as CD69 and HLA-DR as well as the gut homing integrin α4β7, suggests that IBS patients display an activated T cell phenotype 87 compared to healthy controls. Further, an increase in serum antibodies against flagellin has been seen in PI-IBS compared to non-PI-IBS and healthy controls. 88,90 Also, our group has reported comparable levels of B cells in blood of IBS patients and healthy controls, although the frequency of B cells expressing IgG, indicating an activated status of the B cells, was increased in the patients. 91 Furthermore, after bacterial cocktail stimulation of B cells, an impaired expression of costimulatory ligand CD80, thought to lead to restriction of T cell activation, 92 was seen. This suggests that an altered B cell expression of costimulatory molecules could be a potential cause for the increased T cell numbers reported in IBS patients. 91

Still, the evidence linking an increased immune activity and altered gut microbiota composition in IBS is limited 92 and further research is therefore required.

GUT MICROBIOTA RELATED THERAPY AND TREATMENT OF IBS

1. Antibiotics

There is no clear consensus on the benefit of antibiotics to treat symptoms in IBS; however, several studies show that antibiotics alter the composition of the gut microbiota in a potentially deleterious way. 94-97 The reported disruptive effect of antibiotics can diminish protective commensal bacteria populations, making it more likely for expansion of pathogenic species to occur, 98,99 which may lead to dysbiosis and potentially even cause symptoms of IBS. 100-103 However, nonabsorbable antibiotics such as neomycin 104 and rifaximin 105-107 have been seen to have beneficial effects, providing partial alleviation of IBS in general and bloating in particular. Findings showing reduction in IBS symptoms through the use of antibiotics further support the influence microbiota has on gut wellbeing and how the restoration of intestinal microbial normobiosis may help some patients with IBS.

2. Probiotics

The act of directly altering gut microbiota composition through the use of probiotics such as Bifidobacterium spp. and Lactobacillus spp. 108 has been shown to have a positive effect on the symptoms of IBS, though this is not always the case. 115-117 Most studies report no adverse events with the use of probiotics; however, these claims are often not well documented and inconsistent, with some studies even recording worsening of patient discomfort. 115-117 Although probiotics may be beneficial in a subset of IBS patients, further research is required in order to elucidate the full efficacy as well as potential side effects, relevant strain or species cocktail 118 and optimal dose in order to gain the full therapeutic effects in IBS patients. 119

3. Prebiotics

If probiotics are like adding seeds of beneficial bacteria to the gut, prebiotics are the equivalent of fertilizers which affect only the favorable species already colonising the bowels. Commonly used and tested prebiotics are nondigestible oligosaccharides such as fructo-oligosaccharides and galacto-oligosaccharides. Currently, there have not been many randomized controlled trials regarding IBS and prebiotics. Supporting the link between microbiota composition and gut wellbeing, studies on prebiotic use and subsequent mitigation of IBS symptoms tend to show a similar beneficial effect as the use of probiotic strains such as Bifidobacterium spp. and Lactobacillus spp. 120-124 Findings from the aforementioned studies show that the benefit from such treatment is strongly dependant on dose, whereby high levels of prebiotics were revealed to counteractively intensify problems such as bloating and flatulence. 121 These results corroborate with
up and coming research recommending a reduction of FODMAPs (fermentable oligosaccharides, disaccharides, monosaccharides, and polyols) intake for people suffering with IBS.159,160

4. Diet

The composition of the gut microbiota has been shown to be responsive and adaptable to the diet of the host organism.127-131 Since anything not absorbed by the host becomes a source of nutrients for the microbial community residing in the colon, differences have been seen in gut microbiota in favor of those species able to best adapt to and metabolise the primary nutrients like fat, protein, or carbohydrates present in the diet.132 This adaptability thus denotes the types and levels of metabolites produced, e.g., butyrate133 or methane134 and consequent promotion of gut health or IBS symptoms, respectively. Although there is no recommended IBS diet, a reduction in FODMAPs might subdue symptoms associated with IBS.134-137

In conclusion, the findings with regards to therapeutic methods for IBS, e.g., antibiotics, probiotics and prebiotics and restoration of the gut microbiota, adds to the rationale behind the suggested correlation of altered gut microbiota and IBS.138-140 Several studies suggest that fecal microbiota is altered in IBS, and present differences in microbiota composition between healthy controls and IBS patients as well as within the subgroups of IBS patients.141-146 Although the aforementioned endeavours141-146 are interesting and a definite step towards a better understanding of IBS from a microbiological point of view, it must be taken into consideration that the results are usually based on relatively small sample populations. Considering that IBS is a multifactorial disorder with many putative causes and broad symptom presentation, a conjecture could be made that results derived from these studies might not represent the IBS patient population as a whole, but rather a subgroup of patients. Also, microbiota can vary quite extensively even between healthy individuals, making a general inference on the microbiota composition of IBS, let alone the subgroups, a difficult one.

The most easily obtainable material when sampling GI microbiota is fecal matter. For this reason it is used prevalently in gut microbiota research, as well as the less easily obtainable mucosal biopsies. Taken from various locations of the small and large intestine, biopsies can provide a more site specific view of the mucosa adherent microbes inhabiting the gut.

CURRENT KNOWLEDGE ABOUT MICROBIOTA COMPOSITION OF PATIENTS WITH IBS

Today, over 50 bacterial phyla have been defined147 with only 29 of these possible to culture.148 Ten of the known phyla have been discovered in the human gut149 with the majority of species attributed to one of the two largest phyla colonizing the human

| Table 1. Overview of Papers Studying the Microbiota of the Gut with Regards to Irritable Bowel Syndrome |
|-----------------------------------------------|-----------------------------------------------|
| Phyla                                          | Genus                                          | IBS against healthy                          |
| Firmicutes                                     | Lactobacillus                                   | † Tana (2010)154; Carroll (2010)155; Carroll (2011)156 |
|                                               |                                               | ↓ Balsari (1982)157; Kassinen (2007)158       |
|                                               |                                               | ↔ Si (2004)159; Malinen (2005)139; Kerckhoffs (2009)160; Rajilić-Stojanović (2011)161 |
|                                               | Streptococcus spp.                              | † Kassinen (2007)162; Rajilić-Stojanović (2011)163 |
|                                               | Dorca spp.                                      | ↑ Saulnier (2011)164; Rajilić-Stojanović (2011)165 |
|                                               |                                               | Kassinen (2007)166                          |
|                                               | Ruminococcus spp.                               | ↔ Rajilić-Stojanović (2011)167; Jalanka-Tuovinen (2014)168 |
|                                               | Eubacterium                                     | ↔ Lopez-Siles (2014)169                      |
|                                               | Faecalibacterium                                |                                               |
| Actinobacteria                                 | Bifidobacterium                                 | † Kassinen (2007)170                         |
|                                               |                                               | ↔ Balsari (1982)171; Si (2004)172; Kerckhoffs (2009)173; Rajilić-Stojanović (2011)174; Malinen (2005)175; Duboc (2012)176 |
| Bacteroidetes                                  | Bacteroides spp.                                | † Jalanka-Tuovinen (2014)177                |
|                                               |                                               | ↓ Compared to IBD Swidsinski (2005)178       |
| Proteobacteria                                 | Escherichia                                     | † Rajilić-Stojanović (2011)179; Duboc (2012)180; Si (2004)181 |
|                                               | Desulfovibrio spp.                              | ↓ Malinen (2005)182                          |
| Verrucomicrobia                                | Akkermansia                                     | ↑ Saulnier (2011)183                         |
|                                               |                                               | ↔ Rajilić-Stojanović (2011)184               |
| Euryarchaeota                                  | Methanobrevibacter                              | ↑ Rajilić-Stojanović (2011)185; Jalanka-Tuovinen (2014)186; Rana (2009)187 |
gut, Firmicutes and Bacteroidetes. Notably, since the advent and prominent use of culture-independent methods such as 16S sequencing in the last decade, numerous studies have focused on determining the gut microbiota in IBS patients. Table 1 gives a brief overview of the current findings on gut microbiota composition in patients with IBS in comparison to healthy controls with a more detailed review presented below.

1. Firmicutes

The most predominant phylum found in the gut microbiota of healthy individuals,\textsuperscript{159,160} the gram positive Firmicutes, consist of a number of genera which include the commonly known probiotic, Lactobacillus spp.\textsuperscript{106} As early as 1982\textsuperscript{112} studies have presented lowered levels of Lactobacillus spp. within IBS patients compared to healthy controls. However, studies are inconsistent in their results, presenting an increase,\textsuperscript{52,159,151-156} decrease,\textsuperscript{141} or no change\textsuperscript{19,152} in the prevalence of lactobacilli spp. within IBS patients. Also, comparisons between IBS subgroups and healthy controls show either decreased or normal abundance of Lactobacillus\textsuperscript{150,161} in the patients, thus it is still unclear if there are any IBS-subgroup-specific related effects of Lactobacillus. Regardless if Lactobacillus is occurring naturally or from probiotic administration its ability in alleviating symptoms of IBS such as visceral pain,\textsuperscript{108,162} make probiotics, i.e., Lactobacillus, to be of potential benefit to some IBS patients.

Conversely, there might be a positive correlation between some potentially pathogenic species within the phylum of Firmicutes, such as Streptococcus spp. and IL-6 increase in IBS patients.\textsuperscript{142} This finding makes the significantly elevated levels of Streptococcus spp. seen in IBS patients\textsuperscript{141,161} particularly interesting and could potentially serve as evidence of the link between dysbiosis, occurring primarily from specific bacterial genera colonisation, and an altered immune activity in some IBS patients.

Within the Clostridia class, conflicting results have been reported with both an observed increase\textsuperscript{152} and decrease\textsuperscript{142,155,164} in IBS patients. Specifically, Ruminococcus spp. as well as Dorea spp., have both been shown to be increased in patients suffering with IBS.\textsuperscript{141,156,165} although no subgroup preferences have been defined. Interestingly, Rajilić-Stojanović et al.\textsuperscript{161} suggest the nonbutyrate producing phylotypes of Clostridium Group XIVa, related to R. gnavus and R. torques, known mucin degraders,\textsuperscript{156} to be indicators of IBS. These phylotypes are consistently found at elevated levels in IBS fecal samples,\textsuperscript{165} and also found in higher abundance in diarrhea predominant IBS\textsuperscript{167} and PI-IBS,\textsuperscript{142} conversely, however, in lower abundance in alternating IBS.\textsuperscript{151}

Unlike R. gnavus and R. torques other members of Group XIVa, e.g., Roseburia spp.\textsuperscript{160} produce beneficial short-chain fatty acids, such as butyrate. Butyrate is a preferred energy source for colonic epithelial cells and is suggested to reduce inflammation.\textsuperscript{140} Moreover, butyrate helps to maintain normal intestinal barrier function, through regulation of colon epithelial mucin gene MUC2,\textsuperscript{170} and tight junction proteins,\textsuperscript{171} respectively, and might therefore have therapeutic effects in IBS patients.\textsuperscript{172} Although inconsistent, reduced levels in IBS patients\textsuperscript{151,154,173-175} of the butyrate producers Eubacterium, Faecalibacterium and Roseburia spp., known to inhibit the growth of potentially pathogenic species including Campylobacter spp., Salmonella spp., Shigella spp., and E. coli,\textsuperscript{176} could potentially be an ancillary cause for IBS symptom generation in some patients.

At this time, there is no clear consensus on the significance of alterations of Firmicutes in all IBS patients, although evidence suggests that Firmicutes, specifically the family Lachnospiraceae, are increased significantly enough in IBS-D as to make it discernible from other IBS subgroups.\textsuperscript{145} Nevertheless, although no consensus has been agreed upon, a weak tendency for a reduction in the beneficial bacteria of the gut, countered with an increase in pathogenic species is seen in IBS patients. This dysbiosis may potentially have influence on gut function whereby a degradation of the mucus layer by Ruminococcus spp. may allow infiltration of Streptococcus spp. or Staphylococcus aureus,\textsuperscript{43} thus provoking low-grade immune responses in a subgroup of IBS patients.\textsuperscript{177}

2. Bacteroidetes

The second most abundant phylum in the human gut, gram negative Bacteroidetes are found to have a varying higher\textsuperscript{151,154,161} or lower\textsuperscript{143,165,161,178} presence and diversity in the gut microbiota of patients with IBS. Furthermore, increases in Bacteroides spp. have been reported in IBS\textsuperscript{142} although a net decrease in the Bacteroidetes phylum has also been observed.\textsuperscript{141} Our group has demonstrated that a majority of IBS patients had an altered microbiota composition with an increased abundance of Firmicutes and subsequent decrease in Bacteroidetes, whereas the remaining patients had a normal-type gut microbiota composition.\textsuperscript{145}

The Bacteroidetes phylum harbours species with either beneficial or nonbeneficial traits, as shown in the comprehensive review by Wexler.\textsuperscript{179} The abundance of beneficial or nonbeneficial Bacteroidetes species may therefore be important to IBS, under the assumption that more nonbeneficial species might correlate with an increase in symptoms or severity, such as visceral pain.

3. Actinobacteria

Another of the main phyla of the human gut microbiota, the gram positive Actinobacteria includes the probiotic containing genera such as Bifidobacterium and Collinsella. Interestingly a decrease in Actinobacteria has been shown in the gut of patients with IBS\textsuperscript{161} with one study presenting a specific decrease among IBS-D patients.\textsuperscript{142} Notably, the reports on levels of the ubiquitous and recognised probiotic of the GI tract, Bifidobacterium spp., known to aid the gut mucosal barrier,\textsuperscript{181} are mixed. IBS patients are demonstrated to have increased\textsuperscript{156,159,173} or reduced\textsuperscript{156,159,173} levels of bifidobacteria. Although a specific
reduction of *B. catenulatum* has been seen in IBS in some studies, 159,160 Rajilić-Stojanović et al. 161 suggested that it is in fact *B. pseudocatenulatum* which is significantly reduced in IBS. Some studies propose an increase in bifidobacteria in IBS; however, the overall inconsistency suggests that there may potentially be a subgroup of IBS patients where the beneficial traits of these probiotic species might be diminished which could account for symptom generation, this requires further research.

4. Proteobacteria

Increases of Proteobacteria 165 in IBS, notably IBS-D, 143 of the specific family Enterobacteriaceae155,158,151,172 which encompasses many gram negative pathogenic species, including other coliform bacteria151 such as *E. coli* 155 with potential inflammation causing mechanisms, have been associated with IBS. Nevertheless, Malinen et al. 159 recorded lower amounts of sulphate-reducing bacteria, *Desulfovibrio* spp. in IBS-D patients. When considering that species in this genus produce toxic sulphide, one would expect an increased abundance of *Desulfovibrio* spp., rather than reduced levels, as a plausible explanation for symptom generation in some IBS patients.

Since being found in both healthy controls 159 and IBS patients 159 there is no consensus on altered abundance of known, potentially pathogenic, genera such as *Campylobacter* spp. and *Helicobacter* spp. within IBS. Looking at the findings of pathogenic Proteobacteria alone, one could infer that an increase in pathogens could be a single element in the onset of symptoms in a subgroup of IBS patients. Thus, the increase of pathogenic species together with the previously mentioned reduction in probiotic species from genera such as *Bifidobacterium* and *Lactobacillus* and their metabolites, known to keep pathogens at bay, further support that a dysbiosis is occurring in a number of IBS patients.

5. Verrucomicrobia

Increased levels of the not so well documented *Akkermansia* spp., 151 may coincide with IBS. Since its known specialization for mucus degradation 161 *Akkermansia* spp. could compromise the integrity of the mucus layer, and thus hamper the intestinal barrier in the gut. From this assumption, *Akkermansia* spp. may instigate a low grade inflammation in some IBS patients through degradation of the mucus layer, similar to *R. gnarus* and *R. torques*, enabling entry of pathogenic species to the epithelial mucosa.

6. Euryarchaeota

The methane generating archaea Methanogens convert hydrogen produced in the gut into methane. This gas was previously thought to be inert, 185 but has now been shown to reduce gut transit. 131,183 An increase of Methanogens in IBS, especially in those suffering of constipation predominant IBS, could possibly explain the slow gut transit in these patients and why methane is being found at increased volumes in IBS-C patients. 184-186

Also, *Methanobrevibacter smithii* has been suggested to be the predominant Methanogen in IBS-C. 186 These findings support the proposition that at least in some IBS-C patients a dysbiosis favoring Methanogens is potentially occurring which may be causing constipation. However, it might also be hypothesised that a reduction of Methanogens could potentially explain bloating symptoms in IBS, as hydrogen levels would not be reduced as efficiently. Studies which include Methanogens in their research present abundant levels of Methanogens in both healthy controls and IBS patients. 143,151,185,187 Since an abundance of Methanogens is found also in healthy controls, further research is needed to explore the importance of Methanogens for generating IBS symptoms.

### DIVERSITY AND LONG-TERM INSTABILITY OF GUT MICROBIOTA IN IBS

A temporal decreased stability of gut microbiota leading to a state of flux and dysbiosis may provide an explanation for the characteristic symptoms of certain IBS subgroups. The gut microbiota has the potential to affect and also be affected by the physiology of the gut, thus there is bidirectional communication. There are many factors which can potentially alter the normal gut microbiota composition. The use of antibiotics to treat infection is likely to have the side effect of depleting commensal bacterial, thus unintentionally allowing for later pathogenic infiltration of the gut. The consumption of probiotics and prebiotics, however, serve to increase beneficial bacteria of the gut. These beneficial bacteria employ various mechanisms, such as pH regulation, which alters their surroundings as to hinder the growth of noncommensals. The immune system works constantly in order to keep microbial homeostasis. Through this maintenance, alterations to the gut microbiota community occur by removing potentially pathogenic species. Additionally, diet can shape the composition of the gut microbiota through the shift in bacterial species which occurs when a gradual or radical change occurs in the food consumed by the host (Fig. 1). As levels of bacterial species fluctuate to adapt to these changes the shift may eventually favor certain species whereby a subsequent population expansion would likely occur. For example, an expansion of Methanogens in IBS could be linked to symptoms of IBS-C. 160 A few studies have investigated gut microbiota stability through DNA 157 and RNA analysis, 164 showing that IBS patients have an instability of the gut microbiota composition over time as compared to healthy controls. 157 Importantly, it must be acknowledged that the temporal fluctuation in at least some IBS patients, and healthy individuals for that matter, may be partially attributed to the administration of antibiotics. 157

Several studies have demonstrated that IBS patients may have a diminished diversity of the gut microbiota composition, 159,155,178,188 although, when focusing on specific groups such
as Bacteroidetes and Lactobacillus, a broader diversity has been observed in IBS.\textsuperscript{52} In the gut microbiota ecosystem, much like in any other ecosystem, diversity and species richness is required for the system to flourish, where by all niches are filled and kept in check by neighboring beneficial or competing species. However, further studies with larger cohorts and potentially longer time periods are required in order to further investigate gut microbiota composition, instability and diversity in IBS.

**GUT MICROBIOTA AND THE LINK TO IBS SYMPTOMS**

Patients with IBS suffer from symptoms such as pain, constipation, diarrhea, abdominal distension, bloating and even psychiatric problems. The extent to which the gut microbiota influences these symptoms is not fully understood, especially not mechanistically. Interestingly, negatively correlation between beneficial species of the gut and IBS symptoms have been demonstrated\textsuperscript{101} with Rajić-Stojanović \textit{et al.}\textsuperscript{161} reporting a reduction in \textit{Faecalibacterium} spp., producing the anti-inflammatory metabolite butyrate, being associated with an increase in IBS symptoms. Conversely, yet equally as expectable, was a positive correlation between various Firmicute and Proteobacteria species and IBS symptoms.\textsuperscript{161} Also, an increase of \textit{R. torques} has been associated with increased symptom severity such as emotional function, social function, systemic symptoms and bowel symptoms.\textsuperscript{177} Concordantly, IBS patients with \textit{R. torques} in their fecal samples tended to present more frequently with self-reported symptoms.\textsuperscript{177} Moreover, our group has previously discovered that psychological symptoms such as clinically significant depression had a lower prevalence in patients with an altered microbiota profile, i.e., an increase of Firmicutes and subsequent decrease in Bacteroidetes, compared to those with normal microbiota composition.\textsuperscript{161} Additionally, Parkes \textit{et al.}\textsuperscript{161} links altered, though primarily lower, bacterial mass and diversity to increased symptom severity, related to stool frequency, anxiety and pain. Although not fully understood, studies in rats have suggested that fecal bacteria, through specific bacterial metabolites such as sulphides, induce visceral hypersensitivity, which is often demonstrated in IBS patients.\textsuperscript{189} In conclusion, although gut microbiota might explain symptom generation in only a subgroup of IBS patients, this nevertheless helps towards a richer understanding of how symptoms of IBS might occur.

**SUMMARY**

Over the past decade the importance of gut microbiota in IBS has drawn increasing attention. Growing evidence suggest that at least subgroups of IBS patients have an altered gut microbiota composition or dysbiosis. Presented as an altered balance in beneficial or pathogenic bacterial species, dysbiosis is thought
to have a bigger impact on gut wellbeing in IBS patients than previously thought, affecting such processes as intestinal barrier function and immune system regulation (Fig. 2). Therefore the use of therapeutic methods which interact with the microbiota continue to be an interesting option to both increase efficacy in hampering the growth of unwanted species whilst promoting beneficial bacteria in IBS. Improved understanding of the microbiota in respect to IBS may guide future therapeutic strategies with focus on the modulation of gut microbiota composition.

**CONFLICTS OF INTEREST**

No potential conflict of interest relevant to this article was reported.

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