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REVIEW

## Irritable bowel syndrome, the microbiota and the gut-brain axis

Hans Raskov<sup>a</sup>, Jakob Burcharth<sup>b,c</sup>, Hans-Christian Pommergaard<sup>c,d</sup>, and Jacob Rosenberg<sup>c</sup>

<sup>a</sup>Speciallægecentret ved Diakonissestiftelsen, Frederiksberg, Denmark; <sup>b</sup>Department of Surgery, Zealand University Hospital, University of Copenhagen, Copenhagen, Denmark; <sup>c</sup>Centre for Perioperative Optimization, Herlev Hospital, University of Copenhagen, Copenhagen, Denmark; <sup>d</sup>Department of Surgery, Hvidovre Hospital, University of Copenhagen, Copenhagen, Denmark

### ABSTRACT

Irritable bowel syndrome is a common functional gastrointestinal disorder and it is now evident that irritable bowel syndrome is a multi-factorial complex of changes in microbiota and immunology. The bidirectional neurohumoral integrated communication between the microbiota and the autonomous nervous system is called the gut-brain-axis, which integrates brain and GI functions, such as gut motility, appetite and weight. The gut-brain-axis has a central function in the perpetuation of irritable bowel syndrome and the microbiota plays a critical role. The purpose of this article is to review recent research concerning the epidemiology of irritable bowel syndrome, influence of microbiota, probiotics, gut-brain-axis, and possible treatment modalities on irritable bowel syndrome.

### ARTICLE HISTORY

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### KEYWORDS

gut-brain axis; irritable bowel syndrome; microbiota

Irritable bowel syndrome (IBS) is the most common functional gastrointestinal disorder in the Western World. It is a multifactorial condition involving genetics, physiological and psychological responses to stress, diet, age, geographical origin, infections, and use of antibiotics. IBS symptoms are related to gastrointestinal (GI) dysmotility, hypersensitivity, immune activation and changes in composition and function of gut bacteria (the microbiota) and the gut mucosal barrier.<sup>1</sup>

The integrated actions and communication between the microbiota and the autonomous nervous system are central players in the perpetuation of IBS symptoms. This signaling pathway is called the Gut-Brain Axis (GBA). The GBA is a bidirectional neurohumoral communication system that integrates brain and GI functions, such as gut motility, appetite and weight and here the microbiota plays a critical role.<sup>2</sup> Changes in gastrointestinal or central nervous system physiology may result in an altered habitat, which again may cause changes in the composition of the microbiota.

Disruption of the physiologic symbiotic relationship (eubiosis) between the human host and the microbiota is called dysbiosis and is regarded a basic factor for initiating and maintaining IBS in the majority of patients. Current evidence has suggested that

the dysbiosis observed in IBS and the resulting immunological response may drive and perpetuate gastrointestinal symptoms of IBS suggesting that IBS is in fact a disorder of the microbiota and the GBA. It is unclear whether the initiating factor is brain abnormalities that drive the gut changes or if changes in the gut alter brain function through vagal and sympathetic pathways.<sup>3,4</sup> The purpose of this article is to review recent research concerning the influence of microbiota and gut-brain-axis on IBS.

### IBS

It is estimated that approximately 10% of the World population and 15% of the population in the Western World suffer from IBS characterized by a mixture of recurrent abdominal pain, bloating, changing stool consistency such as diarrhea (IBS-D), constipation (IBS-C), or interchanging diarrhea and constipation (mixed-type or IBS-M), mucus secretion, and nausea.<sup>5,6</sup> Community-based data indicate that IBS-M is the most prevalent type followed by IBS-D and IBS-C and that switching among subtypes occurs. Bloating is the most prevalent symptom reported by 96% of patients with IBS of whatever subgroup.<sup>7</sup> In addition to abdominal symptoms, poor sleep, headache,

dyspepsia, fatigue, and depression are frequently observed.

The dysbiosis in the microbiota and subsequent dysfunction in the GBA determines changes in intestinal motility and secretion contributing to visceral hypersensitivity and cellular alterations of the enteroendocrine system and immune system.<sup>8</sup> Evidence has been established that alterations in microbiota with regard to instability and reduced diversity frequently seen in post-infectious IBS often co-exist with small intestinal bacterial overgrowth. Also the beneficial effects of certain probiotics and non-systemic antibiotics are frequent findings in patients with IBS.<sup>9,10</sup> The pathogenesis of visceral hypersensitivity that is presented in IBS is regarded a consequence of dysbiosis in terms of dysregulation of the GBA.<sup>11</sup>

The vast palet of IBS symptoms severely impacts emotional health, functional abilities and consistently carries a negative impact on quality of life (QoL). Patients with IBS visit the doctor more frequently, use more diagnostic tests, consume more medications, have more absence from work, have lower work productivity, and are hospitalized more frequently than patients without IBS. Clinical studies estimate that productivity for patients with IBS is on average 30% lower than healthy workers corresponding to 13.8 hours lost per 40 hours week.<sup>12,13</sup> Patients with IBS and impaired productivity have more extra-intestinal comorbidities (e.g. chronic fatigue syndrome, fibromyalgia, headache, and disease-specific fears and concerns). IBS is also related to psychiatric disturbances such as depression and anxiety. Work productivity is mainly determined by overall measures of symptom intensity such as Visual Analog Scale (VAS), anxiety and other extra-intestinal symptoms, but not to any single bowel symptom.<sup>14,15</sup>

Burden of Illness studies in the US estimate that there are 3.6 million physician visits for IBS annually, and that IBS care consumes over \$ 20 billion in both direct and indirect expenditures. Moreover, patients with IBS consume over 50% more health care resources than matched controls without IBS.<sup>16,17</sup> A national survey among junior and senior high-school pupils in Japan showed that IBS prevalence was 18.6%.<sup>18</sup> No sex difference in prevalence was observed. The prevalence of IBS-D was higher among boys than among girls. Conversely, the prevalence of IBS-C was higher among girls than among boys. The prevalence of IBS increased with increasing school

grade. Furthermore, there was a significant relationship between IBS, disturbed sleep patterns and insomnia, and IBS was significantly associated with poor mental health status and QoL.<sup>18</sup> In this context, IBS not only has a major impact on QoL of the individual afflicted person but may also have a major impact on society in general

Acute bacterial gastroenteritis is the strongest risk factor known for IBS and highly depending on the infecting organism. In 36% of individuals having suffered from infection with both campylobacter jejuni and E. coli 0157:H7 still have IBS symptoms after 2 y caused by a persistent elevation in gut permeability. This subgroup of IBS is called postinfectious IBS (IBS-PI).<sup>9</sup> Acute gastroenteritis, antibiotics and stress are dominant risk factors for intestinal dysbiosis and subsequent IBS in both adults and children.<sup>19</sup> An increase in the inflammation in the bowel wall may result in a chronic, subclinical, low-grade inflammation that is sufficient to alter neuromuscular and epithelial cell function. Dysbiosis may even contribute to the behavioral profile of a patient with IBS.<sup>20</sup> Sedentary work, lack of exercise, western high-carbohydrate diet and processed food are additional well-established risk factors for IBS.<sup>21,22</sup>

IBS is almost twice as prevalent in women,<sup>23</sup> pointing toward a hormonal relationship as well. Of 188,229 subjects the relative risk of IBS in women, compared with men was 1.67. Women with IBS are more likely to suffer from IBS-C and less likely IBS-D.<sup>23</sup> An important factor for the development of IBS is early life stressors, especially maternal separation, which may have long-term effects on the microbiota. This has been documented in several well-established animal models of monkeys and rodents.<sup>24,25</sup> In animal studies stress-induced changes in physiology resulted in dysbiosis and the dysbiotic microbiota was required for stress-induced anxiety-like behavior to manifest itself in the animals.<sup>4,26,27</sup>

### **The microbiota**

The human body is host organism for trillions ( $10^{13}$  –  $10^{14}$ ) of microbes residing in the gastro intestinal (GI) tract covering more than 200 m<sup>2</sup> of mucosa. The number of microbes is 10 times the number of cells in our body and they harbour 150 times as many genes (3.3 mill.) as the human genome.<sup>28</sup> The ecologic system of the diverse commensal microorganisms in the

GI tract is called the microbiota. The genomic profile of the microbiota (the metagenome of the microbiota) is called the microbiome. The human tissues are separated from the microorganisms by only one layer of cells representing the mucosal surface and protected from potential pathogens by the immune system of which the major part of the body's immunological activity is located within the GI tract.<sup>28</sup>

The microbiome has a great influence on the expression of a broad array of human genes. In fact, particular strains of Bifidobacteria, Lactobacilli and *Escherichia coli* influence the gene expression of mucins, Toll-like receptors on macrophages and dendritic cells, caspases, and several nuclear factors leading to an anti-inflammatory response. The interaction between beneficial commensal bacteria and the immune cells results in a downregulation of pro-inflammatory genes, whereas anti-inflammatory genes are upregulated.<sup>29,30</sup> Although identification of the microbiota is ongoing in the Human Microbiome Project, the majority of GI microbiota is still uncharacterized. It is now certain that it consists of a great variety of microbes with a majority of anaerobic bacteria.<sup>31</sup> The current estimation is that there are 500–1000 different bacterial species and more than 7000 individual bacterial strains creating a very delicate community of commensal organisms having evolved over millions of years.<sup>28,32</sup>

The microbiota is dominated by 4 predominant bacterial phylae of which the most abundant are the Gram-positive Firmicutes (among them more than 180 species of *Lactobacillus*), and Actinobacteria (among others the Bifidobacteriae), the Gram-negative Bacteroides (*B. Fragilis* as the most important) and Proteobacteria (*E. coli*, salmonella, yersinia, shigella, vibrio, haemophilus, etc.). The microbiota also includes vast numbers of viruses, protozoa, archaea and fungi.<sup>33,34</sup>

Multidisciplinary approaches for characterizing the gut microbiota show major discrepancies between morphological and molecular studies. It is therefore important to perform multiple techniques to assess the overall bacterial diversity in the gut.<sup>35-37</sup>

Although a state of symbiosis exists between the microbiota and the human body, most microbes are pathogenic if translocated from the gut lumen into the tissues. Bacteroides are found in most anaerobic infections and carry a high mortality.<sup>38,39</sup>

In the colon the fecal (luminal) microbiota differs substantially from the microbiota within the mucine layer of the bowel wall (the (juxta-) mucosal microbiota). The relevance hereof is not yet clear although the mucosal microbiota in one study was predictive of constipation.<sup>40</sup>

Although the microbiota may be partly determined by the genetic profile of the host, the microbiota constantly evolves during the lifespan of the individual. The microbiota is generally stable for long periods of time. With increasing age, the microbiota shifts toward a more pro-inflammatory profile that may be linked to adverse health issues, even tumorigenesis in the elderly host.<sup>1</sup> When looking at individual bacterial strains in relation to colonic tumorigenesis, there appears to be opposing roles for certain Gram-negative (mainly Bacteroides) Gram-positive (Clostridia) in tumor susceptibility. The impact potentially mediated by a balance between protective, butyrate-producing populations and inflammatory, mucin-degrading populations. With age the profile of the microbiota becomes increasingly inflammatory with a reduction in butyrate production and increasing intracolonic pH values creating a hostile environment for colonocytes. From the cecum to the rectum pH increases making a plausible explanation for the increasing susceptibility for tumorigenesis in the left colon and rectum.<sup>41</sup>

The preservation of eubiosis is important for maintaining the integrity of the intestinal epithelium creating an optimal gut barrier to protect against pathogens as well as participating in the uptake of nutrients and production of vitamins. A healthy microbiota is able to distinguish food ingredients into useful, useless and pathogenic substances and sort them accordingly.<sup>2</sup> Dysbiosis favors invasion and growth of pathogenic species and disrupts the homeostasis of the immune system and mucosal barrier.<sup>31</sup>

The microbiota is responsible for the presence of inflammatory and immune cells in the healthy gut wall – the so-called physiologic or controlled inflammation. Also our physical and mental health is highly dependable on the composition and constitution of the microbiota.<sup>42,43</sup> A core-microbiota is shared by approximately 40% of humans, but still we do not have a clear understanding of what a healthy microbiota is.<sup>28</sup> Although each individual has a unique microbiota (microbiological fingerprint), the

abundance and distribution between the bacterial phylotypes is similar among healthy persons.<sup>44</sup>

### **Colonization in early life**

The initial colonizing microbiota is of extreme importance for the development of a healthy microbiota and very early in life there is a critical window during which colonization must take place to secure a normal GBA development.<sup>45,46</sup> This colonization process depends heavily on birth mode. Vaginally born and breastfed infants are initially colonized by Bifidobacteriae, Lactobacilli and Bacteroides, and the full term infant's gut microbiome undergoes rapid maturation over the first year of age. By the age of 3 y the microbiota is securely established in an adult form.<sup>31,47</sup> Cesarean delivery causes colonization by epidermal rather than vaginal bacterial species such as Clostridiae, Staphylococcus, Propionobacteriae, and Corynebacteriae. There is a deficiency of anaerobes such as Bacteroides and Bifidobacterium when compared to vaginally born infants and an overall lower total microbial diversity.<sup>48-50</sup> The presence of a microbiota within the placenta as well as fetal meconium suggests that colonization may begin even before delivery. Recent findings of a non-sterile meconium support this theory.<sup>45,48</sup> The placental microbiota resembles that of the human oral microbiota and can be identified during the third trimester as the fetus matures neurologically and begins to swallow amniotic fluid. Thus, the fetal gut may in turn be colonized by these organisms.<sup>51</sup>

Live bacteria are also found in human milk, including Staphylococcus, Streptococcus, Bifidobacterium, and Lactobacillus.<sup>52</sup> Human breast milk is the most important source of microbes in the neonatal period and mode of delivery also has an important impact on the diversity of the milk microbiota. It is suggested that bacteria from the maternal gut are transported by mononuclear cells to the mammary gland through an endogenous route, the so-called enteromammary pathway and transferred to the infant at a crucial stage for gut immunologic maturation and long-term health status.<sup>53</sup> The mammary microbioma in the human breast milk exercises anti-infective, anti-inflammatory, immunomodulatory, and metabolic properties and provides commensal bacteria to the neonatal gut.<sup>54-56</sup>

### **IBS and the impact of the microbiota**

In IBS, significant changes in major functions of the large bowel are related to microbiome diversity,

immunology, gut barrier integrity and gut-brain signaling. IBS patients express an instability in microbiota composition that may be temporary or permanent and using DNA probing a reduced microbiota diversity can be demonstrated in more than 70% of individuals.<sup>57</sup>

Ten of 50 known bacterial phyla have been discovered in the human gut. Most bacteria belong to the 2 largest phyla, Firmicutes (i.e. Lactobacillus spp., Streptococcus spp. and Clostridium) and Bacteroidetes (i.e., Bacteroides spp). Other phyla like Actinobacteria (i.e. Bifidobacteria) and Proteobacteria (i.e., Escherichia) are also abundant.<sup>58</sup> The common dysbiotic finding in IBS is an increase in Streptococcus spp. (countered by a reduction in Lactobacillus spp) and a reduced abundance of Bacteroidetes, i.e. an overall reduction in beneficial bacteria and an increase in pathogenic species. An increase in the non-butyrate and mucin-degrading Clostridiae group may also play a significant role as well as a decrease in the probiotic Bifidobacterium, a known aid of the mucosal barrier. However, findings are not uniform and observations are conflicting.<sup>59,60</sup>

In a clinical setting, subjects with IBS and who experienced pain, assessed by questionnaire over the 7-week duration of the study, had over 5-fold less Bifidobacteria compared to those without pain.<sup>61</sup> However, the association between specific symptoms and microbiota alterations remains poorly investigated in IBS.<sup>59,60</sup> The most evident findings of dysbiosis are found in PtdIns-IBS where enteric pathogens such as Staphylococcus aureus, Shigella, Clostridium perfringens, Bacillus cereus, and Campylobacter species are often found in large numbers. These species may increase the risk of PI-IBS 6-fold.<sup>62,63</sup>

An interesting study from 2012 including 37 IBS patients reported significantly different IBS subgroups based on microbiota datasets using clustering analysis.<sup>64</sup>

This was the first study to demonstrate an IBS subgroup with no detectable changes in fecal microbiota. The first subgroup (15 patients) clustered with the healthy control samples and had no different microbiota features when compared with non-IBS samples. The second subgroup (22 patients) separated significantly from control samples and consisted of 2 clusters with large microbiota-wide changes, one cluster showed decreased microbial diversity and one cluster had increased diversity. One clinical feature separated the 2 groups: in the first (normal-like IBS) group



depression was more common and the prevalence higher than in the second group in which depression was akin to the rate in the general population. In theory, the IBS patients with a normal microbiota could represent a CNS disorder (and thus the higher prevalence of depression) whereas the IBS with an abnormal microbiome may “just” represent a disturbance in the microbiota.

In conclusion, a decreased diversity in the microbiota can be demonstrated in most but not all cases of IBS. The common findings are an increase in pathogenic species together with a decrease in probiotic species such as *Lactobacillus* and *Bifidobacterium*.<sup>4,65</sup>

### ***IBS, microbiota and inflammation***

In healthy individuals, the inflammatory cells in the lamina propria of the colonized mucosa together with a preservation of the epithelial structure and function reflect a mutually beneficial relationship between the microbiota and the host. In IBS, low-grade inflammation and visceral hyperalgesia are seen in all subgroups. Many studies are linking IBS to low-grade mucosal inflammation challenging the traditional view of IBS as a pure functional disorder.<sup>64,67</sup> In IBS, the mucosal inflammation is dominated by mast cells, eosinophils and intraepithelial lymphocytes, and it is now well established that such inflammation may cause visceral hypersensitivity, epithelial and neuromuscular dysfunction and subsequent altered peristalsis.<sup>68</sup>

The immune system contains an immunologic archive, based on pattern recognition receptors that are able to distinguish potentially pathogenic microorganisms from harmless commensals. Among these receptors are the toll-like receptors (TLR) found on immune cells, enteroendocrine cells and neurons of the enteric nervous system (ENS).<sup>2,69</sup> TLR are necessary to induce increased epithelial cell proliferation and repair. Moreover, TLR signaling enhances barrier function through reinforcement of tight junctions and zonula occludens. Recent studies show that the increased immune response in IBS is characterized by an increased expression and activation TLR (also see section on gut-brain signaling). Intestinal permeability is increased 15–50% in patients with IBS measured by expression in tight junction proteins.<sup>70</sup> The increased permeability in IBS allows the gut microbiota to drive a state of inflammation and may also

influence the CNS via increased levels of circulating cytokines.<sup>71,72</sup>

Potent antigen-presenting cells such as muscular macrophages (MM) located between the different muscular layers in the bowel wall help maintain tissue homeostasis by scavenging and participation in immune responses.<sup>73</sup> During inflammation MM participate in the regulation of gut motility by secretion of inflammatory cytokines and recruitment of inflammatory cells.<sup>74</sup> MM impact on GI motility during normal physiological conditions through the production of BMP2 protein (bone morphogenetic protein 2), which activates BMP receptors expressed by enteric neurons. In turn neurons secrete CSF1 (colony stimulating factor 1) essential for MM development. MM self-maintain locally throughout life with almost no contribution from circulating monocytes.<sup>75</sup> The microbiota regulate both BMP2 and CSF1 expression and thus the cross-talk between MM and enteric neurons.

From animal trials it appears that MMs are sensitive to changes in the microbiota. This is thought to be a result of TLR signaling by enteroendocrine cells and enteric neurons and also from recognition of bacterial lipopolysaccharides and other molecules of microbial origin.<sup>76</sup> Thus, signaling between MM, ENS and gut lumen represent new strategies for the treatment of IBS.

Regarding a possible pathophysiological mechanism, the inflammation-associated permeability driven by proinflammatory cytokines is seen as a result of cytoskeletal contraction of the perijunctional ring increasing paracellular permeability.<sup>4,70,77–80</sup> Serotonin/5-hydroxytryptamin (5-HT) produced by enterochromaffin cells in the intestine and histamine produced by mucosal mast cells act as proinflammatory mediators in the intestine and modulate intestinal permeability.<sup>68</sup> In case of PtdIns-IBS, there seems to be a chronic inflammation-induced upregulation of serotonin signaling that can be related to persistent symptoms.<sup>81</sup>

A proportion of patients with IBS-PI have significant increased number of enterochromaffin cells, which directly correlates to the risk of developing PtdIns-IBS. In addition, serotonin production and upregulation of 5-HT receptors are involved in development of visceral hypersensitivity.<sup>82</sup>

In patients with IBS activated mast cell infiltration in proximity to neurons can often be found in colonic

biopsies and is associated with bloating, pain and severity of symptoms.<sup>83,84</sup> These findings suggest that immune activation may play a crucial role in the pathogenesis of IBS. In addition, psychological stress has been reported to be one of the factors that induce immune activation. However, it remains unknown whether immune activation in IBS patients is dependent on infectious microorganisms.<sup>85</sup>

### **Diet and IBS**

Between 64 and 89% patients with IBS report symptoms to be triggered by meals or specific foods such as wheat/grains, vegetables, milk products, fatty foods, spicy foods, coffee, and alcohol and symptoms most often comprise abdominal pain and gas problems.<sup>86-88</sup>

A western diet enhances the development of a microbiota with a proinflammatory profile. Poorly absorbed and rapidly fermented carbohydrates including fermentable oligo-, di- and monosaccharides and polyols (FODMAPS) have drawn a lot of attention as they may represent some of the dietary culprits in IBS symptoms. Favorable results of low-FODMAP diet in IBS have been reported in recent years. A meta-analysis on low-FODMAP diet from 2015 including 6 randomized controlled trials (RCT) and 16 non-randomized interventional studies found a significant decrease in IBS symptom severity scores for abdominal bloating, pain and diarrhea and significantly improved quality of life scores in 70% of patients.<sup>89</sup> The drawbacks of low FODMAP diets are the high levels of dietary restrictions, the need for monitoring by an expert dietitian to prevent inappropriate weight-loss, over-restrictive or inappropriate diets, and potential nutritional deficiencies.<sup>90</sup>

The major products of FODMAPs fermentation are short chain fatty acids (SCFA), and some major microbial SCFA-producers may be altered in IBS. Low FODMAP diets has been linked to reduced bifidobacterial counts, which seems paradoxical given their potential health benefit.<sup>91</sup> Other studies reported a reduction in total bacterial abundance following the introduction of a low-FODMAP diet compared with habitual diet.<sup>92,93</sup>

In IBS, a high FODMAP diet significantly increases gas volume as well as abdominal symptoms and digestive discomfort. The microbiota develops instability in composition, exhibiting variations in the Bacteroidetes and Firmicutes phylae and a reduction in microbial

diversity. The microbiota of healthy subjects is unaffected by FODMAPs, which points to a strong correlation between diet, symptoms and dysbiosis in patients with IBS.<sup>94</sup> In a small study among children with IBS, a low FODMAP diet significantly decreased abdominal pain severity and frequency. Responders appeared to differ in intestinal microbiota composition, indicating that the efficacy of dietary interventions may be influenced by the microbiota<sup>95</sup> and may also explain why low FODMAP diets are ineffective in some.

A study from 2014 showed that the low-FODMAP diet improved abdominal pain and discomfort but did not alter bowel habits and that low-FODMAP diet cannot be expected to improve diarrhea or constipation.<sup>96,97</sup>

Still, RCTs are needed to prove whether the FODMAP diet is superior to other dietary interventions (e.g., lactose or fructose reduction) or general dietary advice (e.g., the NICE guidelines).<sup>98</sup> Finally, the FODMAP levels vary between studies, countries, and products, which should be taken into account when interpreting study results and recommending low-FODMAP diets.

### **Bile acids and IBS**

Cholic diarrhea and some cases of constipation are thought to result predominantly from disruption of the entero-hepatic circulation of bile acids (BA). It is estimated that approx. Ten% of IBS-D patients have severe BA malabsorption and as many as 25–50% of patients show evidence of some degree of BA malabsorption.<sup>97,99</sup>

BA are produced from cholesterol in hepatocytes with cholic acid and chenodeoxycholic acid is the primary BA. In the liver >90% of BA are conjugated with taurine or glycine to increase solubility and impermeability critical for the formation of micelles surrounding insoluble fatty acids and monoglycerides. The micelles are readily absorbed by enterocytes in the small intestine. 95% of BA, conjugated and unconjugated, are absorbed in the ileum and returned to the liver by the entero-hepatic circulation. The daily production of approx. 600 mg BA is recycled 6–7 times a day.<sup>100</sup>

Intestinal bacteria deconjugate (dehydroxylate) BA to form secondary BA such as lithocholic acid and deoxycholic acid. BA stimulate enterocyte secretion by a decrease in sodium absorption and an increase in chloride secretion. If excess BA reach the colon increased stool water and diarrhea may result.

The negative feed-back mechanism controlling BA production depend on the production-limiting fibroblast growth factor 19 (FGF19).<sup>97</sup> With the reuptake of BA in the ileum the nuclear receptor Farnesoid X receptor (FXR), expressed in enterocytes and in hepatocytes, stimulates the production of FGF19 and release it into the portal circulation. FGF19 inhibits cholesterol 7-hydroxylase and the formation of primary BA.<sup>99,100</sup>

The prosecretory effects of BA depend on bacterial actions and the microbiota has a potential to influence the FGF19 feedback mechanism apparently with bifidobacteria and *e. coli* playing a role.<sup>100,101</sup> Decreased production of FGF19 leads to excessive production of BA and diarrhea.

On the other hand, if a reduction in the amount of BA in the colon occurs, the result often is constipation. It has been shown that the amount of total BA in feces from IBS-C is lower than healthy volunteers, especially with regard to the deoxycholic acid being the most pro-secretory BA.

The signaling pathway of FXR and FGF19 has become a direct target for novel therapeutic products and as these new BA modulators become available they may find a central role in IBS as a proportion of patients have compromised BA metabolism.

### Stress and IBS

Clinical and pre-clinical studies show that psychological stress has a great impact on intestinal sensitivity, motility, secretion, permeability, and mucosal immune activation. These alterations are mediated through the CNS, the peripheral neurons and gastrointestinal microbiota. Stress-induced stimulation of the gut-brain axis can cause symptom flare-ups in IBS.<sup>102</sup>

IBS is a stress-sensitive disorder and the treatment of IBS should also focus on managing stress and stress-induced responses. Over the last decade the characterization of the gut-brain-axis has progressed tremendously and current evidence now points toward multiple mechanisms being involved in the microbiota to brain signaling, including endocrine and neurocrine pathways and that the brain can alter the composition of the microbiota and mental behavior via the autonomic nervous system.<sup>103</sup>

Based on studies using rodents raised in a germ-free environment, the gut microbiota appears to influence the development of emotional behavior, stress- and pain-modulation systems, and brain neurotransmitter

systems. Additionally, microbial perturbations by probiotics and antibiotics exert modulatory effects on some of these functions in adult animals. In humans, pharmacological products such as antidepressants, antipsychotics and serotonin (5-HT) modulators such as 5-HT synthesis inhibitors, selective 5-HT reuptake inhibitors, and specific 5-HT receptor antagonists or agonists have shown effect in IBS management.<sup>102,103</sup>

A healthy microbiota plays a critical role in the development of appropriate stress responses later in life. In early life colonization with beneficial microorganisms and development of a healthy microbiota must take place to ensure a normal development of the core stress axis (hypothalamic-pituitary-adrenal axis, HPA).<sup>46</sup>

Stress induced norepinephrine production may stimulate proliferation of several other strains of enteric pathogens and enhance pathogenicity of *Campylobacter jejuni*.<sup>104</sup> Stressful stimuli may enhance permeability of the intestinal epithelium allowing bacterial antigens to penetrate the gut epithelium and trigger an immune response most often resulting in a low-grade, but sub-clinical inflammation. Stressful stimuli lead to shedding of Lactobacilli and Bifidobacteria, which again may compromise microbiota stability. Studies have shown that stress hormones promote the growth of *E. coli* (both non-pathogenic *E. coli* and pathogenic *E. coli* 0157:H7) with subsequent activation of the HPA. The reduction of Lactobacilli appears to promote the emergence of enteric pathogens such as *campylobacter jejuni*.<sup>3,105,106</sup> Several mechanisms are involved in the stress-induced alteration of the bacterial composition of the GI tract including changes in the epithelial cell function, mucus secretion and gut motility.

The autonomic nervous system modulates mucus secretion and is also likely to have important effects on the thickness and quality of the mucus layer in which the major part of the microbiota resides.<sup>103</sup> The use of oral antibiotics substantially reduces the lactobacillus population, which in turn results in granulocytic (inflammatory) cell activity and release of immunoreactive substance P. Substance P (SP) is a sensory neurotransmitter in the intestinal wall, which results in increased visceromotor activity and visceral pain.<sup>107</sup> In the GI tract, SP is stored in neurons of the intrinsic nervous system, vagal fibers and splanchnic nerves where it stimulates the contraction of smooth muscles.<sup>108</sup> These alterations are associated with increased expression of virulent bacteria. For instance, norepinephrine released during surgery induces the



expression of *Pseudomonas aeruginosa*, which may result in sepsis.<sup>109</sup>

Maternal separation, an early life stressor, caused a significant decrease in Lactobacilli on day 3 after separation. The change returned to baseline 7 d after unification. In germ-free mice the stress response can be fully reversed by *Bifidobacteria infantis* in a time dependant manner.<sup>2</sup> However, in a recent clinical study more patients with IBS (25% versus 7.5%) reported recurrent abdominal pain in childhood, but no difference in separation anxiety scores was found.

Adults with IBS are characterized by somatization, insecure emotional attachment style and recall higher rates of recurrent abdominal pain and symptoms of separation anxiety in childhood.<sup>110</sup> Thus, a personality characterized by social and emotional insecurity, uncertainty and somatization may play an important role in the development of IBS.

### Probiotics and IBS

In 2001, WHO and FAO defined probiotics as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host.<sup>111</sup> Despite evidence for specific health claims there are still controversies with authorities such as European Food Safety Authority (EFSA).<sup>112</sup> Probiotics are present in various dietary components and many already colonize the human intestine in early life. They assist in the maintenance of the mucosal integrity including function, but also protect the mucosa from toxins, allergens and pathogens. Lactobacillus and bifidobacteria are the bacteria most often used in probiotic products. The effect is a result of several mechanisms such as proliferation, cytoprotection, cell migration, anti-apoptosis, protein production and gene expression. In addition, probiotics may directly antagonize pathogens by out-competing them for nutrition and physical space.<sup>113,114</sup> One of the important cytoprotective effects is to secure epithelial tight junction integrity thereby preserving mucosal barrier function. Especially the combination of lactobacillus and bifidobacteria has been shown to restore tight junction barrier integrity, as well as attenuate the HPA axis and autonomic nervous system activity.<sup>8,115,116</sup>

The role of probiotics on the microbiota and bowel function in IBS is supported by the positive effects and lack of adverse effects of probiotics on IBS symptoms in more than 80 trials and more than 10.000 patients.

However, there is conflicting evidence as to the magnitude and clinical significance of the effects of probiotics.<sup>117-119</sup> The effects appear to be strain specific and the most convincing data come from the use of multi-strain probiotics containing both lactobacillus and bifidobacteria.<sup>2</sup> An IBS trial with a multispecies probiotic conducted over 5 months showed a stabilization of the microbiota in conjunction with major benefits for pain and distension.<sup>120</sup> It is now generally accepted that regular intake of certain probiotics relieve IBS symptoms such as bloating, abdominal distension and altered bowel habits.<sup>121</sup> Probiotics increase production of anti-inflammatory cytokines, down-regulate pro-inflammatory pathways and prevent apoptosis. Within minutes, gastric vagal nerve activity was increased following introduction of Lactobacilli into the duodenum.<sup>1,122</sup> Visceral pain secondary to the increase in inflammatory cells during dysbiosis following antibiotics can be reverted by administration of lactobacillus and lactic acid producing bacteria.<sup>123</sup>

In IBS-C, a 4-week intake of *Bifidobacterium lactis* was associated with significant reduction in abdominal distension, increase in transit time and overall symptom improvement.<sup>124</sup> After 8 weeks with *Bifidobacterium infantis*, there was symptom improvement and normalization of the IL10/IL12 ratio in plasma.<sup>3</sup> Lactobacilli upregulate expression of intestinal epithelial transport systems such as the apical anion exchanger (SLC26A3) responsible for the Chlorid-ion absorption by the gut suggesting one of the mechanisms of effect of Lactobacilli in the treatment of diarrhea.<sup>46</sup>

A randomized, placebo-controlled trial reported a beneficial effect of a multispecies probiotic on cognitive reactivity to sad mood measured by the Leiden index of depression scale.<sup>125</sup> During a 4-week intervention trial with non-depressed individuals, probiotics were associated with reduced rumination and aggressive thoughts.<sup>125</sup> In another randomized controlled trial, administration of a probiotic formulation containing Lactobacillus and Bifidobacteria reduced psychological stress in humans.<sup>126</sup> It was suggested that the administration of Bifidobacteria resulted in increased tryptofan levels giving probiotics an antidepressant potential by influencing tryptofan metabolism.<sup>1</sup>

### Gut-brain signaling – The gut brain axis

The bidirectional communication network of the GBA includes the central nervous system (CNS), the

autonomic nervous system (ANS), the enteric nervous system (ENS) and the hypothalamic pituitary adrenal (HPA) axis. Under normal conditions, the communication from the gut to the CNS is autonomous and without notice to the individual. However, under pathological conditions signals may reach the somatic sensory system and cause symptoms such as discomfort, nausea and pain. In return, the CNS output through the autonomic nervous system may lead to gastrointestinal dysfunction (Fig. 1).

The ANS (sympathetic and parasympathetic) conducts afferent signals arising from the gut lumen through enteric, spinal and vagal pathways to CNS. The vagus nerve with its thousands of nerve endings of which 80% are afferent, is the major communication pathway between microbiota and the brain and with the splanchnic nerves being its functional counterpart. The same pathways conduct the efferent signals from CNS to the intestinal wall.<sup>8,127</sup> The HPA axis is a major player in the neuroendocrine system that controls reactions to stress and regulates many body processes, including digestion and the immune system. In addition, it plays a central role in production of corticosteroids in response to environmental stress.

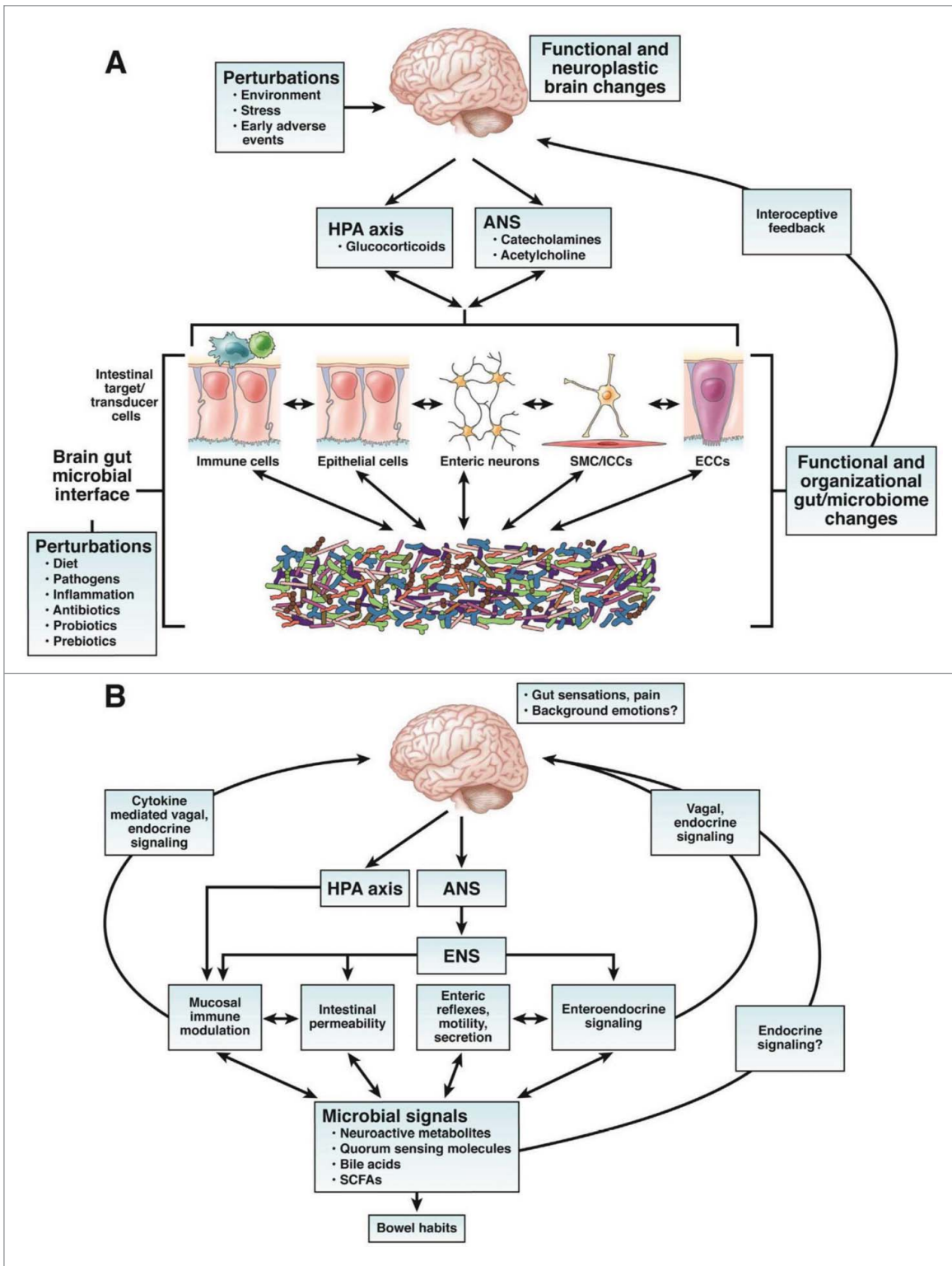
Neural and hormonal communication in combination allows the brain and microbiota to regulate activities of intestinal cells. Signals to and from the microbiota and CNS are dependant on signal transducing cells such as the enterochromaffine cells (EC) and dendritic cells (DC) secreting several transmitter substances such as 5-HT, somatostatin, cholecystokinin and corticotropin-releasing hormone.<sup>3</sup> Efferent signals from CNS are conveyed to the gut by the neuroendocrine transmitters, which are secreted into the gut lumen by neurons, immune cells and EC and can directly modulate microbial behavior. EC function as luminal sensors exposed to the vast diversity of microbes and microbial products in the human gut. Bacteria also possesses neurotransmitter receptors and activation can influence the function and composition of the microbiota.<sup>8</sup> Bacteria are able to communicate with mammal epithelial cells via so-called inter-kingdom signaling through oligo-peptides and monoamines with neurotransmitter- or hormone-like properties.<sup>128</sup> Singalling between the different types of cells and between bacteria and epithelial cells requires the presence of neurotransmitters such as 5-HT, somatostatin, dopamin, neuropeptide Y, peptide YY, cholecystokinin and corticotropin-releasing factor.<sup>14,129</sup>

The ENS is often referred to as the second brain and is located within the wall of the digestive tract and protected from the gut lumen by the mucous barrier. The ENS contains thousands of ganglia and approximately 500 million neurons which are more than any other peripheral organ and 5 times as many as the spinal cord. The ENS is capable of autonomous function and produces more than 30 different neurotransmitters. More than 90% of the body's 5-HT and 50% of the dopamin are produced in the GI tract primarily by EC and to a lesser extent by myenteric neurons and mast cells.

The microbiota plays a critical role in 5-HT regulation and increase levels of colon and blood 5-HT primarily by elevating synthesis by host EC. Especially spore-forming bacteria promote 5-HT synthesis from colonic EC thus modulating GI motility, secretion and immune responses. Gut microbiota act on EC also through SCFA (butyrate and acetate) and increases production of 5-HT primarily in the distal gut.<sup>130</sup> SCFAs are able to stimulate the enteric and sympathetic nervous system<sup>49</sup> and increase gut transit.<sup>8,131</sup> In addition, changes in microbiota can alter levels of 5-HT and other neuroactive substances such as nitric oxide and substance P and probably dysregulation of peripheral 5-HT is involved in the pathogenesis of IBS.

The connections with the CNS are the vagus, pelvic nerves and sympathetic pathways.<sup>132-134</sup> One of the key signaling pathways is via TLR expressed in immune cells of the bowel wall and the neurons of the ENS representing a mechanism for the microbiota to communicate with the CNS.<sup>76</sup> The TLR are pattern recognition receptors detecting lipopolysaccharides (membrane-component of Gram-negative bacteria) and other molecules of microbial origin. TLR signaling through the NF-kappaB pathways result in production of proinflammatory cytokines taking part in the homeostasis of the intestinal epithelium and protecting from epithelial injury.

Probiotic modulation of the microbiota is shown to alter neurons of the myenteric plexus and benefit patients with IBS, maybe through the bacterial neuronal interaction.<sup>135</sup> In IBS-D the majority of patients demonstrated increased expression of TLR in colonic biopsies suggesting communication between the microbiota and the host immune response generating a chronic state of low-grade inflammation.<sup>136,137</sup> In septic ileus the amount of cytokines in response to large amounts of LPS plays an important role in the



**Figure 1.** (A). Components of the gut-brain axis. Signals from the microbiota are transmitted to the brain via multiple afferent signaling pathways, including endocrine and neural (vagal, spinal afferents) pathways. Enterochromaffine (ECC) cells in the gut wall functions as interface between the organism and the gut lumen. SMC – smooth muscle cells; ANS - autonomic nervous system; HPA - hypothalamic pituitary adrenal axis. (B). Functional and symptom-related consequences of brain gut microbial interactions. Intestinal processes relevant to IBS symptoms are modulated by the brain (via the ANS/ENS) and by the microbiota. Microbial molecules signal to the brain via vagal and afferent nerve pathways, by cytokines and neurotransmitters. From EA Mayer. *Gastroenterology*. 2014 May;146(6): 1500–1512. With permission from Elsevier.

mechanism leading to smooth muscle dysfunction and paralysis.<sup>138</sup>

### **IBS, visceral pain and GABA**

A vast array of mediators and pathways impacting the pathophysiology of visceral pain still remains to be elucidated. During visceral inflammation, cytokines and other neurotransmitters mediate pain signals to the central pathways in the spinal cord where increased nociceptive activity and central sensitization are translated into the experience of pain.<sup>139</sup>

$\gamma$ -Aminobutyric acid (GABA) acts as the major inhibitory neurotransmitter in the CNS and without GABA neurons fire too often and too easily. GABA has multiple functions and contribute significantly to homeostasis in both health and disease. Disruption of GABA neurotransmission leads to neurological diseases including epilepsy and general anxiety disorders.

GABA is synthesized from glucose via glutamic acid primarily in the CNS, primarily the cerebral cortex, but also in non-neural tissues using GABA as neurotransmitter such as the endocrine pancreas.<sup>140</sup> Enhancing GABAergic inhibition is useful for the treatment of several pathological situations, including chronic pain, sleep disorders, anxiety, and—most importantly—epilepsy. Two classes of GABA receptors (a and b) have been identified (including a number of different subclasses) in human tissues, and are differentially expressed depending on the tissue type.

GABA's anti-nociceptive effects are believed to be mediated by GABA<sub>B</sub> receptors, which are located in both the brain and spinal cord but also GABA<sub>B</sub> receptors are implicated in other GI functions, such as GI motility and visceral sensation. GABA<sub>B</sub> receptor agonists act as spinal anti-nociceptive agents by inhibiting the release of substance P in the spinal cord and may also act on peripheral receptors. They are able to modulate responses of vagal afferents innervating the GI tract but also possesses undesirable CNS effects, including sedation, respiratory depression and motor deficiency making potential therapeutic use difficult.<sup>141</sup> GABA analogs such as gabapentin and pregabalin have shown efficacy in preclinical models of visceral hypersensitivity.<sup>142,143</sup>

The mostly widely used GABA<sub>B</sub> receptor agonist, baclofen, is used for spasticity resulting from multiple sclerosis and concomitant pain, clonus, and muscular rigidity.

Although baclofen has been shown to inhibit distension sensitive vagal afferents, its anti-nociceptive effects in IBS are untested.<sup>144</sup>

The microbiota is able to produce GABA. The major GABA producing microorganisms are lactic acid bacteria. The production of GABA by bifidobacteria exhibit substantial interspecies variation. *Lactobacillus brevis* and *Bifidobacterium dentium* are the most efficient GABA producers among the commensal intestinal bacteria tested. A wide range of traditional foods produced by microbial fermentation contain GABA. The clinical implications herof still need to be explored.<sup>145,146</sup> New modulators of GABA<sub>B</sub> may provide a novel therapeutics for treatment of visceral pain disorders,<sup>147</sup> Endogenous systems, which also may become new targets in the IBS therapy, include cortico-releasing-factor receptor, neurokinin receptors cannabinoid, and opioid receptors and their ligands.

In IBS, a hypersensitive large bowel is considered a pathognomic feature of all IBS subtypes. Altered rectal perception is documented in >60% of patients with IBS and in some inflammation is the trigger of nociceptive signals. Current scientific evidence points to several mechanisms of visceral hypersensitivity.<sup>148</sup> Among those is sensitization of peripheral and central neurons, as well as altered descending excitatory and inhibitory signals influencing spinal cord nociceptive neurons resulting in misinterpretation of harmless sensations as painful, toxic or dangerous.<sup>149</sup> The hypersensitivity and signal misinterpretation are key factors in IBS.<sup>82</sup>

### **Discussion and conclusion**

It is evident that IBS is a multi-factorial complex of changes in microbiota, immunology, and gut-brain axis signaling with a rather limited success of currently available therapeutic approaches. However, at this time it is reasonable to postulate that the manipulation of the gut microbiota, through probiotics, symbiotics or antibiotics to promote a “healthy” microbiota, may soon become a valuable treatment of irritable bowel syndrome and other functional disorders as well as in patients with anxiety or depression.

It has been known for some time that the microbiota has a great impact on human mental wellbeing. A positive effect of probiotics has been reported on depression and anxiety.<sup>150</sup> Normal sleep patterns can be disrupted after microbiota changes with oral antibiotics.<sup>151</sup> In autistic patients, a greater prevalence of



clostridial species has been shown.<sup>151,152</sup> Other studies hypothesize that late onset of autism is caused by excessive use of antibiotics and an association with trimetoprim and sulfamethoxazole.<sup>153</sup> On the contrary, Vancomycin provided transient relief to children with late-onset autism.<sup>154</sup> In experimental settings introduction of non-invasive pathogens into the cecum rapidly activate brain stem nuclei apparently through afferent vagus nerve and without evidence of bowel inflammation.<sup>155</sup> and in animal trials, abnormal changes in brain chemistry and behavior were reversed by administration of *Lactobacillus*.<sup>156</sup>

As significantly different subgroups of IBS using microbiota clustering has been found further research should address if specific probiotic treatment should be tailored to a particular host's microbiota.

Few medications for the subgroups IBS-D and IBS-C are registered but for the IBS-M group the current advices are mainly dietary and use of dietary supplements. The FODMAP diet has proven effective for a large group of patients but it is cumbersome and cannot be advised for all.<sup>90,91,93</sup> In the future dietary treatments too will be based on structural changes and functional features of an individual's microbiota.

As for dietary fiber it is generally agreed that insoluble fibers have no significant effect on IBS. Two recent meta-analyses provide conflicting data on soluble fibers.<sup>157,158</sup>

For probiotics the results are overall positive for lactic acid producing bacteria such as bifidobacteria and lactobacilli but data are conflicting and authorities are still debating their use.<sup>112</sup> A few natural herbs and other substances are reported effective. The most promising of these is peppermint oil. The oil components of menthol and menthone are very effective in relieving abdominal pain from cramps/spasms and gas.<sup>158-160</sup> Simethicone is ineffective as monotherapy but has been found to produce additive effect on IBS symptoms in combination with other compounds.<sup>161-163</sup> Depending on predominant symptoms, a combination of these compounds can be of value in patients with IBS.

Other targets of interest are the BA and neurotransmitter interventions, which the authors believe will become a mainstay in future treatment modalities together with microbiota modulation following individual profiling.

The fecal microbiota transplantation (FT) is an interesting concept but has still not been elucidated in IBS. Current data show that FT is effective in recurrent or

chronic clostridial infections.<sup>164</sup> We have not identified the effective components of FT and there is no consensus as to the optimal route of FT (naso-duodenal or rectal), the composition or amount of the optimal FT. An optimal donor profile has to be defined and the donor must be screened for contagious diseases before FT. To our knowledge there are no reported adverse effects of FT, but until we know more about benefits and potential harms of FT authorities are reluctant to approve it. With the current speed of microbiological and pharmacological research these issues are likely to be solved in a few years.

In conclusion, human microbiome research continues to expand, and the current data about development and function will assist us in a better understanding of lifelong functional bowel disorders. Our knowledge of the early colonization and genesis of the microbiota, which is primarily influenced by external factors rather than genetic factors, may give us an opportunity to impact the process at an early stage, maybe even before birth with potential interventions that may prevent infections and immunological diseases. Due to the multifactorial etiology of IBS and individual symptom-triggers no single treatment modality is or will be effectful. At this point in time there is no consensus as to the optimal treatment of IBS. Future treatment of IBS will be tailored to each patient based on individual microbial composition, neurotransmitter status and mapping of additional bioactive substances such as BA and SCFA, organ functions, symptoms and severity.

### Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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