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Intestinal Microbiota and its Role in Irritable Bowel Syndrome (IBS)

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Abstract Gut microbiota alterations are increasingly being recognized as an important factor in the pathogenesis and pathophysiology of Irritable bowel syndrome (IBS). The onset of IBS symptoms after a bout of gastroenteritis comprises one of the strongest indications for the importance of gut microbiota for IBS. Moreover, recent studies have identified several susceptibility genes for IBS involved in the innate immunity and recognition of bacteria but also maintaining the integrity of the intestinal barrier. During recent years, it has also been demonstrated that IBS patients, or subgroups thereof, may have an altered microbiota composition relative to healthy individuals, mainly based on the analysis of fecal microbiota. Moreover, a positive effect of treatment with non-absorbable antibiotics and probiotics in IBS provides further indirect support for the relevance of gut microbiota alterations in IBS.

Keywords Irritable bowel syndrome · IBS · Post-infectious IBS · Microbiota · Inflammation · Antibiotics · Probiotics · SIBO

Introduction

Irritable bowel syndrome (IBS) is a functional gastrointestinal (GI) disorder characterized by abdominal pain or discomfort associated with abnormal bowel habit [1]. The prevalence of IBS in the industrialized world is approximately 10–15 % in the general population, which makes IBS one of the most common GI disorders [2]. Individuals suffering from IBS have a reduced quality of life and report more co-morbidities than the general population [3, 4]. IBS is commonly subtyped according to predominant bowel habit into diarrhoea-predominant, constipation-predominant, or mixed/alternating phenotypes (IBS-D, IBS-C, or IBS-M) [1, 5].

The pathogenesis and pathophysiology of IBS is incompletely understood, but abnormal GI motility, visceral hypersensitivity, altered brain–gut function, low-grade inflammation, and psychosocial factors are considered to contribute [6]. During recent years, the evidence for involvement of gut microbiota in the pathogenesis and pathophysiology of irritable bowel syndrome (IBS) has increased, and this is the focus of this review, where direct and indirect evidence for the importance of gut microbiota alterations in IBS will be discussed.

Post-infectious IBS

The onset of IBS symptoms after a bout of gastroenteritis comprises one of the strongest indications for the importance of gut microbiota for the induction of IBS symptoms. There have been numerous studies assessing the risk of developing IBS after a bout of gastroenteritis and meta-analyses have established that there is a six- to sevenfold increased risk of developing IBS after an infectious gastroenteritis [7, 8••]. However, GI infections are extremely common around the world and, in the vast majority of affected people, a full recovery rapidly occurs. Therefore, a bout of gastroenteritis is obviously not enough to develop IBS, and several risk factors are associated with development of post-infectious (PI) IBS. Hence, psychological distress at the time of the gastroenteritis increases the risk of

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developing long-standing GI symptoms, and the same holds true for being of young age and of female gender [9]. Moreover, evidence suggests that factors related to the infectious event or to the inflammatory reaction per se might also be of importance [6, 10].

Immune Activity and Recognition of Luminal Antigens in IBS

The potential role of low-grade inflammation in IBS has been acknowledged during recent years, and a link between altered gut microbiota composition and inflammation in IBS has been suggested [6]. A clinical observation indirectly supporting the potential role of low-grade inflammation in the generation of symptoms compatible with IBS comes from patients with inflammatory bowel disease, i.e. ulcerative colitis and Crohn's disease. A substantial proportion of these patients have IBS-like symptoms when they are in remission, hence when there are no obvious signs of active inflammation [11]. Moreover, IBD patients without signs of active inflammation but with IBS-like symptoms had more severe psychological symptoms and reduced quality of life, pointing indirectly towards an interaction between peripheral and central factors in the genesis of these symptoms, in line with risk factors identified for development of PI-IBS. Also, mucosal dysbiosis in patients with inflammatory bowel disorders has been reported [12], which suggests that this might also be a factor of relevance for remaining symptoms in patients in remission, but this has to be confirmed in future studies.

Also of great interest, several genes involved in the host response to intraluminal antigens or bacterial invasion, or the immune responses to these antigens, are differently expressed in IBS patients, and especially so in patients with PI-IBS [13]. Recent studies have identified several susceptibility genes for IBS-D and PI-IBS involved in the innate immunity and recognition of bacteria but also maintaining the integrity of the intestinal barrier [14]. Interestingly, TNFSF15 encoding the TLA1 protein is identified in both Crohn´s disease and IBS [15, 16•], underlining the possibility of common pathways in these diseases.

Cells of the immune system have specific receptors, for example toll-like receptors (TLR), which recognize danger signals, such as bacterial and viral components (Fig. 1). TLRs are widely expressed, both on the cell surface and within the cytoplasm, by dendritic cells, macrophages, and epithelial cells among others. In a recently published paper, it was reported that IBS-M patients displayed a significant up-regulation of TLR2 and TLR4 on epithelial cells in the colonic mucosa, which resulted in increased secretion of the pro-inflammatory cytokines IL-8 and IL1-β [17]. Additionally, increased levels of mucosal TLR4 and TLR5 and decreased levels of TLR7 and TLR8 in IBS patients have been reported [18]. IBS patients have also been demonstrated to have increased TLR2 expression on monocytes [19], and TLR-induced cytokine secretion patterns are altered in patients relative to healthy individuals [20]. Also, antibodies specific for flagellin, the primary component of bacterial flagellae, are more frequently found in IBS patients than in the healthy population [21]. Taken together, these data support an engagement between gut microbiota and the mucosal immune system, involving both innate and adaptive immunity, which may generate a low-grade inflammatory response that can be of importance for symptom generation in IBS.

Effects of Antibiotics and Probiotics in IBS

The previous understanding has been that antibiotic treatment may induce or worsen IBS symptoms. This view was supported by two observational studies, showing that systemic antibiotic treatment could in fact increase functional GI symptoms [22, 23]. In contrast, recent randomized controlled trials demonstrate improved symptoms in IBS patients after treatment with a non-absorbable antibiotic, Rifaximin [24•, 25]. The therapy seems to be effective, especially for flatulence and bloating, and in the most recent trial a better effect than with placebo was observed during the 10 weeks follow-up period, but the therapeutic advantage over placebo was merely 10 % [24•]. Further studies are needed to evaluate whether Rifaximin should be used for IBS, but the results indicate a potential role for gut microbiota alterations at least in a subset of IBS patients. Another line of indirect evidence for a role of gut microbiota alterations in IBS comes from studies on the effect of treatment with probiotics for IBS patients. A systematic review, comprising 19 randomized controlled trials including more than 1,600 patients with IBS, concluded that probiotics appear to be efficacious in IBS, although the magnitude of benefit and the most effective species and strain are still uncertain [26•]. However, as one plausible effect of probiotics is to alter the gut microbiota composition [27–29], the positive effect of probiotics in IBS, although admittedly modest, supports the involvement of gut microbiota in the genesis of symptoms in IBS.

Gut Microbiota Composition of IBS Patients

In recent years, many research groups have focused on identifying the gut microbiota composition of the large intestine of IBS patients, using modern culture-independent techniques [30]. Several of the available studies demonstrate that IBS patients, or subgroups thereof, have an

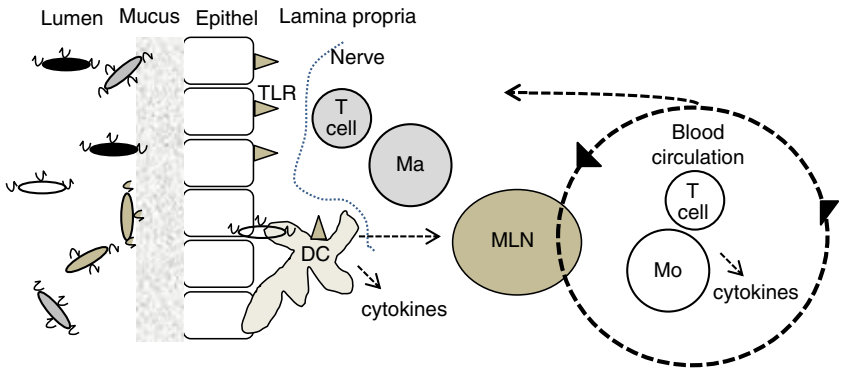

Fig. 1 Mucosal dendritic cells (*DC*) recognize microbial danger signals through pathogen recognition receptors, for example TLRs, migrate to mesenteric lymph nodes (*MLN*), and present danger signals, antigens, to T cells. The T cells leave the blood stream and migrate to the intestinal mucosa to fight the danger. Circulating monocytes (*Mo*) maturating into macrophages (*Ma*), which fagocytose bacteria and are important for clearing infectious agents, are also recruited to the intestinal mucosa in response to pro-inflammatory cytokines produced by DC on site

altered microbiota composition relative to healthy individuals [31, 32•, 33, 34, 35•]. A finding that has been seen in several studies is a gut microbial composition enriched with Firmicutes together with a reduced abundance of Bacteroidetes in the IBS subjects relative to healthy individuals [32•, 35•, 36].

Most studies have been performed in adult patient cohorts, but there are also studies reporting an altered gut microbiota in pediatric IBS patients [37•, 38]. In a study of children with IBS by Saulnier et al., it was reported that the fecal microbiome composition differed between IBS-C and IBS-U patients [37•]. There were not a sufficient number of IBS-D patients in the study to include these in the analysis. Likewise, it has been reported that abundance of bifidobacteria is lower in adult IBS-D patients than in IBS-C patients [39]. In contrast, our group demonstrated no difference of fecal microbiota composition according to IBS subtypes based on Rome criteria [1, 5]. Instead, we could clearly identify two separate clusters of IBS patients, not associated with IBS subtypes, whose microbiota composition was different from healthy individuals. Moreover, a third cluster of IBS patients with normal-like microbiota composition was identified [35•]. In general, the results of current studies on large intestinal microbiota in IBS patients lack consensus. It seems likely that IBS patients, or subgroups of patients, may have an altered microbiota, but it is far from clear whether the microbiota changes are related to IBS subtypes according to the dominant symptoms.

Diversity of Fecal and Mucosal Microbiota

The majority of the hitherto published studies on gut microbiota of IBS patients are based on analysis of fecal microbiota, as a proxy for the intestinal microbial communities. Several studies have demonstrated lower fecal microbial diversity [31, 33] of IBS patients as compared to healthy individuals. Additionally, our group demonstrated that, among the IBS patients with an altered microbiota composition, one cluster of IBS patients had increased phylogenetic diversity whereas another cluster had lower phylogenetic diversity relative to healthy individuals [35•].

It is important to highlight that it is not well known to what extent fecal microbiota reflects the mucosa-adherent microbiota community. Some reports have addressed both fecal and mucosal microbiota composition of IBS patients [31, 40–42]. Differences in both fecal and mucosa-associated gut microbiota between patients and healthy individuals seem to exist, although variations between these two ecological niches are present [31]. Also, lower bifidobacteria counts [42] and higher prevalence of *Pseudomonas aeruginosa* [41] have been described in both fecal and mucosal samples of IBS patients.

Gut Microbiota Associated with IBS Symptoms

There is little direct proof that gut microbiota or their metabolites may control intestinal function in humans, even though several animal studies provide evidence supporting this [43–45]. Hence, there are relatively few reports of association between microbiota abundance and IBS symptoms. However, our group recently reported several clear associations between clinical characteristics and different taxa [35•]. Moreover, in the same study, we demonstrated that the prevalence of depression was increased in IBS patients with normal-like fecal microbiota, whereas the group of IBS patients with an altered fecal microbiota (high Firmicutes:Bacteroidetes ratio) displayed normal prevalence of depression, implicating that symptom generation may be different in these two groups of IBS patients [46]. However, this hypothesis needs to be confirmed in future studies.

Another group has described that stool frequency was negatively correlated with number of mucosa associated bifidobacteria and lactobacilli [39]. The abundance of gammaproteobacteria has also been positively correlated with IBS symptoms [32•]. Additionally, a *Ruminococcus torques*-like phylotype has also been reported to positively correlate with IBS symptom scores, including emotional and social function, and systemic and bowel symptoms [47]. Furthermore, in a recent, relatively large study of IBS patients, correlation analyses of microbial groups and IBS symptoms indicated the involvement of several groups of Firmicutes and proteobacteria in the genesis of IBS symptoms [32•]. In one of the few studies of pediatric IBS patients, an association between high/moderate pain phenotype and taxa belonging to the *Alistipes* genera was identified. Also, taxa of the genera *Akkermansia*, *Parabacteriodes,* and *Ruminococcaceae* were found in children reporting frequent pain episodes [37•]. Interestingly, in a study of healthy Finnish adults, an association between low bifidobacteria counts and pain was described, when the gut microbiota composition and health perception and the occurrence of intestinal symptoms was followed during a 7-week study period [48]. However, even though available studies suggest an association between microbes and symptoms in IBS, the relative importance of different taxa for IBS symptoms is not consistent between existing studies. Further studies in large, phenotypically well-characterized cohorts of IBS patients are needed to clarify this association.

Effects of Bacterial Metabolites on Intestinal Function

Metabolites produced by the gut microbiota community may have effects on intestinal function. For example, short chain fatty acids (SCFA), such as butyrate and acetate, produced by endogenous bacteria, have been demonstrated to activate the ileal motor pattern [49], through specific G protein receptor signaling [50]. It has also been shown that *Lactobacillus paracasei* metabolites modulate contractility of intestinal smooth cells [51], and *E. coli Nissle* secretions modulate contractility of human muscle strips [52]. Moreover, *Lactobacillus acidophilus* and *L. paracasei* have been reported to modulate pain and visceral hypersensitivity perception, respectively [53, 54]. Interestingly, an increased sulfate-reducing microbiota population in the gut of C-IBS patients has been reported, which could lead to enhancement in toxic sulfide production, which in turn could influence gut physiology and contribute to IBS pathogenesis [55]. Also, an altered gut microbiota community producing less SCFA, in an in vitro fermentation system in response to incubations with various carbohydrates and fibers, has been described in patients with D-IBS [56]. Thus, an altered production of metabolites of the colonic bacterial microbiota may be related to the development of gastrointestinal symptoms in patients with IBS.

Small Intestinal Microbiota in IBS

The question whether IBS patients have small intestinal bacterial overgrowth (SIBO) has been extensively debated during the last decade [57••, 58, 59]. Initial reports suggesting an important role for SIBO in IBS [60, 61], have been hard to replicate by other groups [62, 63]. One of the problems with the SIBO hypothesis has been that it is mainly based on demonstrating SIBO with the lactulose hydrogen breath test, which has poor sensitivity and specificity [58]. Moreover, in a recent study using combined oro-caecal scintigraphy and hydrogen breath test, it was demonstrated that the lactulose hydrogen breath test merely reflects rapid oro-caecal transit and not SIBO in IBS patients [64]. Also, potential confounding factors, such as proton pump inhibitors (PPI) [65, 66], and altered motility may explain discrepancies between studies. Further, in a study from our group where we used the gold standard for diagnosing SIBO, i.e. culture of jejunal aspirate, we were unable to find an increased prevalence of SIBO in IBS, even though a mild increase of small intestinal bacteria was more commonly found in patients with IBS than in healthy controls [62]. So, to summarize, the relevance of small intestinal microbiota alterations in IBS remains to be proven, and further studies addressing this controversy, using valid techniques, are much awaited.

Effects of Diet on Gut Microbiota

Dietary factors have effects on the gut microbiota composition, and this might be of considerable importance for symptoms of IBS patients. Unabsorbed dietary components will serve as nutrients, driving the growth of the microbial community. The effect of dietary habits has recently been elegantly demonstrated: the polysaccharide-rich diet of African children from rural areas resulted in enrichment of Bacteroidetes and depletion of Firmicutes, as compared to Italian urban children [67]. Moreover, in another study, using modern culture independent techniques to demonstrate gut microbiota composition and careful determination of food intake, an association between dietary factors and gut microbiota composition was clearly demonstrated [68]. Current data suggest that gut microbiota composition may be a result of food intake, but the genetic background of the host also seems to be an important factor for shaping the gut microbiota community [69].

Knowledge of the impact of food intake on gut microbiota composition in humans may be achieved by

investigating effects of short-term or long-lasting changes in dietary lifestyles. Large intake of fibers and food containing fructans and fructose, and other poorly fermentable dietary carbohydrates (FODMAPs), may give rise to bloating and diarrhea. Therefore, reduction of these food items may have beneficial effects on IBS symptoms [70, 71]. Compared to wheat bran, whole grain cereals increase the numbers of fecal *Bifidobacterium* spp. and *Lactobacillus* spp. in healthy subjects [72]. Low calorie diets containing carbohydrates, decreased *Roseburia* spp. and fecal butyrate concentration in obese subjects [73]. Also, Lactulose acidifies the proximal colon, which has been demonstrated to increase *Bifidobacterium* spp. counts in healthy humans [74] and patients with chronic idiopathic constipation [75]. A fermentable carbohydrate restriction 4-week diet reduced the abundance of bifidobacteria, and also had positive effects on overall symptoms and bloating of IBS patients [76]. However, whether this diet also had major implications on the gut microbiota composition remains to be determined. Moreover, a diet low in FODMAPs was recently shown to change microbiota composition in parallel with improvement in GI symptoms in a group of IBS patients, further highlighting the role of diet for gut microbiota composition [76].

Sample Collection, Preparation and Analyzing Techniques

The majority of the hitherto published studies on the gut microbiota composition of IBS patients comprise relatively low numbers of patients, especially when considering the heterogeneity of this patient group. The inconsistent data regarding the microbiota composition of IBS patients are probably partly due to the lack of uniformity of the patient group. Yet, the diverging results may also be explained by lack of insight into how sample collection and preparation may affect the results. In a recently published report, it was demonstrated that fecal samples exposed to room or deep freezing temperatures for up to 24 h and 6 months, respectively, exhibit a microbial composition and diversity that shares more identity with its host of origin than any other sample [77]. However, the data from that study implied that the microbiota from IBS patients was less stable at room temperature than that of healthy controls. Moreover, the use of different techniques is also a likely reason for the deviating results. The approach to identify gut microbiota has changed dramatically during the last decade. The gold standard for detection and classification has been based on culture methods, although it has been clear that many of the gut-resident microbes are not possible to culture with traditional methods. Development of new culture-independent techniques based on the 16s RNA characterization of microbial communities has rapidly increased our knowledge of the gut microbiota. Classical 16s RNA-based techniques, such as fingerprinting (DGGE and T-RFLP) and PCR amplification of genes, are now complemented by high throughput 16s RNA techniques, allowing more and better powered observations. Thus, thousands of 16s RNA or their respective genes may be identified and quantified in a single experiment [57••].

Conclusion

The knowledge and understanding of the importance of gut microbiota for symptom generation in IBS is rapidly increasing. Today, alterations in gut microbiota composition are considered to be a plausible factor of relevance for symptoms in at least subsets of these patients. However, due to the fact that the heterogeneity of the patient group is large and that many external factors may contribute to the individual microbiota variation, careful studies of large and phenotypically well-characterized groups of patients are needed in order to more clearly demonstrate the contribution of gut microbiota alterations to the heterogeneous symptom pattern in patients with IBS.

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References

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Longstreth GF, Thompson WG, Chey WD, et al. Functional bowel disorders. Gastroenterology. 2006;130:1480–91.
2. Lovell RM, Ford AC. Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. Clin Gastroenterol Hepatol. 2012;10:712–21 e4.
3. Simrén M, Svedlund J, Posserud I, et al. Health-related quality of life in patients attending a gastroenterology outpatient clinic: functional disorders versus organic diseases. Clin Gastroenterol Hepatol. 2006;4:187–95.
4. Drossman DA, Chang L, Bellamy N, et al. Severity in irritable bowel syndrome: a Rome Foundation Working Team report. Am J Gastroenterol. 2011;106:1749–59. Study demonstrating how to assess severity in IBS.

5. Thompson WG, Longstreth GF, Drossman DA, et al. Functional bowel disorders and functional abdominal pain. Gut. 1999;45 Suppl 2:II43–7.
6. Öhman L, Simrén M. Pathogenesis of IBS: role of inflammation, immunity and neuroimmune interactions. Nat Rev Gastroenterol Hepatol. 2010;7:163–73.
7. Halvorson HA, Schlett CD, Riddle MS. Postinfectious irritable bowel syndrome–a meta-analysis. Am J Gastroenterol. 2006;101:1894–9. quiz 1942.
8. •• Thabane M, Kottachchi DT, Marshall JK. Systematic review and meta-analysis: the incidence and prognosis of post-infectious irritable bowel syndrome. Aliment Pharmacol Ther. 2007;26:535–44. *Meta-analysis of incidence and prognosis of post-infectious IBS.*
9. Öhman L, Simrén M. New insights into the pathogenesis and pathophysiology of irritable bowel syndrome. Dig Liver Dis. 2007;39:201–15.
10. Spiller R, Garsed K. Postinfectious irritable bowel syndrome. Gastroenterology. 2009;136:1979–88.
11. Simrén M, Axelsson J, Gillberg R, et al. Quality of life in inflammatory bowel disease in remission: the impact of IBS-like symptoms and associated psychological factors. Am J Gastroenterol. 2002;97:389–96.
12. Lepage P, Hasler R, Spehlmann ME, et al. Twin study indicates loss of interaction between microbiota and mucosa of patients with ulcerative colitis. Gastroenterology. 2011;141:227–36.
13. Aerssens J, Camilleri M, Talloen W, et al. Alterations in mucosal immunity identified in the colon of patients with irritable bowel syndrome. Clin Gastroenterol Hepatol. 2008;6:194–205.
14. Villani AC, Lemire M, Thabane M, et al. Genetic risk factors for post-infectious irritable bowel syndrome following a waterborne outbreak of gastroenteritis. Gastroenterology. 2010;138:1502–13.
15. Swan C, Duroudier NP, Campbell E, et al. Identifying and testing candidate genetic polymorphisms in the irritable bowel syndrome (IBS): association with TNFSF15 and TNFalpha. Gut. 2012, June 8.
16. • Zucchelli M, Camilleri M, Andreasson AN, et al. Association of TNFSF15 polymorphism with irritable bowel syndrome. Gut. 2011;60:1671–7. *Study identifying genetic alterations associated with IBS.*
17. Belmonte L, Beutheu Youmba S, Bertiaux-Vandaele N, et al. Role of toll like receptors in irritable bowel syndrome: differential mucosal immune activation according to the disease subtype. PLoS One. 2012;7:e42777.
18. Brint EK, Macsharry J, Fanning A, et al. Differential expression of toll-like receptors in patients with irritable bowel syndrome. Am J Gastroenterol. 2011;106:329–36.
19. Öhman L, Lindmark AC, Isaksson S, et al. Increased TLR2 expression on blood monocytes in irritable bowel syndrome patients. Eur J Gastroenterol Hepatol. 2012;24:398–405.
20. McKernan DP, Gaszner G, Quigley EM, et al. Altered peripheral toll-like receptor responses in the irritable bowel syndrome. Aliment Pharmacol Ther. 2011;33:1045–52.
21. Schoepfer AM, Schaffer T, Seibold-Schmid B, et al. Antibodies to flagellin indicate reactivity to bacterial antigens in IBS patients. Neurogastroenterol Motil. 2008;20:1110–8.
22. Maxwell PR, Rink E, Kumar D, et al. Antibiotics increase functional abdominal symptoms. Am J Gastroenterol. 2002;97:104–8.
23. Mendall MA, Kumar D. Antibiotic use, childhood affluence and irritable bowel syndrome (IBS). Eur J Gastroenterol Hepatol. 1998;10:59–62.
24. • Pimentel M, Lembo A, Chey WD, et al. Rifaximin therapy for patients with irritable bowel syndrome without constipation. N Engl J Med. 2011;364:22–32. *Study demonstrating improved IBS symptoms after treatment with a non-absorbable antibiotic.*
25. Sharara AI, Aoun E, Abdul-Baki H, et al. A randomized double-blind placebo-controlled trial of rifaximin in patients with abdominal bloating and flatulence. Am J Gastroenterol. 2006;101:326–33.
26. • Moayyedi P, Ford AC, Talley NJ, et al. The efficacy of probiotics in the treatment of irritable bowel syndrome: a systematic review. Gut. 2010;59:325–32. *Meta-analysis of the efficacy of probiotics in the treatment of IBS.*
27. Kajander K, Myllyluoma E, Rajilic-Stojanovic M, et al. Clinical trial: multispecies probiotic supplementation alleviates the symptoms of irritable bowel syndrome and stabilizes intestinal microbiota. Aliment Pharmacol Ther. 2008;27:48–57.
28. Lyra A, Krogius-Kurikka L, Nikkila J, et al. Effect of a multispecies probiotic supplement on quantity of irritable bowel syndrome-related intestinal microbial phylotypes. BMC Gastroenterol. 2010;10:110.
29. Preidis GA, Versalovic J. Targeting the human microbiome with antibiotics, probiotics, and prebiotics: gastroenterology enters the metagenomics era. Gastroenterology. 2009;136:2015–31.
30. Zoetendal EG, Rajilic-Stojanovic M, de Vos WM. High-throughput diversity and functionality analysis of the gastrointestinal tract microbiota. Gut. 2008;57:1605–15.
31. Carroll IM, Ringel-Kulka T, Keku TO, et al. Molecular analysis of the luminal- and mucosal-associated intestinal microbiota in diarrhea-predominant irritable bowel syndrome. Am J Physiol Gastrointest Liver Physiol. 2011;301:G799–807.
32. • Rajilic-Stojanovic M, Biagi E, Heilig HG, et al. Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome. Gastroenterology. 2011;141:1792–801. *Study demonstrating fecal microbiota composition of IBS patients.*
33. Noor SO, Ridgway K, Scovell L, et al. Ulcerative colitis and irritable bowel patients exhibit distinct abnormalities of the gut microbiota. BMC Gastroenterol. 2010;10:134.
34. Kassinen A, Krogius-Kurikka L, Makivuokko H, et al. The fecal microbiota of irritable bowel syndrome patients differs significantly from that of healthy subjects. Gastroenterology. 2007;133:24–33.
35. • Jeffery IB, O'Toole PW, Öhman L, et al. An irritable bowel syndrome subtype defined by species-specific alterations in faecal microbiota. Gut. 2012;61:997–1006. *Study demonstrating fecal microbiota composition of IBS patients.*
36. Krogius-Kurikka L, Lyra A, Malinen E, et al. Microbial community analysis reveals high level phylogenetic alterations in the overall gastrointestinal microbiota of diarrhoea-predominant irritable bowel syndrome sufferers. BMC Gastroenterol. 2009;9:95.
37. • Saulnier DM, Riehle K, Mistretta TA, et al. Gastrointestinal microbiome signatures of pediatric patients with irritable bowel syndrome. Gastroenterology. 2011;141:1782–91. *Study demonstrating fecal microbiota composition of pediatric IBS patients.*
38. Rigsbee L, Agans R, Shankar V, et al. Quantitative profiling of gut microbiota of children with diarrhea-predominant irritable bowel syndrome. Am J Gastroenterol. 2012;107:1740–51.
39. Parkes GC, Rayment NB, Hudspith BN, et al. Distinct microbial populations exist in the mucosa-associated microbiota of subgroups of irritable bowel syndrome. Neurogastroenterol Motil. 2012;24:31–9.
40. Carroll IM, Chang YH, Park J, et al. Luminal and mucosal-associated intestinal microbiota in patients with diarrhea-predominant irritable bowel syndrome. Gut Pathog. 2010;2:19.
41. Kerckhoffs AP, Ben-Amor K, Samsom M, et al. Molecular analysis of faecal and duodenal samples reveals significantly higher prevalence and numbers of Pseudomonas aeruginosa in irritable bowel syndrome. J Med Microbiol. 2011;60:236–45.
42. Kerckhoffs AP, Samsom M, van der Rest ME, et al. Lower Bifidobacteria counts in both duodenal mucosa-associated and fecal microbiota in irritable bowel syndrome patients. World J Gastroenterol. 2009;15:2887–92.

43. Barbara G, Stanghellini V, Brandi G, et al. Interactions between commensal bacteria and gut sensorimotor function in health and disease. Am J Gastroenterol. 2005;100:2560–8.
44. Abrams GD, Bishop JE. Effect of the normal microbial flora on gastrointestinal motility. Proc Soc Exp Biol Med. 1967;126:301–4.
45. Husebye E, Hellstrom PM, Sundler F, et al. Influence of microbial species on small intestinal myoelectric activity and transit in germ-free rats. Am J Physiol Gastrointest Liver Physiol. 2001;280: G368–80.
46. Jeffery IB, Quigley EM, Öhman L, et al. The microbiota link to Irritable Bowel Syndrome: an emerging story. Gut Microbes. 2012;3:572–6.
47. Malinen E, Krogius-Kurikka L, Lyra A, et al. Association of symptoms with gastrointestinal microbiota in irritable bowel syndrome. World J Gastroenterol. 2010;16:4532–40.
48. Jalanka-Tuovinen J, Salonen A, Nikkila J, et al. Intestinal microbiota in healthy adults: temporal analysis reveals individual and common core and relation to intestinal symptoms. PLoS One. 2011;6:e23035.
49. Kamath PS, Phillips SF, Zinsmeister AR. Short-chain fatty acids stimulate ileal motility in humans. Gastroenterology. 1988;95:1496–502.
50. Tazoe H, Otomo Y, Kaji I, et al. Roles of short-chain fatty acids receptors, GPR41 and GPR43 on colonic functions. J Physiol Pharmacol. 2008;59 Suppl 2:251–62.
51. Verdu EF, Bercik P, Bergonzelli GE, et al. Lactobacillus paracasei normalizes muscle hypercontractility in a murine model of postinfective gut dysfunction. Gastroenterology. 2004;127:826–37.
52. Bar F, Von Koschitzky H, Roblick U, et al. Cell-free supernatants of Escherichia coli Nissle 1917 modulate human colonic motility: evidence from an in vitro organ bath study. Neurogastroenterol Motil. 2009;21(559–66):e16–7.
53. Eutamene H, Lamine F, Chabo C, et al. Synergy between Lactobacillus paracasei and its bacterial products to counteract stress-induced gut permeability and sensitivity increase in rats. J Nutr. 2007;137:1901–7.
54. Rousseaux C, Thuru X, Gelot A, et al. Lactobacillus acidophilus modulates intestinal pain and induces opioid and cannabinoid receptors. Nat Med. 2007;13:35–7.
55. Chassard C, Dapoigny M, Scott KP, et al. Functional dysbiosis within the gut microbiota of patients with constipated-irritable bowel syndrome. Aliment Pharmacol Ther. 2012;35:828–38.
56. Treem WR, Ahsan N, Kastoff G, et al. Fecal short-chain fatty acids in patients with diarrhea-predominant irritable bowel syndrome: in vitro studies of carbohydrate fermentation. J Pediatr Gastroenterol Nutr. 1996;23:280–6.
57. •• Simrén M, Barbara G, Flint HJ, et al. Intestinal microbiota in functional bowel disorders: a Rome foundation report. Gut. 2013;62:159–76. *Exhaustive review of the importance of gut microbiota for functional bowel disorders*.
58. Simrén M, Stotzer PO. Use and abuse of hydrogen breath tests. Gut. 2006;55:297–303.
59. Vanner S. The small intestinal bacterial overgrowth. Irritable bowel syndrome hypothesis: implications for treatment. Gut. 2008;57:1315–21.
60. Pimentel M, Chow EJ, Lin HC. Eradication of small intestinal bacterial overgrowth reduces symptoms of irritable bowel syndrome. Am J Gastroenterol. 2000;95:3503–6.
61. Pimentel M, Chow EJ, Lin HC. Normalization of lactulose breath testing correlates with symptom improvement in irritable bowel syndrome. a double-blind, randomized, placebo-controlled study. Am J Gastroenterol. 2003;98:412–9.
62. Posserud I, Stotzer PO, Björnsson ES, et al. Small intestinal bacterial overgrowth in patients with irritable bowel syndrome. Gut. 2007;56:802–8.
63. Walters B, Vanner SJ. Detection of bacterial overgrowth in IBS using the lactulose H2 breath test: comparison with 14C-D-xylose and healthy controls. Am J Gastroenterol. 2005;100:1566–70.
64. Yu D, Cheeseman F, Vanner S. Combined oro-caecal scintigraphy and lactulose hydrogen breath testing demonstrate that breath testing detects oro-caecal transit, not small intestinal bacterial overgrowth in patients with IBS. Gut. 2011;60:334–40.
65. Spiegel BM, Chey WD, Chang L. Bacterial overgrowth and irritable bowel syndrome: unifying hypothesis or a spurious consequence of proton pump inhibitors? Am J Gastroenterol. 2008;103:2972–6.
66. Verdu E, Viani F, Armstrong D, et al. Effect of omeprazole on intragastric bacterial counts, nitrates, nitrites, and N-nitroso compounds. Gut. 1994;35:455–60.
67. De Filippo C, Cavalieri D, Di Paola M, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci U S A. 2010;107:14691–6.
68. Wu GD, Chen J, Hoffmann C, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science. 2011;334:105–8.
69. Ottman N, Smidt H, de Vos WM, et al. The function of our microbiota: who is out there and what do they do? Front Cell Infect Microbiol. 2012;2:104.
70. Dear KL, Elia M, Hunter JO. Do interventions which reduce colonic bacterial fermentation improve symptoms of irritable bowel syndrome? Dig Dis Sci. 2005;50:758–66.
71. Shepherd SJ, Gibson PR. Fructose malabsorption and symptoms of irritable bowel syndrome: guidelines for effective dietary management. J Am Diet Assoc. 2006;106:1631–9.
72. Costabile A, Klinder A, Fava F, et al. Whole-grain wheat breakfast cereal has a prebiotic effect on the human gut microbiota: a double-blind, placebo-controlled, crossover study. Br J Nutr. 2008;99:110–20.
73. Duncan SH, Belenguer A, Holtrop G, et al. Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations of butyrate and butyrate-producing bacteria in feces. Appl Environ Microbiol. 2007;73:1073–8.
74. Bouhnik Y, Attar A, Joly FA, et al. Lactulose ingestion increases faecal bifidobacterial counts: a randomised double-blind study in healthy humans. Eur J Clin Nutr. 2004;58:462–6.
75. Bouhnik Y, Neut C, Raskine L, et al. Prospective, randomized, parallel-group trial to evaluate the effects of lactulose and polyethylene glycol-4000 on colonic flora in chronic idiopathic constipation. Aliment Pharmacol Ther. 2004;19:889–99.
76. Staudacher HM, Lomer MC, Anderson JL, et al. Fermentable carbohydrate restriction reduces luminal bifidobacteria and gastrointestinal symptoms in patients with irritable bowel syndrome. J Nutr. 2012;142:1510–8.
77. Carroll IM, Ringel-Kulka T, Siddle JP, et al. Characterization of the fecal microbiota using high-throughput sequencing reveals a stable microbial community during storage. PLoS One. 2012;7:e46953.