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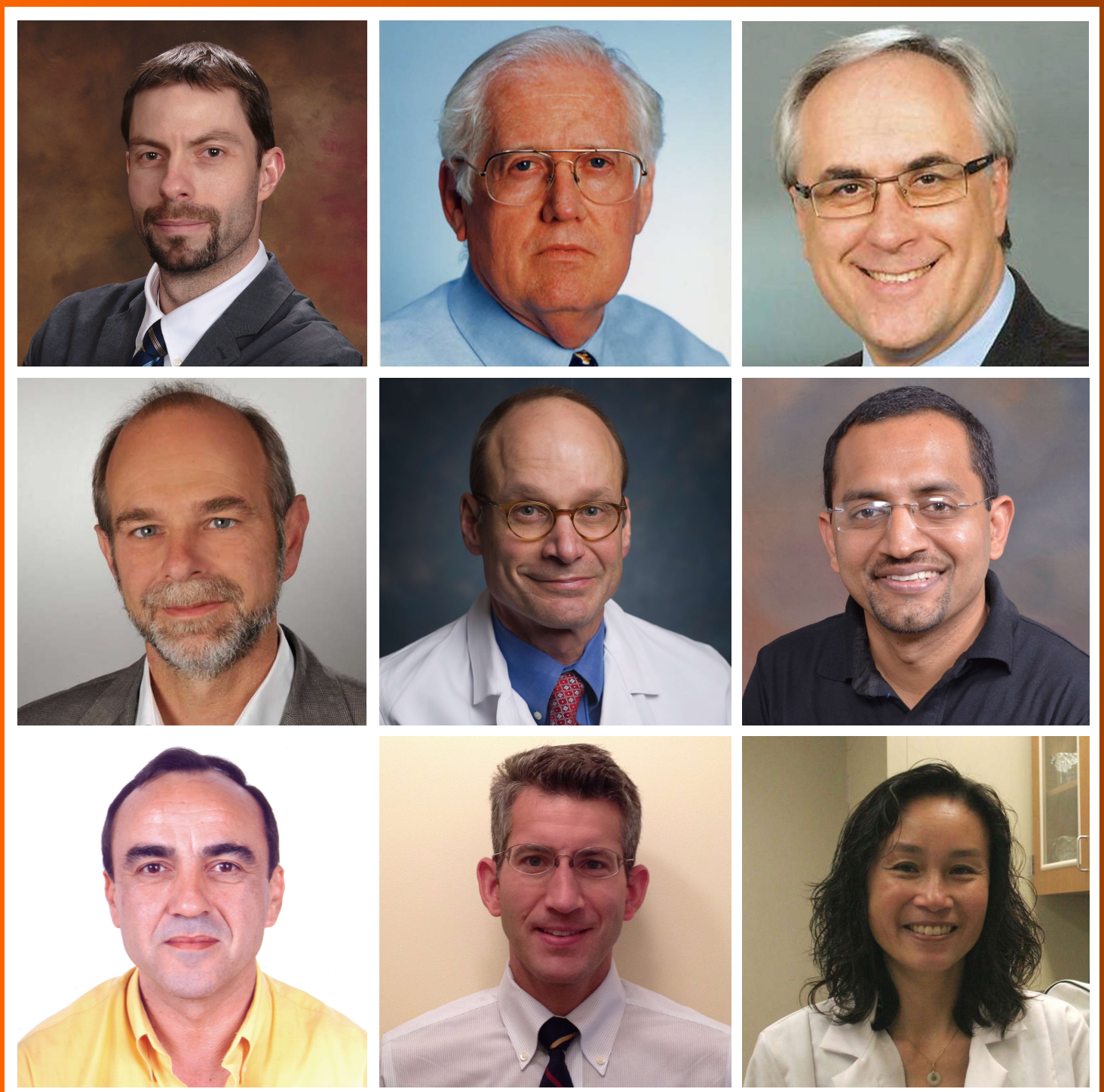

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WCIBS World Clinical Irritable Bowel Syndrome

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Oliver Grundmann, MSc, PhD, Assistant Professor; David Y Graham, MD, Professor; Peter Christopher Konturek, MD, Director, Professor; Thomas Bock, PhD, Head, Professor; C. Mel Wilcox, MD, Doctor, Professor; Pankaj Garg, MD, MNAMS, MNAMS, Associate Professor; Miguel Angel Plaza, PhD, Associate Professor; Daniel E Freedberg, MD; Michele Terrell Yip-Schneider, PhD, Associate Professor.

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EDITOR
Oliver Grundmann, PhD, Clinical Associate Professor, Director graduate online programs in Pharmaceutical Chemistry and Clinical Toxicology, Department of Medicinal Chemistry, College of Pharmacy, Department of Adult and Elderly Nursing, College of Nursing, University of Florida, Gainesville, FL 32610, United States.

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Baishideng Publishing Group Inc
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Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
http: //www.wjgnet.com

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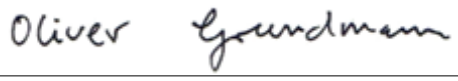
Core progress in clinical management of irritable bowel syndrome in 2014

The first edition of the *World* series publication of core progress in clinical management of irritable bowel syndrome, commonly known as IBS, summarizes the progress made in our understanding and clinical management of this widespread disease through laboratory and clinical research efforts that were published in 2014. To address the ever-growing need for accurate differential diagnosis and the increasing recognition of the importance of not only treating symptoms but the underlying pathophysiologies as well, the articles chosen for inclusion in this first edition compendium represent diverse IBS-related topics and management approaches. All 34 articles were originally published in the *World Journal of Gastroenterology* in 2014, and highlight the growing awareness and intensified research efforts to unravel the pathophysiology, advance diagnostic approaches, and provide comprehensive treatment approaches to address IBS as a chronic disorder and which can significantly impact a patients' quality of life.

Considering that between 5% and 20% of the world's population is affected by IBS, it is crucial to deepen our knowledge of this disorder. The collective information presented in this compendium indicates that IBS is not defined by a single causative pathophysiological mechanism, but is rather a multidimensional intestinal disorder. It appears that the brain-gut axis, also known as the enteric nervous system, is affected in a majority of IBS patients, suggesting the requirement of an integrative treatment approach combining pharmacotherapy, psychotherapy, and complementary treatments such as yoga or acupuncture. Complementary therapies can often alleviate symptoms when applied in conjunction with pharmacotherapy, this combined treatment approach is gaining popularity among many practitioners. Another important impact on the field of IBS over the past few years is the advanced understanding that has been gained for the interaction of the intestinal microbiota with intestinal barrier function and the immune system. Therefore, although IBS may not present with a significant inflammatory component, it appears that the endocrine system of IBS patients has undergone unique changes, such as steroid hormone fluctuations and genetic polymorphisms related to immune defense and intestinal motility.

I am pleased to share this first edition of the *World Clinical Irritable Bowel Syndrome* (ISBN 978-0-99144 30-6-2) with our colleagues to address the current state of IBS research and serve both as a guide for clinicians in their everyday practice as well as an indicator for researchers to advance our understanding of this disorder and benefit IBS patients.



Oliver Grundmann, PhD, Clinical Associate Professor

Oliver Grundmann, University of Florida, Colleges of Pharmacy and Nursing, 1345 Center Drive, Gainesville, FL 32610, United States

February 1, 2015

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WJG 20th Anniversary Special Issues (4): Irritable bowel syndrome

Complementary and alternative medicines in irritable bowel syndrome: An integrative view

Oliver Grundmann, Saunjoo L Yoon

Oliver Grundmann, Department of Medicinal Chemistry, College of Pharmacy, University of Florida, FL 32610, United States
Oliver Grundmann, Saunjoo L Yoon, Department of Adult and Elderly Nursing, College of Nursing, University of Florida, FL 32610, United States
Author contributions: Grundmann O and Yoon SL performed research and wrote the paper.
Correspondence to: Oliver Grundmann, PhD, Department of Medicinal Chemistry, College of Pharmacy, University of Florida, PO Box 100485, Gainesville, FL 32610, United States. grundman@ufl.edu
Telephone: +1-352-2464994 Fax: +1-352-3929455
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Abstract

Irritable bowel syndrome (IBS) is a common gastrointestinal disorder with a high incidence in the general population. The diagnosis of IBS is mainly based on exclusion of other intestinal conditions through the absence of inflammatory markers and specific antigens. The current pharmacological treatment approaches available focus on reducing symptom severity while often limiting quality of life because of significant side effects. This has led to an effectiveness gap for IBS patients that seek further relief to increase their quality of life. Complementary and alternative medicines (CAM) have been associated with a higher degree of symptom management and quality of life in IBS patients. Over the past decade, a number of important clinical trials have shown that specific herbal therapies (peppermint oil and Iberogast®), hypnotherapy, cognitive behavior therapy, acupuncture, and yoga present with improved treatment outcomes in IBS patients. We propose an integrative approach to treating the diverse symptoms of IBS by combining the benefits of and need for pharmacotherapy with known CAM therapies to provide IBS patients with the best treatment outcome achievable. Initial steps in this direction are already being considered with an increasing number of practitioners recommending CAM therapies to their patients if pharmacotherapy alone does not alleviate symptoms sufficiently.

Key words: Irritable bowel syndrome; Complementary and alternative medicine; Hypnotherapy; Cognitive behavioral therapy; Herbal therapy; Peppermint

Core tip: Irritable bowel syndrome is a prevalent gastrointestinal disorder that interferes with daily living in 5%-20% of the population. The current review summarizes the most widely used complementary and alternative medicine (CAM) approaches that have proven to be effective and have been endorsed by professional organizations. The review encourages the use of both pharmacotherapy and CAM approaches in an integrative setting to provide the best outcome and quality of life to patients.

Original sources: Grundmann O, Yoon SL. Complementary and alternative medicines in irritable bowel syndrome: An integrative view. *World J Gastroenterol* 2014; 20(2): 346-362 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i2/346.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i2.346

INTRODUCTION

Irritable bowel syndrome (IBS) is among the most common gastrointestinal disorders with a prevalence ranging from 5%-20% in the general population worldwide[1,2]. IBS is more commonly diagnosed in women than in men and in people younger than 50 years[1,3,4]. The high prevalence of diagnosis also results in a significant so-

cioeconomic burden through decreased work productivity, increased direct and indirect healthcare costs, and - depending on the severity - a reduction in quality of life for IBS patients and their caregivers[5-8]. The estimated indirect and direct healthcare costs related to IBS in the United States have been steadily increasing and amount to $1.35 billion dollar as of 2003[9]. The worldwide health costs associated with IBS are estimated to exceed $200 billion United States dollars[10]. The International Classification of Diseases (ICD) of the World Health Organization in its latest revision, ICD-10, classifies IBS as a functional digestive disorder with the ICD-10 classification 58.9 with sub-classifications as irritable colon or spastic colon[11]. This classification does not distinguish between the Rome-Ⅲ criteria and the consensus of many professional medical organizations that have divided IBS into four different subgroups based on the primary symptom presentation as constipation-predominant IBS (IBS-C), diarrhea-predominant IBS (IBS-D), mixed or alternating IBS, and unspecified IBS[2,12]. The diagnosis of IBS is mainly dependent on the absence of pathophysiological and morphological indicators and therefore remains an exclusion diagnosis concentrated on symptom presentation[13]. There have been indications in recent research studies that IBS may be the result of a low-grade inflammatory process within the lower intestinal tract but definitive and validated biochemical markers have not emerged as of yet[14-17]. There also remains a gap in our understanding of the underlying pathophysiology and what causes IBS. A few hypotheses have linked genetic predisposition, post-infectious small bowel bacterial overgrowth, and certain diets with a higher incidence for developing IBS[18-20]. However, a unified understanding of the pathophysiology that may result in a feasible and causal treatment approach has not emerged. In defense of this deficit, similar knowledge gaps exist for a wide range of conditions for which symptomatic treatment to date provides the only therapeutic approach.

Because current pharmacological treatment approaches for IBS are solely based on symptom reduction, many patients remain undertreated and dissatisfied with their quality of life. In addition, many pharmacological treatment approaches are associated with side effects that result in a smaller benefit to the patient in terms of treatment outcomes[18,19]. The treatment also depends on the specific subtype of IBS. While IBS-C patients mainly suffer from abdominal pain because of slow bowel movement and less frequent bowel release, patients with IBS-D suffer from a social stigma due to the frequent bowel release that requires a bathroom in close proximity as well as bloating and increased flatulence[8,19,21,22]. Comorbidities between IBS with depressive and anxiety disorders have been well defined although it remains unknown which of these is the cause and the effect[23-26]. A common treatment for all subtypes of IBS are antidepressants which may indicate that certain depressive and anxiety disorders play a role in the pathophysiology of IBS[25,27-29]. Another emerging field of research is the investigation of the gut-brain axis also referred to as the enteric nervous system (ENS). It has been established that interconnected sensing of afferent and efferent neurons in the ENS influences gut motility based mainly on serotonergic and cholinergic nerve innervations[30,31]. The 2 major serotonin receptors present in the intestinal tract, 5-HT_3 and 5-HT_4, and a serotonin reuptake transporter are either differently expressed or even present with mutations in certain IBS populations[27,28,32-34]. This correlates well with the current pharmacological treatment approaches of using 5-HT_3 receptor antagonists and 5-HT_4 receptor agonists in IBS patients to reduce both visceral pain perception and regulate gastrointestinal motility[35-37]. Considering that the neurotransmitter and hormone serotonin is involved in both intestinal motility and mood regulation may serve as an indicator that changes in the ENS neurotransmission are involved in the comorbidity between IBS with depressive and anxiety disorders.

As mentioned, current pharmacological treatment approaches provide limited symptomatic relief to IBS patients. This has resulted in a significant increase in self-medication and the use of complementary and alternative medicines (CAM) by patients and even healthcare providers to bridge the gap and increase quality of life[38-42]. This review will summarize the current knowledge of CAM alone and in conjunction with pharmacological treatments as an integrative approach to manage patients with IBS and improve their quality of life. Although the review is not comprehensive in addressing all aspects of CAM and integrative medical approaches to treating IBS, it is intended to provide practitioners with the most commonly used and most widely recommended CAM approaches that have shown repeated success in clinical trials over the past decades. It is important to point out that pharmacological treatment should not be abandoned by patients and their providers in lieu of CAM approaches but rather an integrative approach considered that provides both maximum relief of symptoms and increased quality of life.

LITERATURE SEARCH

This article reviews current research regarding the most commonly used CAM therapies for IBS in the United States, which are single or combination herbal products, acupuncture, yoga, hypnotherapy, and cognitive behavioral therapy. The literature search covered the period from January 1996 to June 2013 using Medline and PubMed with the search terms "irritable bowel syndrome" in combination with "yoga", "hypnotherapy", "cognitive behavioral therapy", "CBT", "CAM", "acupuncture", "herbal therapy", and "integrative medicine". Out of 714 total articles retrieved, 243 were excluded because they were reviews or protocols, 215 were not in English, and 102 were duplicates or did not relate to IBS. A total of 154 articles were selected for inclusion in this review (Figure 1).

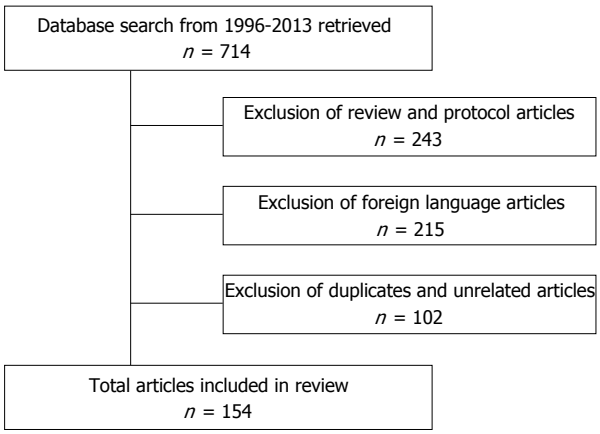

Figure 1 Flow chart illustrating the selection criteria for inclusion of articles.

PHARMACOLOGICAL TREATMENT APPROACHES

The current approach to treating IBS is symptomatic and consists of a regimen of first line pharmacological treatment options often coupled with lifestyle adjustments followed by potential off-label use of a number of other medications that are not specifically indicated for IBS if symptom management is insufficient[2,20,43]. The current leading guidelines have been developed by the Task Force on Irritable Bowel Syndrome of the American College of Gastroenterology (ACG) and the British Society of Gastroenterology[2,12]. Both associations recognize that symptomatic treatment of IBS is associated with a significant placebo effect which has been confirmed in a number of studies as well as in a now well-known unblinded study where patients were told that they were receiving placebo and still showed significant improvements in symptoms[44]. These findings support the hypothesis of an underlying connection between the brain and the gut and the potential interplay of emotions and mood disorders affecting the severity of IBS symptoms.

Considering the increased incidence of comorbid depressive and anxiety disorders in patients with IBS, the use of antidepressants, mainly tricyclic (TCA) and selective serotonin reuptake inhibitor (SSRI) antidepressants, have shown improvements in IBS symptoms and there seems to be an indication that such medication may also provide symptom relief in IBS patients without comorbid psychiatric disorders[45-48]. The effectiveness of low dose TCA and SSRI antidepressants as well as benzodiazepines is yet another indication that the enteric nervous system is influenced by mood and that this in turn affects the innervation by serotonergic and cholinergic neurons in the gastrointestinal (GI) tract[49,50].

The initial step in treating IBS is to consider supportive treatments that may help to alleviate mild gastrointestinal symptoms by increasing fiber and probiotics consumption, regular exercise regimens, and eliminating certain food items that may be linked to an allergic reaction (often lactose intolerance). These supportive treatments can be considered integrative in nature since many IBS patients will remain on a specialized diet even after initiating pharmacotherapy if their symptoms are moderate to severe[40,51,52]. Patients with moderate IBS symptoms often require a first line treatment to reduce symptoms and may also benefit from CAM therapy, especially cognitive behavior therapy (CBT) or hypnotherapy[53-57]. A number of publications indicate that the patient-practitioner relationship can have a significant influence on treatment outcomes[58,59]. Practitioners should try to communicate clearly with patients about the diagnostic process, the potential treatment options, setting realistic goals for outcomes and improvement, and providing an atmosphere that is caring and supportive.

The most commonly used pharmacological interventions for symptomatic relief of moderate to severe IBS constitute prokinetics and antispasmodics for IBS-C patients and opioid agonists, anticholinergics, and 5-HT$_3$ antagonists for relieving IBS-D symptoms[19,20]. Prokinetics are not specific to IBS and increase gastrointestinal motility in general by acting *via* dopamine and 5-HT$_3$ receptors as antagonists or 5-HT$_4$ receptors as agonists[60,61]. Tegaserod is to date the only Food and Drug Administration approved prokinetic drug specific for the treatment of IBS-C but has been significantly limited in its use due to an increased cardiovascular risk[62,63]. Lubiprostone, a 5-HT$_4$ agonist, has been recently approved to treat IBS-C in women through activation of chlorine channels leading to increased water secretion into the lumen which decreased transit time and associated visceral pain in patients[61]. The common use of 5-HT$_3$ receptor antagonists such as ondansetron and granisetron to reduce visceral pain perception in IBS-D patients has shown some benefits but is also limited by side effects. The best risk-to-benefit ratio has been shown for the 5-HT$_3$ antagonist alosetron to date[36,64,65].

The common use of antispasmodics in both IBS-C and IBS-D patients serves to reduce abdominal pain and cramping but requires close monitoring especially in IBS-C patients because of further slowing of GI motility[66-68]. All of the currently available antispasmodics are not specific to IBS and act as anticholinergics which are associated with a number of side effects including hyposalivation and cardiovascular events[20,69].

The use of opioid agonists to reduce GI motility in IBS-D patients is another off-label use that can help to improve quality of life and reduce pain perception. The most commonly used opioid agonists are diphenoxylate and loperamide although there is a risk for dependence development which needs to be monitored[70,71].

Low-grade inflammation has been observed in some IBS patients, especially those with post-infectious IBS and small intestinal bacterial overgrowth (SIBO) which has led to the use of antibiotics and glucocorticoids[72-74]. An important consideration in the treatment of low-grade inflammatory processes is the choice of agents since the anti-inflammatory effects should remain localized and not influence the systemic immune system.

So far, the salicylate derivative mesalazine which is also used to treat Crohn's disease with a known inflammatory component has shown an increase in quality of life and symptom reduction in IBS-D patients[75]. Glucocorticoids may provide benefits in IBS although this has only been shown in animal models to date and is based on the observation that patients on oral glucocorticoids show a lower incidence of IBS[76,77]. Rifaximin remains the only antibiotic that has been tested in IBS patients and has shown a moderate improvement in GI symptoms and quality of life whereas other antiinfectives such as nystatin and tetracyclines did present with unacceptable systemic side effects and a low responder rate[78-81].

A number of new targets and accompanying drugs are being developed and tested which may provide additional benefits in the treatment of IBS[19,82]. The coming years will show if they are effective and associated with less side effects compared to the currently available pharmacological options.

Aside from the pharmacological treatments which are often limited by significant side effects, up to 50% of patients are self-medicating using herbals and dietary supplements or other CAM approaches to improve their quality of life and reduce IBS symptoms[38,83,84]. Further increases in the use of CAM are a result of underdiagnosed or misdiagnosed IBS since the differential diagnosis of IBS can be complicated and delayed[40,85,86]. This review addresses the current state of CAM use in IBS and how both CAM and conventional therapeutic treatment can be used in synergy as an integrative approach to treating IBS and providing patients with the best possible quality of life.

COMPLEMENTARY AND ALTERNATIVE MEDICINES

The wide variety of CAM approaches includes basic changes in diet and lifestyle such as increased fiber intake or regular exercise as well as specific use of herbal medicines, combination products, mechanical interventions (acupuncture or massage), or behavioral therapy (cognitive behavioral therapy, relaxation techniques, and hypnotherapy)[43,84]. CAM is often used for chronic health conditions either alone or in conjunction with pharmacological treatment options. About half of IBS patients reported using at least one CAM treatment alone or in addition to their prescription medicine[42,87]. A lack of evidence-based clinical trials often limits the available knowledge about the efficacy of CAM in disease conditions, which is why the ACG Task force on IBS reports that CAM have not consistently demonstrated a strong positive outcome[2]. Recent systematic reviews, however, show indications that various CAM modalities may benefit IBS patients and increase their quality of life[42,43,84,88]. Since IBS can present with varying symptoms and severity even with daily or weekly fluctuations within the same patient, the effectiveness of CAM may at times appear inadequate or questionable by patients themselves. The most commonly used CAM interventions that have been evaluated in clinical trials, are dietary changes, use of probiotics, exercise, single herbal extracts, herbal combination products, hypnotherapy, acupuncture, and relaxation techniques. All of these approaches are discussed in more detail.

Diet and lifestyle modifications

Diet modifications are not generally considered CAM and usually are the first step in reducing IBS symptom severity even before pharmacotherapy is initiated (Table 1). However, exclusion diets can often be supplemented with CAM to reduce specific symptoms such as bloating and distension[2,89]. Exclusion diets may benefit patients with a known allergy and those in post-infectious IBS or SIBO[40,51,52] and consists of removing wheat, dairy products, eggs, coffee and caffeinated beverages, yeast, potatoes, and citrus fruits[52]. Despite the reported successes with exclusion diets, they may, at times, be hard to follow thereby resulting in bouts of increased IBS symptom severity. While dietary restrictions may benefit some IBS patients, entirely skipping meals actually has shown to worsen IBS symptoms[52,90].

Some contribution to intestinal symptoms may come from a diet that is high in fat, carbohydrates, and sugar alcohols. It has been shown that increased fat consumption is linked to increased stool numbers and diarrhea and therefore should be considered as a factor in worsening IBS-D[90,91]. Fructose-rich food and beverage items (soft drinks, baked and packaged goods, cereals) can also aggravate flatulence, abdominal discomfort, and diarrhea and should therefore be monitored in IBS patients[51,52]. Especially poorly absorbed sugar alcohols that are present in diet soft drinks and low carbohydrate foods can exacerbate GI symptoms[51,92]. Together with general restrictions on carbohydrate intake, lactose intolerance and malabsorption appear to be more prevalent in IBS patients. If lactose is not absorbed from the GI tract it is metabolized *via* the gut bacteria and leads to increased bloating, distension, and diarrhea which can aggravate IBS symptoms[93-95].

Fiber is often recommended as a dietary change to reduce global IBS symptoms but the clinical data to date are less clear. It has been shown that soluble fiber can lower GI symptoms in IBS-C although the data supporting it is highly variable both in the amount of fiber consumed (ranging from 5-30 g/d) and the duration of the trials (3-16 wk)[51,91,96,97]. Fiber, regardless soluble or insoluble, was not able to reduce pain perception in IBS patients and specifically insoluble fiber such as nuts and whole grains may exacerbate IBS symptoms overall[97].

An important lifestyle adjustment that should be recommended to IBS patients is regular exercise. Mild exercise or physical activity has been shown to reduce IBS symptoms and alleviates bloating and gas production in several studies[98,99]. Since regular exercise also helps to increase gastrointestinal motility it is beneficial in IBS-C patients with primary low GI movement and hard stools[100]. As part of exercise, yoga has been investigated due to its low impact on joints and its relatively targeted postures

Table 1 Clinical trials on diet and exercise interventions for irritable bowel syndrome

Intervention	Study design	Sample size	Outcome	Ref.
Acceptability questionnaire	Anonymous survey	256	Most acceptable were tablets (84%), diet and lifestyle changes (82%), yoga (77%); less acceptable were acupuncture (59%) and suppositories (57%)	[40]
Food elimination	Open label pilot study	20	Significant improvements in stool frequency ($P < 0.05$), pain ($P < 0.05$), and IBS-QOL ($P < 0.001$)	[89]
Diet and lifestyle	Cross-sectional study	1717	Significant difference between IBS and non-IBS participants in regards to residential type (OR = 1.27) and frequency of meals (OR = 1.69)	[90]
Diet and lifestyle	Questionnaire	983	BMI was associated with abdominal pain and diarrhea, healthier diet and physical activity were associated with fewer GI symptoms	[91]
Diet	3-way cross-over study	22	IBS-D patients showed significant increase in small bowel and mucosal permeability for mannitol and lactulose sugars compared to healthy controls	[92]
Diet	Questionnaire	1978	Potential for higher lactose intolerance incidence in patients with IBS compared to healthy patients	[93]
Diet	Case-control study	177	Symptomatic lactose intolerance more frequent in patients with IBS than healthy subjects, but incidence of lactose intolerance not different between groups	[94]
Diet	Case-control study	120	Lactose intolerance resulted in more frequent self-reported symptoms in patients with IBS-D than controls ($P < 0.001$, OR = 6.25), IBS-D patients consumed significantly less dairy products ($P = 0.04$)	[95]
Exercise	Randomized, controlled trial	56	No difference in quality of life between exercise and usual care groups, exercise group presented with significant less symptoms of constipation after 12 wk intervention	[98]
Exercise	Cross-over study	8	Gas retention during rest was associated with significant abdominal symptoms in IBS patients ($P < 0.01$), symptoms improved during exercise ($P < 0.05$) compared to rest	[99]
Exercise	Descriptive comparative study	89	Women with IBS report less physical activity ($P < 0.05$), women with IBS who were physical active reported significantly less symptoms of fatigue ($P = 0.003$) compared with the ones with IBS who were physically inactive	[100]
Yoga	Randomized cross-over study	25	Lower functional disability ($P = 0.073$) and anxiety levels ($P = 0.09$) in the yoga group compared to the waitlist group, significantly lower GI symptoms ($P < 0.01$)	[101]
Yoga	Randomized parallel design	21	Similar reductions in symptoms after 2 mo for yoga and the group receiving loperamide in IBS-D patients	[102]

IBS: Irritable bowel syndrome; QOL: Quality of life; BMI: Body mass index; IBS-D: Diarrhea-predominant IBS; GI: Gastrointestinal.

that can help to reduce GI symptoms[101,102]. Pranayama yoga administered twice daily has been shown to increase sympathetic tone and may benefit IBS-D patients that present with decreased sympathetic activity to the same degree than daily loperamide administration in the control group[102].

Herbal medicines and supplements

Aside from diet and lifestyle changes, another commonly used CAM intervention that is often self-administered is the use of herbal supplements either as single herbs or in combination products (Table 2). A few well-designed clinical studies have been performed on a number of such herbal supplements but in general the current knowledge remains limited to make a definitive judgment about their effectiveness in treating IBS symptoms. Many of the commonly used supplements have evolved from folk and traditional applications as remedies for gastrointestinal disorders.

The use of peppermint extracts has been studied in a number of clinical trials which evaluated the administration of enteric coated peppermint oil capsules to IBS patients[103-107]. The duration of the trials ranged from 4-8 wk and was not divided into specific IBS subtypes. The trials showed a significant reduction in abdominal pain and severity compared to placebo after 4 wk and a significant increase in quality of life although the effects did not last once peppermint was discontinued[108-110]. The spasmolytic effects of peppermint oil have been well known in folk medicines and mainly been attributed to the presence of mono- and sesquiterpenes[110]. Peppermint oil has also been recommended by the American Academy of Pediatricians as well as received a positive evaluation from the Task force on IBS of the ACG[2,111] although caution is advised for its use in young children due to its side effects of causing respiratory depression and heartburn[111]. Peppermint oil appears to be more potent in exerting the spasmolytic effects than aqueous extractions such as teas since it allows for a more concentrated dose of the proposed active ingredients[112].

Hydroalcoholic extracts from artichoke leaves have also been used to reduce IBS symptoms and evaluated in at least two clinical studies[113,114]. Artichoke has long been used as a digestive aid which aims to reduce bloating, abdominal pain and cramps, as well as reducing both diarrhea and constipation through normalization of GI motility[115]. Both studies were conducted as part of a post-marketing surveillance which may limit the credibility of the results due to limited influence on the study design (no double-blinding, no placebo control) and potential consumer bias. Both trials report a significant improvement in IBS symptoms, specifically in normalizing GI motility and reducing bloating as well as relieving distension and abdominal pain and cramps[113]. Since the change in IBS symptom severity was compared between baseline and follow-up, the results provide limited evidence of the effectiveness compared to placebo or standard pharmacological treatment. Given the high placebo responder rate,

Table 2 Clinical trials on Herbal medicines and supplements for irritable bowel syndrome

Intervention	Study design	Sample size	Outcome	Ref.
Peppermint oil	Randomized, double-blind, placebo-controlled study	99	Peppermint oil (Colpermin®) group showed significant symptom improvement ($P < 0.05$) compared to placebo group after 1 mo	[104]
Peppermint oil	Randomized, placebo-controlled study	18	Peppermint oil significantly reduced GI symptoms ($P < 0.01$) after 3 wk compared to placebo	[106]
Peppermint oil	Randomized, double-blind. Placebo-controlled study	57	Total IBS severity score was significantly decreased after 4 wk of treatment ($P < 0.009$) and after 2 mo ($P < 0.01$) in the peppermint oil group compared to placebo	[108]
Peppermint oil	Randomized, double-blind, placebo-controlled study	90	Significant reduction in IBS symptoms, no abdominal pain in more patients in the peppermint oil group compared to placebo ($P < 0.001$), less severe abdominal pain in peppermint oil group ($P < 0.05$) in peppermint oil group after 2 mo	[109]
Peppermint oil	Randomized, double-blind, placebo-controlled study	65	Significant reduction in abdominal pain in peppermint oil group compared to placebo group ($P < 0.001$), but pain score increased 2 wk after completion of trial	[110]
Artichoke leaf	Post-marketing surveillance	279	Significant reduction ($P < 0.05$) in overall IBS symptoms after 6 wk of treatment	[113]
Artichoke leaf	Post-marketing surveillance in IBS with concomitant dyspepsia	209	Significant reduction in Nepean Dyspepsia Index after 2 mo ($P < 0.001$) and normalization of bowel pattern ($P < 0.001$)	[114]
Turmeric	Partially blinded, randomized, two-dose pilot study	207	Reduction in IBS prevalence in both treatment groups (1 or 2 tablets) compared to baseline ($P < 0.001$) after 2 mo intervention, no significant differences between groups	[116]
Curcuma and fumitory	Randomized, double-blind, placebo-controlled study	106	No significant differences between curcuma, fumitory, and placebo groups in abdominal pain ($P = 0.81$) and distension ($P = 0.48$) after 3 mo	[117]
STW5	Randomized, double-blind, placebo-controlled study in patients with dyspepsia	137	Significant decrease in gastrointestinal symptom score between STW5 and placebo ($P < 0.001$)	[118]
STW5	Randomized, double-blind, placebo-controlled multicenter study in patients with functional dyspepsia	315	Significant decrease in gastrointestinal symptom score between STW5 and placebo ($P < 0.05$) after 2 mo intervention	[119]
STW5	Randomized, double-blind, placebo-controlled multicenter study	203	Significant reduction in abdominal pain scores for STW5 ($P = 0.009$) and STW5-Ⅱ ($P = 0.005$) and IBS-SSS ($P = 0.001$ for STW5 and $P = 0.0003$ for STW5-Ⅱ) compared to placebo after 4 wk intervention	[120]
Padma Lax	Randomized, double-blind, placebo-controlled pilot study	61	Significant improvement in global IBS symptom scores compared to placebo ($P < 0.05$) following 3 mo intervention	[123]
TCM	Randomized, double-blind, placebo-controlled study	119	No significant improvements in IBS global symptom score between TCM and placebo group at week 8 ($P = 0.38$) and week 16 ($P = 0.62$)	[129]

IBS: Irritable bowel syndrome; TCM: Traditional chinese medicine; SSS: Symptom severity scale.

artichoke leaf extracts will require additional trials that are more rigorous in terms of study design.

Turmeric, a spice traditionally used in Asian cuisine where it often provides both for taste and color improvement, was evaluated in IBS patients and indicated decreases in IBS symptoms and increased quality of life if given in two different doses of 72 and 144 mg per day over 8 wk[116]. However, this study again lacks a double-blinded and placebo-controlled design which reduces the strength of the presented data. Another double-blind placebo controlled study compared curcuma extract from which turmeric is derived with a placebo and a fumitory extract[117]. Both curcuma and fumitory extracts did not show any significant improvements in abdominal pain and distension compared to the placebo group.

All single herbal supplements discussed have a strong folkloric and traditional background as digestive aids and it is therefore not surprising that to date trials with such herbal supplements are focused on these single extracts.

Combination products of herbal extracts add to the wide range of available supplements used in the self-treatment of IBS. The first combination product which has received some interest from patients and healthcare providers alike is Iberogast®, a mixture of nine herbal plant extracts that was originally mainly used for functional dyspepsia in Germany[118,119]. The product has been on the market for more than 30 years. Iberogast® for use in IBS symptoms was investigated in 208 patients with various IBS subtypes in the United States[120]. This study adheres to the clinical trial standards by utilizing a randomized, double-blind, placebo-controlled study protocol over a 4 wk period. The extract consists of liquid extracts from chamomile flowers, bitter candytuft, angelica root, caraway fruits, milk thistle, lemon balm leaves, greater celandine, licorice root, and peppermint leaves[121]; all of which have been used in folklore and traditional medicines to aid in digestive disorders. The study indicates that Iberogast® significantly improves quality of life and reduces abdominal pain in IBS patients[122] which appears to be mediated through influences on serotonin, acetylcholine, and opioid receptors in the GI tract[121]. Despite the positive outcome, further research is required to support the findings of this

Table 3 Clinical trials on mechanical complementary and alternative medicines interventions for irritable bowel syndrome

Intervention	Study design	Sample size	Outcome	Ref.
Physical activity	Randomized study	75	Significant decrease in IBS-SSS between physical activity and placebo group (P = 0.003)	[133]
Reflexology	Randomized, single-blind, placebo-controlled study	34	No significant difference between foot reflexology and non-reflexology massage group	[134]
Acupuncture	Randomized, single-blind, placebo-controlled study	230	Acupuncture and sham acupuncture significantly improved IBS-GIS scores compared to waitlist group (P = 0.001), no difference between acupuncture and sham acupuncture during 3 wk intervention	[143]
Acupuncture	Randomized, single-blind, placebo-controlled study	43	Significant improvements (P = 0.022) in quality of life for both acupuncture and sham acupuncture compared to baseline after 10 intervention sessions (5 wk), no differences between acupuncture and sham acupuncture	[144]
Acupuncture/ moxibustion	Randomized, single-blind, placebo-controlled study	29	Significant reduction in IBS-SSS in acupuncture/moxibustion group after 4 wk compared to sham acupuncture/moxibustion group (P = 0.01)	[146]
Yoga	Observational pilot study (adolescents)	20	Decrease in pain frequency (P = 0.031 for 8-11 yr old and P = 0.004 for 12-18 yr old) and pain intensity (P = 0.015 in 8-11 yr old) after 10 yoga sessions compared to baseline, decrease in pain frequency was maintained for 3 mo following intervention (P = 0.004 for 8-11 yr old)	[149]

IBS: Irritable bowel syndrome; GIS: Global improvement scores; SSS: Symptom severity scale.

study and a few case reports from patients and healthcare providers alike. Iberogast® has been recognized by the ACG task force on IBS as a potential complementary treatment to reduce certain IBS symptoms[2].

A Tibetan preparation of twelve different plant extracts, commonly referred to and marketed as Padma Lax, was tested in IBS-C patients over the course of three months in a randomized and double-blinded observational study and showed significant improvements over a placebo in reducing constipation, abdominal pain, and flatulence[123]. The dose had to be adjusted for some of the patients since they developed loose stools that bordered on diarrhea indicating that this combination herbal product may have significant potential as a laxative. The ingredient list includes the known laxatives rhubarb root, cascara bark, and nux vomica seeds which have been used as strong laxatives in severe constipation in traditional medicines across the world[124]. It has been proposed that some of the herbs exert the laxative effect through activity on cholinergic receptors as antagonists to reduce GI contractility[124,125].

Traditional Chinese medicine (TCM) has provided a range of different treatment approaches over the centuries, among them a number of TCM herbal mixtures that are specifically formulated based on the patients' symptoms[126]. Such individualized medicine is not uncommon but has the obvious limitation of resisting standardization and fitting the rigorous clinical trial design that is used as a major determinant of effectiveness in Western medicine. *Tong Xie Yao Fang* (TXYF) is one such TCM that has been studied in a few clinical trials but often adjustments were made to the herbal combination based on the predominant IBS symptom presentation[127-129]. A review of 12 studies with modified TXYF preparations reached the general conclusion that the extracts improved IBS symptoms, in particular abdominal pain, distension, flatulence, and diarrhea[128]. However, the study design and end points were diverse between these studies complicating a direct comparison of outcomes. A more streamlined and standardized approach to TXYF trials is warranted.

Overall, the use of single and combination herbal supplements appears promising but should be approached with caution given the lack of rigorous larger clinical trials. The strongest evidence for the use of herbal medicines is currently available for peppermint oil preparations and the herbal combination product Iberogast®.

Mind-body therapies

Since there is evidence of the involvement of the enteric nervous system or brain-gut axis in the pathophysiology of IBS, the use of mind-body interventions as CAM treatments may provide benefits in relieving symptoms. Mind-body therapies are interventions that primarily "focus on the interactions among the brain, mind, body, and behavior with the intent to use the mind to affect physical functioning and promote health"[130]. Yoga, Tai-chi, meditation, hypnotherapy, deep-breathing exercises, relaxation techniques, and acupuncture all fall under this definition. While yoga, relaxation techniques, and acupuncture are commonly used as CAM therapies, they involve both a physical and psychological component with a focus on influencing physical functions through mechanical interventions. Meditation, hypnotherapy, and CBT do not involve a mechanical component but rather seek to change physiological function through psychological reprogramming entirely[131,132]. Mind-body therapies have been evaluated for their potential application as CAM in IBS. Clinical evidence supporting the use of yoga, relaxation, acupuncture, hypnotherapy, and cognitive behavior therapy is indicating that these CAM interventions show improvement in IBS symptoms and overall quality of life.

Mechanical CAM interventions

Mechanical interventions are based on direct body interventions such as massage, acupuncture, yoga, and physical exercise (Table 3)[131]. While some interventions may

not benefit patients with IBS because of the significant physical involvement that may cause a temporary worsening of symptoms, low impact physical exercise such as aerobic training, bike riding, and muscle strengthening have shown benefits in maintaining GI function and reducing flatulence and gas production[90,99,100,133]. One study indicated that strenuous exercise shows an inverse relationship with IBS symptoms[91] while another study pointed to reduction of constipation in IBS-C patients with mild exercise levels[98]. Johannesson and colleagues evaluated the impact of regular exercise on IBS symptoms severity scores compared to a control group and found a significant reduction in symptoms over the course of 12 wk[133]. It is therefore important to emphasize that a patient should start off with low impact and light exercise regimens that are not exhausting or causing increases in abdominal pain or overall IBS symptoms. As such, mild physical exercise may be considered a lifestyle change rather than an actual CAM intervention, but guided assistance may benefit IBS patients who seek counseling and advice on respective exercise regimens for their condition.

To date there is little information about the potential impact of massage and reflexology treatments on IBS symptoms. One study compared the use of foot reflexology massage to non-reflexology foot massage and could not find any significant difference in the small study of 34 patients[134]. However, case reports have indicated that gut-directed massage may relieve specific symptoms such as bloating and chronic constipation although such reports were not specific to IBS patients[135-137]. It therefore remains unknown if reflexology or massage techniques can provide benefits to IBS patients.

A number of trials investigated the effect of gut-directed and general acupuncture on symptom relief in IBS patients. It is well known that acupuncture can affect physiological functions in a number of conditions through regulating various neurotransmitter systems. It has been shown that application of acupuncture in IBS patients targets serotonergic, cholinergic, and glutamatergic pathways, can lower blood cortisol levels related to stress, and can increase the concentration of endogenous opioids to reduce visceral and global pain perception[138-140]. A complicating factor in the study of acupuncture effects are adequate comparison groups. One commonly used comparison group is sham acupuncture which uses needles as well so to suggest to patients that they are being treated when the practitioner does not utilize the specific acupuncture points and only superficially applies needles[141,142]. What appears to be a contributing factor to the effectiveness of acupuncture is the patient-practitioner relationship, especially in IBS patients where an underlying psychological contribution to IBS symptoms can be suspected[58,59]. In one study, a sample of 230 IBS patients were randomly assigned to receive either acupuncture, sham acupuncture, or remain on a waitlist for the duration of the trial[143]. Initially both the true acupuncture and sham acupuncture groups received only sham acupuncture for 3 wk followed by 3 wk of true acupuncture in half of these patients while the other half continued receiving sham acupuncture. Both groups showed significant improvement in global IBS symptoms compared to the waitlist control group, but the patients receiving true acupuncture did not differ from the sham acupuncture group thus complicating interpretation of results related to the actual acupuncture points being used. Other studies using sham acupuncture as a comparator have also found that improvements in quality of life and IBS symptoms did not differ from true acupuncture points thus potentially indicating that acupuncture for IBS is primarily a placebo response[144,145]. When combined with moxibustion, acupuncture has shown significant improvements in IBS symptoms with reduced abdominal pain, gas and bloating being reported in one study including 29 subjects over a 4 wk, eight session intervention[146]. A Cochrane meta-analysis of studies including acupuncture suggests that further studies are warranted to confirm the beneficial effects of such treatments in IBS patients[147].

A mechanical intervention that has been studied in IBS is yoga as a specific form of exercise and focused relaxation technique combined[148]. Yoga consists of different poses accompanied by a specific breathing pattern to focus attention on muscle contraction and relaxation. There are certain poses that can be utilized to focus on GI tract motility and abdominal pain perception. There are only few trials conducted with yoga as the intervention but a number of indicators suggest that yoga may provide relief of IBS symptoms. One study in 22 male patients with confirmed IBS-D compared yoga poses and breathing techniques to conventional treatment over the course of 8 wk[102]. Overall GI symptoms were reduced in both groups at the end of the study but the yogic intervention group showed a higher parasympathetic reactivity leading to improved IBS symptom outcomes whereas the control group presented with increased gastric activity. Another small clinical trial compared the use of yoga in adolescents to a wait list group over the course of 8 wk[101]. In the first 4 wk, the yoga intervention group received instructions on daily yoga practices and continued the poses after the first 4 wk. The control wait list group received yoga intervention after 4 wk for another month at which point both groups were compared. This preliminary study indicates that yoga intervention over the course of a 2 mo period improves GI symptoms in adolescent IBS patients and is well received by youth. Another small yoga intervention study conducted in 20 children aged 8-18 years with IBS or functional abdominal pain indicates that yoga does decrease both pain frequency and pain intensity at the end of intervention and that this effect also persisted for at least another 3 mo after the intervention has ended[149]. In addition, adolescents in this trial reported increased quality of life throughout the intervention and during follow-up. Although not many studies have been conducted using yoga as a CAM intervention, the current data suggests that it may provide benefits in IBS patients by alleviating both pain and GI symptoms.

Table 4 Clinical trials on psychological complementary and alternative medicines interventions for irritable bowel syndrome

Intervention	Study design	Sample size	Outcome	Ref.
Hypnotherapy	Pre- and post-assessment	23	Normalized hypersensitivity pain threshold in hypersensitive group (P = 0.04) after 12 wk of treatment, no significant change in hyposensitive and normosensitive groups	[154]
Hypnotherapy	Randomized controlled trial in children with functional abdominal pain or IBS	53	Significant reduction in pain scores in hypnotherapy group (P < 0.001) compared to standard medical therapy at 1-yr after intervention	[159]
Hypnotherapy	Questionnaire	83	69% of patients were either satisfied or very satisfied with hypnotherapy following 12 wk intervention, overall improvement in quality of life and GI symptoms	[160]
Hypnotherapy	Randomized, placebo-controlled study	138 in two studies (90 and 48)	Significant reduction in IBS symptoms in hypnotherapy groups (P < 0.05) compared to supportive therapy after 3 mo of intervention	[161]
Hypnotherapy	Randomized, placebo-controlled study	90	Significant improvement in overall IBS symptoms in gut-directed hypnotherapy and medical treatment group compared to medical treatment group alone (P = 0.046) after 12 wk; improvement remained up to 12 mo after intervention in hypnotherapy group (P = 0.004) compared to medical treatment alone	[162]
Hypnotherapy	Pre- and post-assessment	75	Group hypnotherapy decreased symptom severity significantly (P < 0.01) at 3, 6, and 12 mo post-intervention	[163]
Hypnotherapy	Retrospective analysis	208	Significantly higher use of hypnotherapy (P < 0.001) by initial responders *vs* non-responders at 2-7 yr follow-up, in total 87% of participants reported hypnotherapy to be useful	[164]
Cognitive behavior therapy	Randomized-comparator-controlled study in patients with functional bowel disorders	431	CBT was more effective than education (P = 0.0001) and desipramine was more effective than placebo (P = 0.01) after 12 wk of treatment as assessed by treatment satisfaction	[170]
Cognitive behavior therapy	Randomized, placebo-controlled study in patients with functional bowel disorders	397	No significant differences between treatment arms for desipramine, cognitive behavior therapy, and placebo groups	[171]
Cognitive behavior therapy and mindfulness training	Randomized controlled trial	195	Internet-delivered cognitive behavior therapy resulted in adequate relief of IBS symptoms that was significant compared to internet-delivered stress management at 6 mo follow-up (P = 0.004)	[173]
Cognitive behavior therapy	Randomized controlled trial	149	Significant reduction in symptom severity scores in CBT plus mebeverine group compared to mebeverine alone at post-treatment and 3, 6, and 12 mo follow-up (regression P = 0.001)	[174]
Psychotherapy [cognitive behavior therapy]	Randomized controlled trial	50	Rome-Ⅱ scores significantly decreased (P = 0.001) in patients receiving CBT in conjunction with standard medical care compared to standard medical care alone after 2 mo intervention	[175]
Cognitive behavior therapy	Randomized controlled trial	28	Psychosocial functioning was significantly improved (P = 0.004) in patients receiving CBT in addition to standard medical care compare to standard medical care alone at 3 mo follow-up	[176]
Cognitive behavior therapy	Randomized controlled trial	76	Cognitive behavior therapy presented with significant improvements compared to stress management and attention control groups in reducing visceral sensitivity (P < 0.0001) compared to baseline at 3 mo follow-up	[177]
Cognitive behavior therapy	Randomized, placebo-controlled study	85	Internet delivered CBT reduced several IBS symptom parameters (total pain, diarrhea, bloating primary symptoms all P < 0.001) after 10 wk of intervention compared to discussion board control group; quality of life and visceral sensitivity were also significantly improved (P < 0.001) after 3 mo follow up	[179]
Mindfulness training	Randomized controlled trial	75	Women in mindfulness training group showed significant reduction (P = 0.006) in IBS symptom severity compared to support control group after 8 wk of intervention which remained significant at 3 mo follow-up (P = 0.001)	[180]
Cognitive behavior therapy	Retrospective analysis	75	Long-term follow-up after 15-18 mo of original intervention resulted in lasting significant reductions in visceral sensitivity (P < 0.05), increase in quality of life (P < 0.05), and gastrointestinal symptoms (P < 0.05)	[181]

IBS: Irritable bowel syndrome; CBT: Cognitive behavior therapy; GI: Gastrointestinal.

Psychological CAM interventions

While mechanical interventions can provide IBS symptom relief, compliance can often be an issue as well as limited physical ability to follow the treatment (especially with exercise and yoga). Other mind-body CAM approaches are solely based on psychological interventions with hypnotherapy and cognitive behavior therapy showing the most clinical evidence of effectiveness (Table 4).

Hypnotherapy is based on initiating a suggestive state for the patient similar to sedation but without loss of consciousness that allows for heightened senses and control over body functions affecting mood, pain perception, cardiovascular responses, and gastrointestinal motility[150,151]. Hypnosis and hypnotherapy have been used for various applications foremost for the treatment of acute and chronic pain conditions but also to improve mood or change certain undesirable behaviors[152,153]. It has been shown that gut-directed hypnotherapy can alleviate IBS symptoms comparable to current pharmacological treatment approaches[154-156]. Several clinical studies and meta-analysis indicate that 8-12 weekly hypnotherapy sessions can improve pain, GI motility, mood (improving depressive and anxiety disorders), and overall quality of life of IBS patients significantly even in the absence of pharmacological treatment[157-161]. Interestingly, in a number of studies during follow-up the beneficial effects of hypnotherapy remained for at least 10 mo even if patients did not continue therapy[55,155,162-164]. A Cochrane database review examined available studies and found a positive effect associated with hypnotherapy although the effect size could not be determined due to the heterogeneity of study designs and the relatively small sample sizes[54]. Although there is clinical evidence for the use of hypnotherapy in treating IBS symptoms, further research utilizing a homogenous study design needs to be emphasized to support its benefits.

In contrast to hypnosis where the patient is being influenced and subjected to a subconscious suggestive state that influences physiological processes, CBT takes a more direct approach in influencing the conscious awareness of IBS symptoms and attempts to enable the patient to overcome the negative attitudes they may have towards their chronic condition[165-167]. Similar to hypnosis, patients are enabled to change a negatively perceived situation into a positive outlook which then affects the actual symptoms. CBT has been shown to be effective in improving symptoms in a number of chronic disorders such as obesity, chronic pain, insomnia, or depressive disorders[166,168,169]. While hypnosis can be utilized to directly affect perception of symptoms, CBT is more geared towards enabling patients themselves to change their behavior and thought processes about their condition. In IBS patients, symptom aggravation may often occur when patients worry about the condition and focus their thoughts around them[170,171]. CBT has been shown to improve both the quality of life and reduce symptom severity in IBS patients, especially in regards to pain perception and comorbid depressive and anxiety disorders[170,172,173]. Comparing the use of CBT as a CAM approach to treating IBS with conventional pharmacological treatment indicates that it does not only improve the direct IBS symptoms such as pain and GI dysmotility, but in addition also benefits mood and coping with the condition[174-177]. Although the effects of CBT lasted past the intervention period in some of the trials, the beneficial effects seemed to wane over time which may indicate a long-term supportive treatment with CBT at least in frequent intervals following the initial treatment period. Currently, an ongoing irritable bowel syndrome outcome study evaluates the long-term effects of self-versus clinician-administered CBT on IBS symptoms and the economic benefit of this intervention[178].

Closely related to CBT as a CAM intervention is mindfulness exercises that promote acceptance instead of control of IBS symptoms[179,180]. This technique is often delivered in conjunction with CBT and is more patient-centered to increase quality of life through coping mechanisms. In conjunction with CBT, mindfulness exercises have shown a reduction in IBS symptoms beyond the intervention period up to 3 years[173,181].

INTEGRATIVE APPROACHES AND CLINICAL IMPLICATIONS TO TREATING IBS

Most of the clinical trials that have been discussed focus on the comparison between CAM approaches and standard care with pharmacological therapies in treating IBS symptoms. This separation often creates a painful choice for patients that need the immediate relief with medication but also seek long-term alleviation of the symptoms through a pro-active approach. Integrating both conventional pharmacological care and CAM treatments to provide the best symptom relief and highest quality of life to IBS patients should therefore be considered by healthcare providers. A number of clinical trials - although small in sample sizes and number - have shown that a combination of pharmacotherapy with a CAM treatment is superior to either treatment alone. As long as the CAM treatment does not interfere or interact with the pharmacological treatment, both can coexist in the treatment of IBS symptoms.

A study evaluating the use and cost of CAM by patients with functional bowel disorders (including IBS patients) reported a number of interesting results[85]. The most common types of CAM were ginger, massage therapy, and yoga with a median yearly cost of $200, which was considered significantly less compared to standard pharmacotherapy. Furthermore, the use of CAM was associated with female gender, higher education, and comorbid anxiety disorders. Although most of the patients using CAM (35% of 1012 participating patients) were not referred by their healthcare provider, those who received a recommendation from their primary care physician followed the advice. This indicates the important role of the healthcare provider as a mediator and facilitator for patients to seek CAM treatments. Several of the CAM approaches discussed in this review have been recommended or given at least positive consideration by current professional organizations (ACG and the British Society for Gastroenterology) to be considered in the treatment of IBS in addition to pharmacotherapy[2,12]. Another important point that the study reveals is that CAM use is not based on dissatisfaction with pharmacotherapy but seems to be rather linked to higher comorbidity with de-

pressive and anxiety disorders as well as somatization of their condition[85]. CAM users in this study also showed a higher symptom severity on the IBS-SSS as well as gastrointestinal distension. The authors of the study conclude that CAM use can benefit patients with various functional bowel disorders and should be made more widely available potentially through providing insurance coverage. Medical professionals should recommend CAM approaches such as hypnotherapy, yoga, cognitive behavior therapy, or herbal supplements that have shown benefits to IBS patients that do not experience adequate symptom relief with pharmacotherapy alone.

In a study that evaluated a gap in effectiveness between current treatment approaches and treatment outcomes conducted in the United Kingdom, 32% of general practitioners reported an effectiveness gap for IBS patients in their practice[86]. The most common reasons given for the effectiveness gap were lack of effective treatments, adverse effects of current treatments, and unacceptability of available treatments by patients. These findings are supported by other studies which indicate a lack of treatment effectiveness in a significant percentage of IBS patients[40,43,182]. The use of CAM has been shown in randomized controlled trials as well as in systematic reviews to decrease or close the effectiveness gap thereby increasing quality of life and treatment outcomes for IBS patients. The authors of this study conclude that there is a discrepancy between available evidence for the effectiveness of CAM in various chronic conditions and its recommendation by current practice guidelines which often omit such approaches. In addition, patients appear to be more favorable towards trying CAM approaches than practitioners thereby most referrals are initiated by patients themselves or without including the healthcare provider when considering CAM treatments in addition to conventional care[86,183,184].

The integrative medical approach has gained significant traction in the last decade with the growth of the Consortium of Academic Health Centers for Integrative Medicine[185] to which many well respected United States institutions belong (Yale University, Stanford University, University of California, Johns Hopkins University, among others). Integrative medicine as a subdivision of medical practice is seeking to emphasize on patient-centered care and improve wellness and quality of life rather than limiting the treatment outcome to a specific disorder. The definition of integrative medicine remains somewhat elusive to date ranging from simply adding CAM treatments to conventional care to a more holistic healthcare approach overall. While the conventional approach to treating IBS is primarily founded on evidence-based clinical trials and extensive knowledge, the CAM approach to treating IBS also claims a long tradition of use and an increasing body of research supporting the benefits of CAM treatments in IBS. The debate over integrative medicine continues with defenders of conventional medicine indicating that such a definition should not exist because treatments with proven benefits will eventually become the standard of care no matter where they originated from. However, proponents of integrative medicine argue that excluding CAM or preventing patients from seeking CAM treatments in addition to conventional care will result in reduced quality of life and worse treatment outcomes. As mentioned above, this has been supported by various studies. It has been argued that even if a CAM treatment is not supported by sufficient evidence-based clinical trials, as long as it does not cause adverse effects or interfere with conventional therapy it should not be denied to patients seeking such treatments. Instead, physicians and healthcare providers should seek training or knowledge in integrative medicine to best support their patients. This applies especially to chronic conditions such as IBS that present with a high placebo response and where a number of CAM treatments - herbal therapies, probiotics, dietary changes, acupuncture, yoga, hypnotherapy, and cognitive behavior therapy - have shown benefits.

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Diagnostic utility of faecal biomarkers in patients with irritable bowel syndrome

Jan Däbritz, Jason Musci, Dirk Foell

Jan Däbritz, Gastrointestinal Research in Inflammation and Pathology, Murdoch Children's Research Institute, The Royal Children's Hospital Melbourne, Parkville 3052, VIC, Australia
Jan Däbritz, Jason Musci, Department of Paediatrics, Melbourne Medical School, University of Melbourne, Parkville 3052, VIC, Australia
Jan Däbritz, Dirk Foell, Department of Paediatric Rheumatology and Immunology, University Children's Hospital Münster, 48149 Münster, Germany
Author contributions: Däbritz J and Musci J contributed equally to this work; Däbritz J, Musci J and Foell D analysed and interpreted data; Däbritz J created tables and figures; Däbritz J and Musci J wrote the paper; Foell D critically reviewed and submitted the manuscript; Däbritz J, Musci J and Foell D read and approved the final version of the manuscript.
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Correspondence to: Dirk Foell, MD, Professor, Director, Department of Paediatric Rheumatology and Immunology, University Children's Hospital Münster, Röntgenstr. 21, 48149 Münster, Germany. dfoell@uni-muenster.de
Telephone: +49-251-8358178 Fax: +49-251-8358104
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Abstract

Irritable bowel syndrome (IBS) is a common functional gastrointestinal (GI) disorder characterized by unspecific symptoms. In clinical practice it is crucial to distinguish between non-inflammatory functional problems and inflammatory, malignant or infectious diseases of the GI tract. Differentiation between these involves the use of clinical, radiological, endoscopic, histological and serological techniques, which are invasive, expensive, time-consuming and/or hindered by inaccuracies arising from subjective components. A range of faecal markers now appears to have the potential to greatly assist in the differentiation of inflammatory bowel disease (IBD) and IBS. Faecal markers of neutrophil influx into the mucosa are reliable indicators of intestinal inflammation and their role has been mainly studied in discriminating IBD from non-IBD conditions (including IBS) rather than organic from non-organic diseases. Phagocyte-specific proteins of the S100 family (S100A12, calprotectin) are amongst the most promising faecal biomarkers of inflammation. Faecal leukocyte degranulation markers (lactoferrin, polymorphonuclear elastase and myeloperoxidase) have also been suggested as diagnostic tools for the differentiation of IBD and IBS. More recently, additional proteins, including granins, defensins and matrix-metalloproteases, have been discussed as differential diagnostic markers in IBD and IBS. In this review, some of the most promising faecal markers, which have the potential to differentiate IBD and IBS and to advance diagnostic practices, will be discussed.

Key words: S100A12; Calprotectin; Lactoferrin; M2-pyruvate kinase; Polymorphonuclear elastase; Defensins; Granins; Irritable bowel syndrome

Core tip: Faecal markers of intestinal inflammation represent a practicable, non-invasive, inexpensive and objective diagnostic tool to differentiate organic [inflammatory bowel disease (IBD)] and functional [irritable bowel syndrome (IBS)] gastrointestinal diseases. Faecal markers have the potential to be incorporated into standard clinical practice for the routine assessment of IBS and IBD. Neutrophil-derived faecal biomarkers show a high diagnostic accuracy in the differentiation of IBD *vs* IBS. They can provide reassurance to the physicians that the clinical diagnosis of IBS is correct. Future progress in our knowledge about the biology of these proteins and the underlying pathogenesis of IBS will help translate IBD/IBS research into patient care.

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IRRITABLE BOWEL SYNDROME

Irritable bowel syndrome (IBS) is one of the most common gastrointestinal (GI) disorders, with a reported prevalence of approximately 10% to 15% worldwide[1]. The exact pathogenesis of IBS is only partially understood but seems to be multifactorial. There is evidence that heritability and genetics, environment and social learning, dietary or intestinal microbiota, low-grade inflammation and disturbances in the neuroendocrine system of the gut play a central role[2]. There is no medical therapy established to alter the natural history of IBS and most traditional therapies (*e.g.*, bulking agents, antidiarrheals, antispasmodics) focus on improving individual symptoms. However, these symptom-based therapies have limited efficacy and as such novel and emerging therapies have been developed based upon the evolving understanding of the pathophysiology of IBS[3,4].

Though a variety of GI and extraintestinal symptoms and presentations are associated with IBS, it is primarily characterized by symptoms of abdominal pain or discomfort associated with an altered bowel function in the absence of any organic cause. Patients commonly report abnormal defecation ranging from diarrhoea to constipation, including a combination of the two, the degree of which can vary in both severity and duration[5,6]. Four subtypes of IBS were recognized: IBS with constipation (IBS-C), IBS with diarrhea (IBS-D), mixed IBS (IBS-M), and unsubtyped IBS (IBS-U). IBS presents a challenge to gastroenterologists, with several groups having attempted to define a set of standardized symptom-based criteria for the diagnosis of IBS. Although no symptom-based criteria have ideal accuracy for diagnosing IBS[7], the third iteration of the Rome criteria (Rome Ⅲ) and the Manning criteria are widely used by clinicians to diagnose IBS[8,9].

Since many GI disorders present with symptoms similar to IBS, it is important to exclude other causes. The diagnosis of IBS should be made using symptoms based on clinical criteria rather than excluding underlying organic disease by exhaustive investigation. Routine laboratory studies are normal in IBS and thus only a limited number of diagnostic studies are used to rule out other likely conditions. However, patients with alarm symptoms (*e.g.*, fever, weight loss, blood in stools, nocturnal of progressive abdominal pain), laboratory abnormalities, abnormal physical findings, and/or a family history of inflammatory bowel disease (IBD) or colorectal cancer (CRC) require more extensive evaluation (*e.g.*, imaging studies and/or colonoscopy)[2,3,10]. Otherwise, a limited number of diagnostic studies can rule out organic illness in the majority of patients and a sizeable number require no testing at all. However, whilst alarm symptoms ("red flags") may have a relatively modest predictive value for identifying organic disease, their presence as exclusion criteria would result in many missed cases of IBS[11]. It is this large symptomatic overlap between functional and organic disease, in conjunction with the current lack of a biochemical, histopathological, or radiological diagnostic tests for IBS, which engenders the need for more definitive diagnostic tools[2].

FAECAL MARKERS OF INTESTINAL INFLAMMATION

A simple, reliable, reproducible and non-invasive test, with the ability to differentiate IBD from other GI condition, such as IBS, would be of substantial clinical utility. Serological markers (*e.g.*, C-reactive protein, erythrocyte sedimentation rate) reflect the presence and intensity of a (systemic) inflammatory process and are not specific for intestinal inflammatory disease. Radiological and endoscopic techniques are invasive, time-consuming and/or expensive. Clinical disease (activity) scores are hindered by inaccuracies arising from subjective components. Faecal markers, however, offer a non-invasive approach to objectively measuring intestinal inflammation with the ability to differentiate organic and functional GI diseases. Stool markers are inexpensive, easily measured and therefore suitable for extensive use. Faecal markers include a heterogeneous group of substances that either leak from or are generated by the inflamed intestinal mucosa. The inflamed hyper-permeable gut mucosa is associated with increased protein cytokines and markers of neutrophil activation in faecal samples. Faecal markers of neutrophil influx into the mucosa are promising indicators of intestinal inflammation and their role has been mainly studied in discriminating IBD from non-IBD conditions (including IBS) rather than organic from non-organic diseases (Figure 1). Lactoferrin, polymorphonuclear (PMN) elastase and myeloperoxidase (MPO) are faecal markers of neutrophil degranulation. Of the proteins stored in neutrophilic granules, lactoferrin is the most accurate marker of intestinal inflammation. Importantly, lactoferrin, MPO and PMN elastase are not only expressed in neutrophils and show limited stability in stool samples at room temperature. Other faecal markers including alpha 1-antitrypsin, tumour necrosis factor alpha, lysozyme, and markers of eosinophil degranulation (*e.g.*, eosinophil protein X, eosinophil cationic protein) have also been described as markers of intestinal inflammation but their clinical utility and/or diagnostic accuracy is inferior and data on their role in differentiating IBD from IBS are lacking or very limited[12-20]. The utility of other faecal markers [*e.g.*, granins, defensins, matrix-metalloproteases (MMP)] in differentiating organic from functional disease has not been widely studied. More recently, neutrophil-derived S100 proteins have been identified as faecal markers for differentiating IBD and IBS. Proteins of

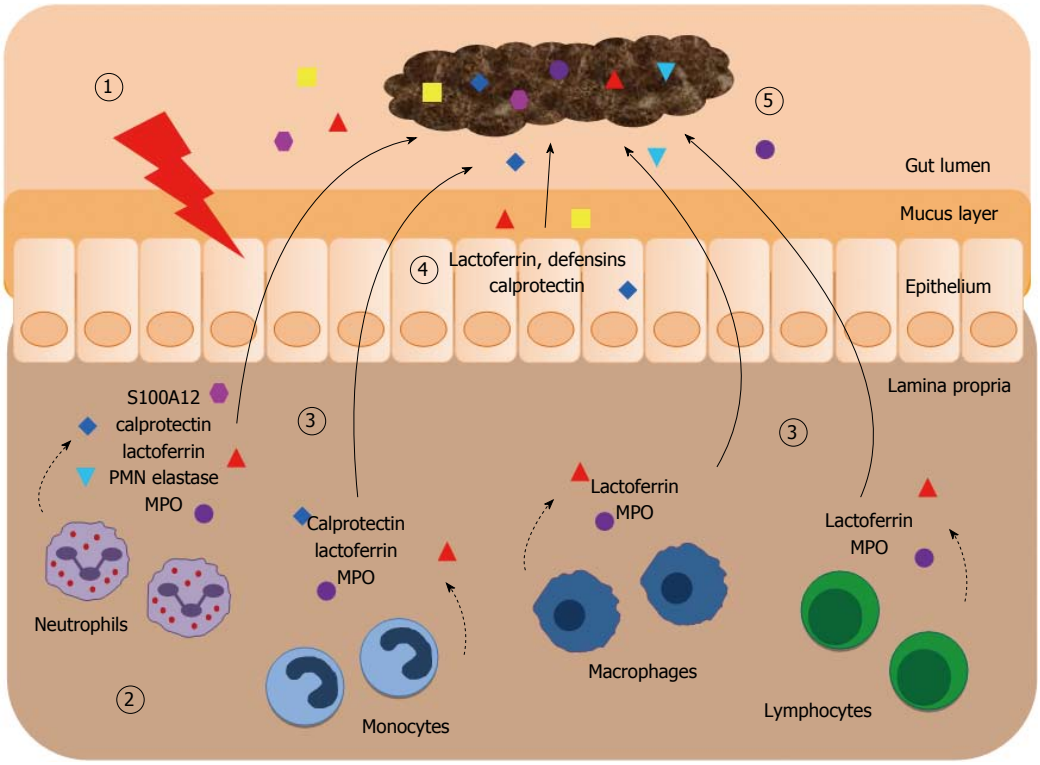

Figure 1 Faecal markers of intestinal inflammation. (1) Initially, unidentified triggers affect the epithelium and lead to an activation of the intestinal immune system; (2) The initiated immune response involves the influx of different innate immune cells (*e.g.*, granulocytes, monocytes, macrophages) and cells of the adaptive immune system (*e.g.*, T cells) into the affected mucosa. These cells actively secret inflammatory mediators or release granule proteins by cell degranulation. The contents of neutrophil granules [▲ lactoferrin, ▼ polymorphonuclear (PMN) elastase, ● myeloperoxidase (MPO)] have antimicrobial properties. The cytosol is the source of the damage associated molecular pattern proteins S100A8/A9 (◆ calprotectin) and S100A12 (●); (3) During early stages of intestinal inflammation these released proteins spill over from the mucosa into the gut lumen; (4) Some of these factors (including ■ defensins) are also released from the epithelium and the mucus layer; (5) In direct contact with the intestinal mucosa, the faecal stream contains the specific proteins of mucosal disease. The detection of these markers in faeces indicates the presence and degree of intestinal inflammation.

the S100 family [S100A8/A9 (calprotectin), S100A12] are molecules released from the cytosol by activated or damaged cells under conditions of cell stress, followed by pro-inflammatory activation of pattern recognition receptors. S100 proteins are remarkably resistant to degradation by faecal bacteria, making them suitable markers for gut wall inflammation[14]. Faecal S100A12 and calprotectin are highly sensitive and specific markers of intestinal inflammation and exert a strong influence upon the pathogenesis of IBD[21]. In this review, some of the most promising faecal markers, which have the potential both to differentiate IBD and IBS and to advance diagnostic practices, will be discussed (Figure 1).

CALPROTECTIN

S100A8 [also known as calgranulin A and myeloid-related protein 8 (MRP8)] and S100A9 (calgranulin B, MRP14) are members of the S100 calcium-binding protein family. Both proteins are linked to the innate immune system and expressed in granulocytes, monocytes/macrophages and epithelial cells (Figure 1)[14]. The two proteins exist in multiple isoforms, the most abundant of which is the S100A8/S100A9 heterodimer ("calprotectin")[22,23]. Calprotectin constitutes 60% of cytosolic protein in neutrophils and the influx of these neutrophils into the GI mucosa during inflammation is therefore proportional to the amount of measured faecal calprotectin[24,25]. Furthermore, calprotectin has not only shown resistance to degradation in faeces and stability at room temperature, but has also been reported to correlate well with ^{111}Indium-labelled granulocyte scintigraphy[24,26]. It is these favourable characteristic prerequisites for the validity of a faecal biomarker that have witnessed the emergence of calprotectin as one of the most studied faecal biomarkers for intestinal inflammation[27].

Elevated faecal calprotectin levels have been reported in multiple organic GI diseases when compared with functional GI diseases (Table 1). In a large-scale study, Tibble *et al*[28] determined that at a cutoff value of 10 mg/L, faecal calprotectin had a sensitivity of 89% and a specificity of 79% for detecting organic disease, which performed better than the respective values for a positive Rome I criteria diagnosis (85% and 71% respectively). Following this, Costa *et al*[29] discussed the value of setting a cutoff point determined by the collective results of complete GI investigations on all patients with chronic abdominal pain and diarrhoea. For example, by using a cutoff of 60 μg/g they were able to produce their optimal diagnostic accuracy, with a sensitivity of 81% and a specificity of 88%[29]. In another study, for patients presenting with lower GI symptoms, D'Incà *et al*[30] reported

Table 1 Studies investigating faecal markers in the differentiation of inflammatory bowel disease or healthy controls *vs* irritable bowel syndrome

Study	Marker	Cutoff value	Se	Sp	PPV	NPV	Subjects (*n*)	CD (*n*)	UC (*n*)	IBS (*n*)	HC (*n*)	Other (*n*)	Other diagnosis (*n*)	Verification
Kaiser *et al*[35] 2007	S100A12	> 0.8 mg/kg	86%	96%	98%	76%	195	32	27	24	24	88	Bacterial (65) and viral (23) enteritis	Endoscopy/histology; immunohistochemistry
	CP	> 50 mg/kg	63%	86%	90%	51%								
Sidler *et al*[42] 2008	S100A12	> 10 mg/kg	97%	97%	97%	97%	61	30	1	14	0	16	Reflux esophagitis (6), juvenile polyp (2); eosinophilic GI disorder (3), others (5)	Endoscopy/histology
	CP	> 50 mg/kg	100%	67%	75%	100%								
Tibble *et al*[26] 2000	CP	> 30 mg/L	100%	97%	-	-	276	31	0	159	56	30	Microscopic colitis (6), polyps (3), CRC (2), diverticulosis (19)	Radiology and/or colonoscopy
Tibble *et al*[28] 2002	CP	> 10 mg/L	89%	79%	76%	89%	602	102	87	339	0	74	Coeliac disease (12), diarrhea (14), CRC (7), colitis (6), small bowel enteropathy (21), diverticulosis (14)	Radiology and/or colonoscopy
Carroccio *et al*[32] 2003	CP	> 50 μg/g	66%	84%	83%	68%	158	18	0	55	20	65	Cow's milk/food intolerance (22), coeliac disease (23), CRC/polyps (3), diverticulosis (4), colitis (2), CD (9), giardiasis (2)	Endoscopy/histology (in selected patients only)
		> 100 μg/g	46%	93%	90%	59%								
Fagerberg *et al*[33] 2006	CP	> 50 μg/g	95%	93%	95%	93%	36	10	7	5	0	14	Indeterminate colitis/IBD (3), polyps (1), proctitis (1), food intolerance (4), others (5)	Endoscopy/histology
Sydora *et al*[47] 2012	CP	> 150 μg/g (desk top device)	56% -100%	100%	-	-	42	7	9	7	19	0	-	Mayo clinic or harvey bradshaw index
Dolwani *et al*[91] 2004	CP	> 60 μg/g	100%	79%	60%	100%	138	25	0	24	26	63	Symptoms of diarrhea and/or abdominal pain (63)	Barium follow through
Costa *et al*[29] 2003	CP	> 50 μg/g	83%	82%	90%	71%	239	49	82	48	34	26	Intestinal neoplasms (26)	Colonoscopy and/or radiology
Canani *et al*[31] 2006	CP	> 95 μg/g	93%	89%	93%	89%	45	17	10	8	0	10	Food allergy (5), infectious enterocolitis (4), familial Mediterranean fever (1)	Endoscopy, histology and radiology
Summerton *et al*[40] 2002	CP	> 50 mg/kg	82%	73%	-	-	134	4	10	7	28	85	CRC (8), upper-GI lesions (44), diverticulosis (15), polyps (12), colon adenoma (6)	Upper or lower endoscopy
Dai *et al*[92] 2007	LF	> 24 μg/g	100%	100%	-	-	177	18	59	25	34	41	Bacteria infectious bowel disease (41)	Colonoscopy
Walker *et al*[70] 2007	LF	> 7.25 μg/mL	84%	97%	99%	55%	170	79	62	7	22	0	-	Endoscopy and/or radiology (in selected patients only)
Kane *et al*[68] 2003	LF	> 4 μg/g	86%	100%	100%	87%	271	104	80	31	56	0	-	Clinical, radiographic, endoscopic, and histological criteria, as appropriate
Sidhu *et al*[71] 2010	LF	> 7.25 μg/g	67%	96%	87%	87%	465	104	126	137	98	0	-	Colonoscopy (in selected patients only), questionnaires
Schoepfer *et al*[25] 2008	CP	> 50 μg/mL	83%	100%	100%	74%	136	36	28	30	42	0	-	Endoscopy/histology
	LF	> 7 μg/mL	87%	96%	98%	77%								
D'Inca *et al*[30] 2007	CP	> 50 mg/kg	78%	83%	86%	-	144	31	46	20	0	47	CRC (8), polyps (26), diverticulosis (11), CD (2)	Colonoscopy/histology
	LF	> 0.04 OD	80%	85%	87%									
Otten *et al*[41] 2008	CP	> 50 mg/kg	96%	87%	65%	99%	114	6	5	91	0	12	Unspecified colitis/IBD (12)	Colonoscopy/sigmoidoscopy
	CP FRT	> 15 mg/kg	100%	95%	82%	100%								
	CP FRT	> 60 mg/kg	61%	98%	88%	91%								
	LF	> 7.25 mg/mL	78%	90%	67%	94%								
	LF FRT	> 128 ng/mL	78%	99%	95%	95%								
Schröder *et al*[38] 2007	CP	> 24 μg/g	93%	100%	100%	91%	76	25	20	31	0	0	-	Endoscopy/histology; clinical disease activity indices
	LF	> 8.9 μg/g	82%	100%	100%	80%								
	PMNE	> 19 ng/g	84%	87%	91%	79%								
Langhorst *et al*[36] 2008	CP	> 48 μg/mL	82%	84%	-	-	139	43	42	54	0	0	-	Clinical disease activity indices; endoscopy
	LF	> 7.05 μg/mL	87%	77%										
	PMNE	> 0.062 μg/mL	77%	77%										

Langhorst *et al*[37] 2009	HBD2	Median UC: 107 μg/g IBS: 76 μg/g HC: 30 μg/g	-	-	-	-	100	0	30	46	24	0	-	Endoscopy/histology; immunohistochemistry; faecal CP and LF
Ohman *et al*[87] 2012	CgB	< 0.48 nmol/g	78%	69%	-	-	111	0	0	82	29	0	-	Faecal CP; rectal sensitivity; colon transit time; questionnaires
	Sg II	> 0.16 nmol/g	80%	79%										
Annaházi *et al*[86] 2013	MMP-9	> 0.245 ng/mL	85%	100%	-	-	94	0	47	23	24	0	-	Clinical and endoscopic Mayo score; faecal CP
Silberer *et al*[39] 2005	CP	> 18.6 μg/g	62%	95%	-	-	119	21	18	40	40	0	-	Endoscopy/histology
	LF	> 6.64 μg/g	33%	95%										
	PMNE	> 124 ng/g	80%	95%										
Jeffery *et al*[82] 2009	M2PK	> 4 U/mL	67%	88%	47%	94%	199	9	1	91	94	4	Collagenous colitis (1), CRC (1), stricture (1), coeliac disease (1)	Colonoscopy (n = 87) or radiology (n = 4)
	CP	> 50 μg/g	93%	92%	62%	99%								
Chung-Faye *et al*[81] 2007	M2PK	> 3.7 U/mL	73%	74%	89%	57%	131	31	50	43	0	7	CRC (7)	Endoscopy/histology
	CP	> 25 μg/g	80%	74%	87%	65%								

Se: Sensitivity; Sp: Specificity; PPV: Positive predictive value; NPV: Negative predictive value; CD: Crohn's disease; UC: Ulcerative colitis; IBS: Irritable bowel syndrome; HC: Healthy control; CP: Calprotectin; LF: Lactoferrin; PMNE: Polymorphonuclear elastase; MMP: Matrix-metalloprotease; HBD: Human β-defensin; Cg: Chromogranin; Sg: Secretogranin; MPO: Myeloperoxidase; M2PK: M2-pyruvate kinase; IBD: Inflammatory bowel disease; GI: Gastrointestinal; CRC: Colorectal cancer; FRT: Faecal rapid test.

a sensitivity, specificity and diagnostic accuracy of 78%, 83% and 80% respectively for diagnosing inflammatory disease, irrespective of diagnosis. Similar results have also been obtained in the paediatric population[31-33]. Carroccio *et al*[32] reported specificities which were in line with previous studies, but the sensitivities were far lower. This was attributed to a combination of a higher potential number of referrals for possible coeliac patients (due to their hospital being a tertiary centre for food intolerance), and the reported high frequency of negative calprotectin results for patients with coeliac disease. Furthermore, they highlighted the association between false-positive results for faecal calprotectin and both nonsteroidal anti-inflammatory drug use and liver cirrhosis, believed to be due to the mucosal abnormalities associated with each[34].

In efforts to highlight the potential of faecal calprotectin to distinguish between IBD and IBS specifically, a number of further studies have been performed[26,32,35-40] (Table 1). Langhorst *et al*[37] confirmed that faecal calprotectin was significantly raised (104 μg/g) in patients with active ulcerative colitis (UC) compared to faecal levels in patients with IBS (19 μg/g). In a slightly smaller prospective study, Schröder *et al*[38] reported that faecal calprotectin had a sensitivity of 93% and a specificity of 100% when differentiating IBD from IBS (cutoff 24.3 μg/g), though the diagnostic accuracy of calprotectin was not statistically significant superior to that of faecal lactoferrin or polymorphonuclear (PMN) elastase. The distinctly high diagnostic values found in this study were potentially due to a selection bias (their hospital represents a referral centre for IBD), which was supported by the exceptionally high number of patients suffering from IBD compared to IBS[38]. The comparative diagnostic accuracies between faecal calprotectin and other faecal markers have also been studied extensively[25,30,35,36,38,39,41,42]. Silberer *et al*[39] have reported a high and similar diagnostic accuracy of faecal calprotectin and PMN elastase for the differentiation of chronic IBD and IBS, which was superior to that of other leukocyte proteins in the faeces including lactoferrin and myeloperoxidase (MPO). In another such study, Schröder *et al*[38] reported that any combination of calprotectin, lactoferrin and PMN elastase did not improve their diagnostic accuracy in distinguishing between IBD and IBS, a result supported by other studies[36,38,41].

Correlation of faecal calprotectin with endoscopically and histologically assessed disease has always been the "gold standard" to ascertain its true prognostic value. Schoepfer *et al*[25] were able to demonstrate good correlation of faecal calprotectin with endoscopically assessed severity of disease in both Crohn's disease (CD) and UC. These findings were confirmed by a recent study reporting a significant correlation of faecal calprotectin levels and endoscopic disease activity in 126 included patients with IBD and 32 patients with IBS[43]. Following from this, the role of faecal calprotectin in being able to distinguish between IBD patients in remission with or without IBS symptoms has been investigated. Faecal calprotectin tends to be increased in subgroups of IBS-positive patients with IBD in remission, regardless of diagnosis[44,45]. Keohane *et al*[45] reported that both CD and UC patients with IBS-like symptoms had significantly higher faecal calprotectin levels than those without, most likely indicating the presence of ongoing subclinical inflammation rather than coexisting functional disease. Berrill *et al*[46] have reported that there is no statistical difference between the faecal calprotectin levels of patients with IBD

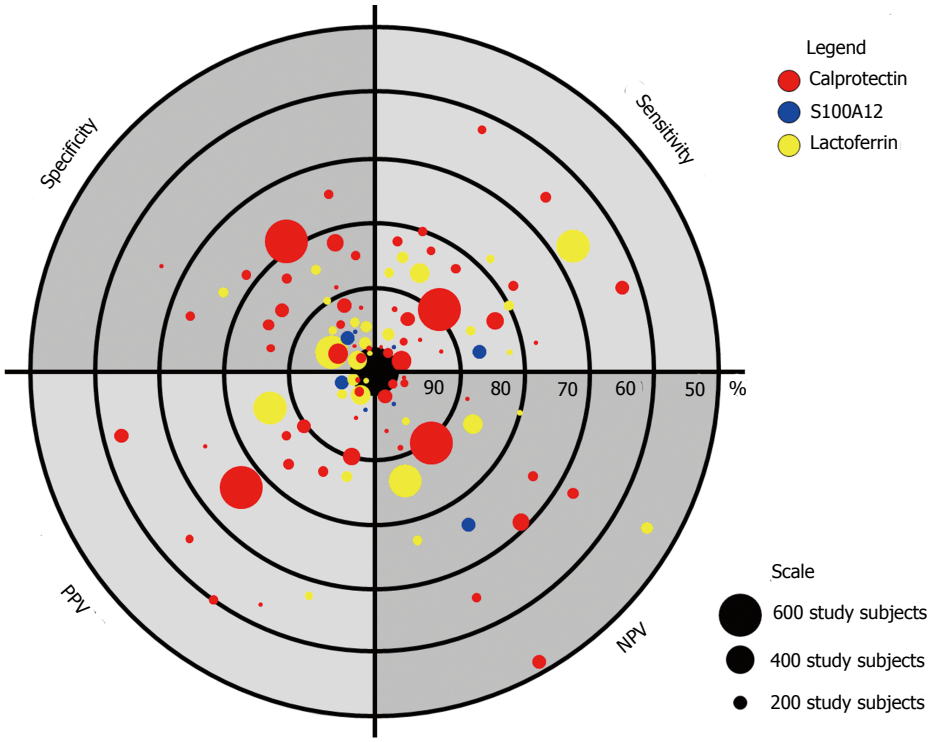

Figure 2 Diagnostic accuracy of faecal markers in the differentiation of organic gastrointestinal disease *vs* irritable bowel syndrome. The figure illustrates statistical measures of the diagnostic performance of different studies on the role of faecal markers in the diagnosis of irritable bowel syndrome. Sensitivities, specificities, positive predictive values (PPV), and negative predictive values (NPV) of different biomarker studies are represented with highest values close to the center of the "dartboard" (*i.e.*, 100%). Each dot represents a biomarker study and different colors represent the type of the faecal marker (see legend). The size of each dot represents the number of included study subjects (see scale).

Table 2 Overall diagnostic accuracy of faecal markers in the differentiation of inflammatory bowel disease *vs* irritable bowel syndrome in relation to the size of study cohorts

	Se	*n*	Sp	*n*	PPV	*n*	NPV	*n*	Ref.
Calprotectin	85%	2984	85%	2984	81%	2274	82%	2130	[25,26,28-33,35,36,38-42,47,81,82,91]
S100A12	89%	256	96%	256	98%	256	81%	256	[35,42]
Laktoferrin	78%	1811	94%	1811	91%	1376	82%	1232	[25,30,36,38,39,41,68,70,71,92]
M2-PK	69%	330	82%	330	64%	330	79%	330	[81,82]

Se: Sensitivity; Sp: Specificity; PPV: Positive predictive value; NPV: Negative predictive value; n: Number of study subjects; M2PK: M2-pyruvate kinase.

in clinical remission with IBS-type symptoms compared with those without. While faecal calprotectin may be useful as a noninvasive marker to distinguish patients with IBD in need of intensified follow-up, the utility of faecal calprotectin as an aid to discriminate between inflammatory and functional symptoms in IBD patients remains uncertain.

There have also been some interesting results of other faecal calprotectin analysis techniques. Otten *et al*[41] reported that faecal rapid testing of calprotectin had an associated sensitivity and specificity of 100% and 95% respectively, at a cutoff value of 15 mg/kg. Interestingly, these results outperformed those of the standard enzyme-linked immunosorbent assay (ELISA) faecal calprotectin test (Table 1)[41]. Similarly, Sydora *et al*[47] found that "desk top" faecal analysis devices reported sensitivities of 56%-100% and specificities of 100% when differentiating between IBD and IBS (cutoff 150 μg/g). However, these data were generated from a very small cohort (Table 1), and though showing promise should nonetheless be treated with caution at present. In addition, it was recently reported that different faecal calprotectin ELISA kits show a between-assay variability[48].

Since many disorders present with symptoms similar to IBS, it is important to exclude other causes like IBD. Overall, calprotectin is the most widely studied faecal marker for the differentiation between IBD and IBS and a sensitive and specific marker of inflammatory activity in the gut (Table 2, Figure 2). Because of its high diagnostic accuracy in ruling out intestinal inflammation, many clinicians use faecal calprotectin as a noninvasive screen for IBD in their patients with IBS symptoms[49].

S100A12

S100A12, also known as calgranulin C or EN-RAGE (extra cellular newly identified receptor for advanced

glycation end-products), is another member of the S100 calcium-binding protein family. In contrast to calprotectin, S100A12 is expressed almost exclusively by neutrophils (Figure 1) and does not form heterodimers with either S100A8, S100A9 or associate with the heterodimer S100A8/S100A9[50]. S100A12 was reported to function as a pro-inflammatory molecule, the binding of which to RAGE on endothelial cells, mononuclear phagocytes, and lymphocytes leads to upregulation of pro-inflammatory cytokines[51]. More recently, it was shown that S100A12 is a ligand of Toll-like receptor 4, amplifying monocyte activation and thus contributing to organ-specific as well as systemic inflammation[52]. Not surprisingly, S100A12 has been implicated in multiple inflammatory disorders[53-55]. S100A12 is strongly upregulated during chronic active IBD[21] and its release from intestinal mucosal specimens correlates to the intestinal inflammation status[56].

More recently, the value of S100A12 as a faecal biomarker of inflammatory conditions within the bowel has been investigated[42,57-62]. de Jong *et al*[62] showed that S100A12 was equally distributed in faeces, as well as being temperature stable for up to 7 d. Furthermore, in their study of 48 children, they reported that faecal S100A12 had a sensitivity of 96% and a specificity of 92% (cutoff 10 mg/kg) when distinguishing between healthy controls and the IBD group (mainly CD)[62]. In the wake of these findings we assessed the correlation between faecal S100A12 levels with endoscopic and histological findings in patients with IBD and IBS[35]. We demonstrated a sensitivity of 86% and a specificity of 96% (cutoff 0.8 mg/kg) when differentiating active IBD from IBS. Our study also showed a strong correlation between faecal S100A12 levels and endoscopically and histologically confirmed intestinal inflammation in both CD and UC. Our head-to-head comparison of faecal S100A12 and faecal calprotectin showed that faecal S100A12 was superior in distinguishing active IBD from IBS[35]. Similarly, in a prospective study of a paediatric population presenting with GI symptoms, Sidler *et al*[42] investigated the utility of faecal S100A12 compared to faecal calprotectin as a marker for intestinal inflammation. Children diagnosed with IBD (n = 31) had elevated faecal S100A12 (median 55.2 mg/kg) and faecal calprotectin (median 1265 mg/kg) levels when compared to 30 children without IBD (median S100A12 1.1 mg/kg; median calprotectin 30.5 mg/kg). The sensitivity and specificity of faecal S100A12 for the diagnosis of IBD (cutoff 10 mg/kg) were both 97%, whereas faecal calprotectin had a sensitivity of 100% and a specificity of only 67% (Table 1).

Though more recent studies into the role of S100A12 for diagnosis, prediction of outcomes and monitoring of disease responses for other GI diseases (including necrotizing enterocolitis and CRC) have been undertaken[58,59,63], further prospective studies into the role of S100A12 in distinguishing organic from functional disease are required to consolidate promising initial data (Table 2, Figure 2).

LACTOFERRIN

Lactoferrin is a multifunctional iron binding glycoprotein that is found in the secretions of most mucosal surfaces including tears, saliva, human breast milk, synovial fluid and serum[64]. Lactoferrin has been shown to exert bacteriocidal activity and is a major component of secondary granules released during the degranulation of polymorphonuclear neutrophils in response to inflammation[65,66]. In the intestinal lumen, the presence of inflammation triggers polymorphonuclear neutrophils to infiltrate the intestinal mucosa, causing a proportional increase of faecal lactoferrin levels (Figure 1)[67]. Lactoferrin demonstrates reasonable stability in faeces; it is unaffected by multiple freeze-thaw cycles, though it has been reported that after 48 h at room temperature, stool concentrations of lactoferrin declined slightly to 90% of their original levels[13,39,68].

Several studies have attempted to elucidate the utility of lactoferrin as a marker for intestinal inflammation, with variable outcomes[69]. Results were more variable when assessing the capabilities for lactoferrin as a distinguishing marker between IBS and IBD (Table 1). Compared to other proteins stored in neutrophilic granules such as PMN elastase, MPO, and human neutrophil lipocalin, Sugi *et al*[13] reported that lactoferrin was a superior faecal marker of neutrophil-derived intestinal inflammation. D'Incà *et al*[30] were able to quantify that, in colonoscopy referrals for lower GI symptoms, results of faecal lactoferrin assays yielded an overall sensitivity, specificity, positive predictive value (PPV), and diagnostic accuracy of 80%, 85%, 87% and 81% respectively in identifying intestinal inflammation. Similarly, Walker *et al*[70] reported that all of their included patients with IBS (n = 7) had normal levels of faecal lactoferrin (cutoff 7.25 μg/mL) and that the sensitivity, specificity, PPV and negative predictive value (NPV) for distinguishing individuals with IBD from those without IBD, were 84%, 97%, 99% and 55% respectively. Furthermore, in a recent meta-analysis, Gisbert *et al*[69] calculated the mean sensitivities and specificities of faecal lactoferrin in the diagnosis of IBD to be 80% and 82% respectively. Silberer *et al*[39] found that calprotectin and PMN elastase, but not lactoferrin, correlated with the severity of inflammation determined by ileocolonoscopy and were able to differentiate chronic IBD from IBS. When comparing receiver operating characteristic (ROC) curves calculated for healthy controls and patients with IBD, the areas under the curve (AUCs) for PMN elastase and calprotectin were 0.916 and 0.872 respectively, whilst that for lactoferrin was 0.693[39]. On the other hand, our recent review of studies on faecal markers of intestinal inflammation revealed that the diagnostic accuracy of faecal lactoferrin in the differentiation of IBD *vs* IBS had sensitivities and specificities between 56%-100% and 61%-100% respectively, with PPVs and NPVs of 59%-100% and 78%-99% respectively[14] (Table 1). In a more recent study, Sidhu *et al*[71] were further able to demonstrate that patients with inactive IBD had significantly higher median faecal lactoferrin levels than those with IBS. Of particular interest were the results of Otten *et al*[41] showing that new faecal rapid testing techniques for evaluating faecal lactoferrin in the primary care setting were

at least comparable to the more standard ELISA tests when testing 114 patients referred for lower GI endoscopy for investigation of abdominal complaints (bloating, change in defecation frequency or consistency, or blood and mucus in the faeces) (Table 1).

Considering these positive results, the main disadvantages of faecal lactoferrin stem from its non-specificity to any particular organic disease and by the fact that it is not solely expressed by degranulated neutrophils. Lactoferrin is secreted endogenously by several mucosal epithelial cell types and can therefore act as a non-inflammatory induced source of faecal lactoferrin[72]. Furthermore, it has been reported that the use of non-steroidal anti-inflammatory drugs may increase the amount of lactoferrin detected in faeces, probably due to an associated induced enteropathy[32,73,74].

Similarly to S100 proteins, it should be emphasized that lactoferrin itself is not a marker of any specific organic disease, but rather of neutrophilic intestinal inflammation[75]. A negative faecal lactoferrin test, therefore, should only be seen as the absence of significant neutrophilic intestinal inflammation. It has consequently been proposed that faecal lactoferrin may have a role in excluding underlying inflammatory conditions thus removing the need for colonoscopy in patients presenting undifferentiated diarrhoea with no alarm symptoms[76]. In studies designed to compare IBD patients with healthy controls or IBS, direct comparison of calprotectin and lactoferrin revealed comparable levels of diagnostic accuracy (Tables 1 and 2)[25,30,36,38,39,41]. These conclusions support the notion that although lactoferrin may be of limited use in the direct classification or diagnosis of organic disease, it may yet have utility in IBD diagnosis.

M2-PYRUVATE KINASE

The glycolytic enzyme M2-pyruvate kinase (M2-PK) is a multifunctional protein, involved in several nonglycolytic pathways influencing cellular physiology including immunological responses, cellular growth and apoptosis[77]. The dimeric isoform of M2-PK (tumor M2-PK) is present in undifferentiated and proliferating tissues and M2-PK is upregulated in a range of GI malignancy[78]. The determination of M2-PK in stool samples was proposed as a new promising screening tool for CRC[79]. The usefulness of faecal M2-PK for the detection of intestinal inflammation was also studied in patients with IBD since these patients have increased cell turnover in the GI tract. The PK stool test requires a single, small and random faecal sample whilst the enzyme is stable for two days at room temperature[80]. Czub *et al*[80] have reported that faecal M2-PK could potentially be a useful marker for IBD activity with a better correlation for UC patients. Likewise, Turner *et al*[61] showed that faecal M2-PK reflects severity of paediatric UC by having very high faecal values. Furthermore, the authors demonstrated that faecal M2-PK has, in contrast to other faecal biomarkers (calprotectin, lactoferrin, S100A12), the best ability to predict steroid refractoriness in severe paediatric UC, but is still inferior to a clinical disease activity index[61]. Importantly, it has also been shown that faecal M2-PK is able to differentiate between patients with IBD or IBS (cutoff 3.7 U/mL) and that M2-PK and faecal calprotectin are highly significantly correlated[81]. In this study 67% of included patients (n = 88) had organic GI disease and faecal M2-PK had a sensitivity of 73%, specificity of 74%, PPV of 89%, and a NPV of 57% for IBD and CRC. These results were comparable to the diagnostic accuracy of faecal calprotectin (cutoff 25 μg/g) in the same patients with a sensitivity of 80%, specificity of 74%, PPV of 87%, and a NPV of 65% (Table 1). Jeffery *et al*[82] showed that, in a setting of a low prevalence or organic bowel disease, faecal M2-PK is able to differentiate organic disease from functional bowel disease (cutoff 4 U/mL) with a sensitivity of 67%, specificity of 88%, PPV of 47% and a NPV of 94%. In this study the incidence of functional bowel disorder was much higher (87% of included patients; n = 91) than in the aforementioned study (33% of included patients; n = 43) and the results showed that M2-PK does not perform as well as calprotectin (cutoff 50 μg/g; sensitivity 93%; specificity 92%, PPV 62%, NPV 99%) (Table 1)[82]. The authors concluded that use of calprotectin and M2-PK may be particularly advantageous as a rule-out test in clinical populations with a similar disease prevalence.

POLYMORPHONUCLEAR NEUTROPHIL ELASTASE

PMN elastase is a neutral serinproteinase, which is released from leucocyte granules as a mediator of inflammation by activation of neutrophils. Elastase is stable for four days in faeces at room temperature[39]. Silberer *et al*[39] showed that faecal PMN elastase levels in patients with IBS (n = 40) were in the range of healthy persons (n = 40). Faecal PMN elastase and calprotectin correlated with endoscopically classified severity of intestinal inflammation and yielded similar AUCs when ROC curves were calculated for healthy persons and patients with IBD (n = 39). The authors concluded that faecal PMN elastase and calprotectin are able to differentiate between chronic IBD and IBS. Similarly, Langhorst *et al*[36] showed that faecal PMN elastase, calprotectin and lactoferrin differentiate IBD and IBS. Patients with IBS (n = 54) demonstrated significantly lower levels of PMN elastase in stools when compared to patients with endoscopically active IBD (n = 60) and, interestingly, when compared with endoscopically inactive IBD (n = 25). The specificity and overall diagnostic accuracy of PMN elastase in patients with IBS were each 82% and slightly lower than for faecal lactoferrin (83%), faecal calprotectin (87%), and serum CRP (91%). Schröder *et al*[38] prospectively evaluated the diagnostic accuracy of faecal PMN elastase alone (cutoff 62 ng/g) and in combination with faecal calprotectin (cutoff 15 μg/g) and/or lactoferrin (cutoff 7.3 μg/g) to detect intestinal inflammation in patients with IBD (n = 45) and IBS (n = 31)[38]. The sensitivity, specificity, PPV, and NPV

of faecal PMN elastase in distinguishing between IBD and IBS was 84%, 87%, 91% and 79%, respectively, and increased to 96%, 100%, 100% and 94%, respectively, when combined with faecal calprotectin ± lactoferrin. The odds ratio for having intestinal inflammation with an elevated faecal PMN elastase was 37 (95%CI: 12-116). However, the results of the study indicate an advantage of calprotectin over lactoferrin and PMN elastase in the detection of intestinal inflammation.

HUMAN β-DEFENSIN-2

Defensins belong to the class of protective antimicrobial peptides and play an important role in the host innate defense at the mucosal surface of the GI tract (Figure 1). Human β defensins (HBD) are expressed in the colon by epithelial cells and plasma cells. HBD-2 plays a crucial role in determining innate immune responses to bacteria in the gut. Cumulating evidence suggests a special role for HBD-2 as a marker for intestinal inflammation in IBD[83]. Interestingly, Langhorst *et al*[84] reported that elevated faecal levels of HBD-2 indicate an activation of innate immunity not only in IBD but also in IBS[37,84]. Faecal HBD-2 levels of patients with IBS (n = 46) were significantly elevated compared with health controls (n = 24) and similar to those in patients with active UC (n = 30), whereas faecal levels of calprotectin and lactoferrin did not differ between healthy controls and patients with IBS. These findings suggest a pro-inflammatory activation of the mucosal innate immune system in patients with IBS in the absence of endoscopic or histologic signs of inflammation. These results support the idea that IBS could be a (low-grade) inflammatory disorder though the functional significance remains to be established.

MYELOPEROXIDASE

MPO is another lysosomal protein that is released from granules of neutrophil granulocytes during inflammation (Figure 1). MPO produces oxygen radicals during the neutrophil's respiratory burst, which are important in the killing of bacteria. MPO is stable for at least four days in feces at room temperature[39]. To date, MPO has shown to be of only limited utility as an inflammatory marker for IBD[85]. Thus, the use of MPO in the differentiation between IBS and IBD has not been widely studied (Table 1). In addition, Silberer *et al*[39] found that MPO separated healthy controls (n = 40) and patients with IBS (n = 40) from patients with chronic IBD (n = 39) less effectively than PMN elastase or calprotectin.

MATRIX-METALLOPROTEASE 9

MMPs are a family of zinc-dependent endopeptidases capable of degradation of extracellular matrix proteins. MMPs are secreted by various cell types including tumor cells and several immune cell types. MMP-9 is released from neutrophils and elevated in colonic biopsies, urine, and blood plasma of patients with UC[86]. Annaházi *et al*[86] compared faecal MMP-9 levels in patients with UC (n = 47) with those of patients with diarrhea predominant IBS-D (n = 23) and healthy controls (n = 24). Healthy controls and patients with IBS-D showed very low faecal MMP-9 levels compared with faecal levels of patients with UC. The sensitivity and specificity of faecal MMP-9 in distinguishing between UC and IBS-D was 85% and 100%, respectively (cutoff 0.245 ng/mL). Faecal MMP-9 levels correlated significantly with faecal calprotectin levels. The authors suggested that faecal MMP-9 could be a novel marker to help in the differential diagnosis of patients with diarrhea and abdominal pain. However, this is the first published study on the diagnostic role of faecal MMP-9 in IBD and IBS and further studies are needed to confirm these findings.

GRANINS

Granins are proteins expressed by cells of the enteric, endocrine, and immune system, and may broadly reflect activity of these systems. Chromogranins (Cg) and secretogranins (Sg) are precursors of several bioactive peptides and regulate a number of cellular functions. Öhman *et al*[87] assessed the association between faecal levels of Cg and Sg with IBS. The results showed that, compared to healthy controls (n = 29), IBS patients (n = 82) demonstrated higher levels of CgA, SgⅡ, and SgⅢ, but lower levels of CgB. Thus, faecal levels of SgⅡ, SgⅢ and CgB may be used to discriminate between IBS patients and healthy individuals. However, there was no disease control group included in this study, which therefore precludes the proper evaluation of faecal granins as diagnostic biomarkers. Faecal granins are however unlikely to be specific IBS markers since other diseases (*e.g.*, coeliac disease) also manifest increased Cgs[88]. Furthermore, faecal calprotectin levels were not associated with the faecal concentrations of granins. Finally, the study design cannot differentiate whether the increased faecal levels of granins cause IBS or its symptoms, or merely reflect the phenotype of IBS. Elevation of faecal granins may serve as a marker for guiding medical treatment of IBS. However, the lack of specificity of faecal granins does not support the use of these proteins as positive biomarkers for IBS.

CONCLUSION

Extensive diagnostic tests in the evaluation of patients with typical symptoms of IBS and the absence of alarm features are not necessary[89]. A positive diagnostic strategy based on symptom-based criteria and simple blood tests is not inferior to a strategy of exclusion of organic disease with multiple unnecessary, expensive, and potentially harmful diagnostic tests and procedures[90]. Faecal surrogate markers of intestinal inflammation represent a practicable, inexpensive and objective diagnostic tool to differentiate organic and functional GI diseases. Neutrophil-derived faecal biomarkers show a high diagnostic accuracy in the differentiation of IBD *vs* IBS (Table 2) and could be useful in reducing unnecessary invasive investi-

gations. Thus, these markers can provide reassurance to physicians that their clinical diagnosis of IBS is correct. Further studies are required to more comprehensively define and compare the role of these faecal proteins in the diagnosis and pathogenesis IBS. Nonetheless, faecal biomarkers have the potential to be incorporated into standard clinical practice for the routine assessment of IBS and IBD.

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Molecular basis of the irritable bowel syndrome

Anna Vaiopoulou, Georgios Karamanolis, Theodora Psaltopoulou, George Karatzias, Maria Gazouli

Anna Vaiopoulou, Maria Gazouli, Department of Basic Medical Science, Laboratory of Biology, School of Medicine, University of Athens, 11527 Athens, Greece
Georgios Karamanolis, George Karatzias, Gastroenterology Unit, 2nd Department of Surgery, "Aretaieio" University Hospital, School of Medicine, University of Athens, 11527 Athens, Greece
Theodora Psaltopoulou, Department of Hygiene, Epidemiology and Medical Statistics, School of Medicine, University of Athens, 11527 Athens, Greece
Author contributions: Karamanolis G and Gazouli M designed the study; Vaiopoulou A, Psaltopoulou T and Karatzias G performed research at the literature; Vaiopoulou A and Gazouli M analyzed data; Vaiopoulou A, Karamanolis G and Gazouli M wrote the paper.
Correspondence to: Maria Gazouli, Assist Professor, Department of Basic Medical Science, Laboratory of Biology, School of Medicine, University of Athens, Michalakopoulou 176, 11527 Athens, Greece. mgazouli@med.uoa.gr
Telephone: +30-210-7462231 Fax: +30-210-7462231
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Abstract

Irritable bowel syndrome (IBS) is a functional disorder characterized by abdominal pain, discomfort and bloating. The pathophysiology of IBS is poorly understood, but the presence of psychosocial basis is now known. There is an increasing number of publications supporting the role of genetics in IBS. Most of the variations are found in genes associated with the brain-gut axis, revealing the strong correlation of brain-gut axis and IBS. miRNAs, which play critical roles in physiological processes, are not well studied in IBS. However, so far there is found an involvement of alterations in miRNA expression or sequence, in IBS symptoms. IBS phenotype is affected by epigenetic alteration and environment. Changes in DNA and histone methylation are observed in patients who suffered childhood trauma or abuse, resulting in altered gene expression, such as the glucocorticoid receptor gene. Finally, diet is another factor associated with IBS, which may contribute to symptom onset. Certain foods may affect on bacterial metabolism and epigenetic modifications, predisposing to IBS.

Key words: Irritable bowel syndrome; Gastrointestinal diseases; Genetics; Epigenetics; Diet

Core tip: Irritable bowel syndrome (IBS) is a multifactorial disease, whose development and phenotype are related to both genetic and epigenetic factors. Gene polymorphisms and epigenetic modifications affect the function of brain-gut axis and are responsible for many of the symptoms of the disease. The relationship between environmental factors and IBS shows the effect of environment on gene expression alteration by epigenetic modification.

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INTRODUCTION

Irritable bowel syndrome (IBS) is amongst the most widely recognized functional gastrointestinal disorders and is remarkably prevalent in the general population, affecting as many as 5%-20% of people worldwide[1]. The prevalence of IBS is slightly higher in women, with a variable influence of age across studies. Symptom based criteria is applied to diagnose the entity. The presence of chronic or recurrent abdominal pain or discomfort, relieved by defecation and associated with an altered bowel habit, in the absence of any underlying structural or bio-

Table 1 Genetic alterations on irritable bowel syndrome

Gene	Polymorphism	Ref.
Serotonergic system		
SERT promoter	5-HTTLPR, deletion	[13-17]
	rs25531	[81]
HTR3A	-42C > T	[50]
HTR3B	386A > C	[82]
HTR3C	489C > A	[83]
HTR3E	rs62625044	[50]
Adrenergic and opioidergic system		
α2-adrenergic receptor	α2C del 322-325, deletion	[19]
	α2A-1291C > G	[19,84]
COMT	α2A-1291 C > G	[20]
	$Val^{158}Met$	[21,22]
CNR1	(AAT)n triplet repeat	[23]
	rs806378	[24]
CRH-R1	rs7209436	[27]
	rs242924	[27]
BDNF	$Val^{166}Met$	[85]
OPRM1	118A > G	[85]
Cytokines		
IL-10	-1082 A > G	[28-30]
	396 T > G	[30]
	-819T > G	[34]
TNF alpha	-308G > A	[28]
	-238G > A	[86]
GNβ3	825C > T	[32]
TLR9^{+}	-1237T > C	[84]
	2848 G> A	[84]
IL1R	Pst- I 1970C > T	[86]
IL4	-590C > T	[84,87]
	-33T	[87]
IL6	-174G > C	[84,86]

SERT: Serotonin reuptake transporter; COMT: Catechol-*O*-methyltransferase; CNR1: Cannabinoid receptor 1; CRH-R1: CRH receptor 1; IL: Interleukin; TNF: Tumor necrosis factor.

chemical abnormalities, identifies patients with IBS[2].

The syndrome has been subdivided into different subgroups based on the predominant bowel habit; diarrhea-predominant (D-IBS), constipation-predominant (C-IBS), or a mixture of both diarrhea and constipation (M-IBS). The use of these subgroups has received acceptance by most clinical investigators, as it commonly dictates symptomatic pharmacological management[3]. However, the value of this categorization is under consideration, knowing that each IBS patient could switch from one subgroup to another over time.

There is a significant variability in the clinical presentation of patients with IBS and they could differ by predominant stool type, severity and frequency of pain/discomfort and comorbidities including psychological distress and somatic complaints[4]. Moreover, IBS symptoms can fluctuate over time. The severity and intensity of IBS symptoms vary from very mild in patients who do not seek medical attention to very severe one that may significantly affect quality of life with the same degree of impairment as major chronic disorders. Despite the fact that a minority of IBS patients chooses to consult a physician, IBS is a clinical problem of considerable cost for the health care system because of its high prevalence and the chronic or recurrent nature of symptoms[5].

The pathophysiology of IBS is largely unknown and it is generally considered a multifactorial disorder. Among the putative mechanisms involved in the pathogenesis of IBS, there is evidence to support the key role of heritability and genetics factors. It is recognized that psychological factors and stress appear to be the primary drivers of symptoms in IBS patients. There is a hypothesis that IBS patients have a certain personality with predisposition to develop the disease. Dimensions of personality that are important in clinical practice include response to stress, attitude toward illness, health and medical treatment. These constitutional features may have genetic origins that may be influenced by early environmental experiences.

GENETICS AND IBS

Gene polymorphisms

IBS, as a multifactorial disorder, is also associated with altered brain-gut axis[6]. A recent study showed that corticotrophin-releasing hormone (CRH) is involved in stress-related pathophysiology of IBS and in the inflammation of the intestinal mucosa[7]. Polymorphisms in genetic factors may influence these mechanisms, and affect brain-gut interrelations[8-10]. Polymorphisms involve the serotonergic, adrenergic and opioidergic systems, and genes encoding proteins with immunomodulatory and/or neuromodulatory features[9,10].

Serotonergic system

Serotonin [5-hydroxytryptamine (5-HT)] controls gastrointestinal secretion, motility, and visceral perception by activating at least five types of receptors[10]. Alterations in 5-HT levels and signaling are present in IBS patients which may induce diarrhea, nausea, and vomiting[11,12]. So far, only a few gene polymorphisms are associated with IBS. Polymorphisms in promoter of serotonin reuptake transporter (*SERT*) gene effect on transcription activity and influence 5-HT reuptake efficiency. In a recent study, among 9 polymorphisms in promoter region of SERT, only one polymorphism (insertion/deletion polymorphism) was associated with diarrhea in women with IBS. The deletion polymorphism decreases expression of the sodium-dependent serotonin transporter and, thus, reduces reuptake of serotonin[13]. Another study showed a lower prevalence of the SS genotype (homozygosity for deletion) in IBS and, particularly, in D-IBS, but this was only observed in male patients[14] (Table 1).

This polymorphism is also correlated with behavioral traits and psychiatric disorders and IBS patients homozygous for the deletion present significantly higher risk for depressive episodes[15]. Another study also associated insersion/deletion polymorphism with anxiety. Long allele (insertion) in females is implicated with negative emotion but acts contrary in males[16]. This allele influences the efficacy of tegaserod treatment. IBS patients

carrying the long allele respond poorly to treatment[17].

Adrenergic and opioidergic systems

Autonomic system has an important role in gastrointestinal motility, acting *via* adrenergic receptors. Genetic variations in α_2-adrenergic receptor may change sensory and motor function in IBS[18]. α2C Del 322-325 deletion, a variation resulting in a loss-of-function phenotype, is associated with C-IBS (constipation IBS)[19]. The α2A -1291 C>G is associated with D-IBS, but no with C-IBS[20] (Table 1).

A polymorphism (Val^{158}Met) in catechol-*O*-methyltransferase, an enzyme metabolizing catecholamines, showed association with IBS[21]. Patients carrying this polymorphism have a reduced response to pain[22] (Table 1).

Alterations in cannabinoid receptor genes are also analysed and associated with IBS. A polymorphic (AAT)n triplet repeat in the 3-flanking region of the cannabinoid receptor 1 (*CNR1*) gene is related with IBS and severity of abdominal pain in IBS[23] (Table 1).

Additionally, single nucleotide polymorphisms (SNPs) in CRH receptor 1 (CRH-R1), which plays a critical role in stress-induced pathophysiology of IBS, were studied for moderating IBS phenotype and negative emotion in IBS patients (Table 1). Findings of this study showed association between SNPs and IBS moderation, but no association was found with negative emotion[24]. Genetic variation rs806378 in CNR1 is associated with colonic transit in D-IBS and sensation rating of gas[25] (Table 1). This polymorphism is also correlated with treatment effectiveness of nonselective cannabinoid receptor agonist, dronabinol[26,27].

Cytokines

Several studies have reported cytokine gene polymorphisms in IBS. Interleukin (IL)-10-1082 G/G, a high producer IL-10 genotype, correlated with lower risk for developing IBS[28,29] (Table 1). Gene SNPs of IL-8 and IL-10 were also analyzed by Romero-Valdovinos *et al*[30] and an association between alleles IL-8$^+$ 396G and IL-10-1082A and IBS was found. These findings were confirmed by other study[31] (Table 1). TNF alpha (-308 G/A) polymorphism and IBS are correlated, and G/G genotype may increase risk of IBS. G/A genotype has a protective role[28] (Table 1). A study evaluating GNβ3 825C>T polymorphism in IBS showed significant interactions between gastrointestinal infection and T allele in the development of IBS, suggesting gene-environment interactions[32] (Table 1). However, another study replicated none of these results[33]. Another IL-10 polymorphism associated with IBS is IL-10-819 T>C. The frequency of IL-10 -819 CC genotype was significantly higher in D-IBS[34] (Table 1).

miRNAs and IBS

miRNAs are small (21-23 nucleotides) single-stranded RNA molecules[35,36]. miRNAs are not translated into proteins and have regulatory function, such as translational repression of targeted mRNAs[37]. miRNAs form RNA-induced silencing complex, which can prevent the expression of proteins, either by activating endonuclease that degrades mRNAs or by blocking translation[38]. miRNAs are connected with physiological processes such as cell division and death[39], cellular metabolism[40], intracellular signaling[41], immunity[42] and cell movement[43]. Thus, altered miRNA expression can affect these critical processes, and as a result, lead to various pathological and occasionally malignant outcomes.

Cancer is one of human diseases clearly associated with miRNA regulation. miRNAs may involve in tumor development as tumor suppressors or oncogenes. They also play roles in tumor invasion and metastasis. Down-regulation of miR-15 and miR-16 is correlated with the pathogenesis of B-cell chronic lymphocytic leukemia[44]. In addition, miR-125b, miR-145, miR-21 and miR-155 expression is associated with the increased risk of breast cancer[45]. The implication of miRNAs in immune-related diseases, such as multiple sclerosis (MS), systemic lupus erythematosus (SLE), and type Ⅰ / Ⅱ diabetes is also known. In MS, miR-34a, miR-155 and miR-326 are overexpressed[46]. In SLE, increased risk of disease development is associated with decreased expression of miR-46a[47]. Several studies show that miRNAs regulate critical pathways in inflammation, such as pathways correlated with nuclear factor kappa beta. miR-155 and miR-146 are the best characterized miRNAs which are implicated in immune-diseases[46,48,49].

The role of miRNAs in IBS is not well studied. The first association of miRNAs and IBS was from Kapeller *et al*[50]. This study showed that the variation c.*76G>A (rs62625044) in the 3' untranslated region (UTR) of the serotonin receptor type 3 subunit genes *HTR3E* correlates with D-IBS. This functional variation is located in the miRNA-510 target site of the gene. The co-localization of HTR3E and miR-510 in enterocytes of the gut epithelium and the presence of cis-regulatory variation show the regulation of serotonin receptor gene expression by miRNA.

Next evidence came from Zhou *et al*[51], who evaluated the miRNA expression in blood microvesicles (circular membrane fragments that are shed from the cell surfaces and accompanies cell activation) and gut tissues in D-IBS patients and IBS patients with normal membrane permeability. They found that miR-29a expression was increased in blood microvesicles in the small bowel and colon tissues of IBS patients with increased permeability. miR-29a is complementary in the 3' UTR of the glutamine synthetase gene. These results suggest the role of glutamine synthetase in the intestinal membrane permeability and the role of miR-29a in regulation of glutamine synthetase and intestinal membrane permeability.

EPIGENETICS AND IBS

Phenotype is the combination of DNA sequence, epigenetic DNA modifications and environmental factors.

The presence of epigenetic changes in monozygotic twins, leading to phenotypic alterations, suggests a potential role of epigenetics in IBS[52]. DNA methylation and histone modification are the most common epigenetic mechanisms. DNA methylation usually silences gene expression[53]. However, histone acetylation or methylation may activate or not gene transcription[54].

IBS is associated with early life trauma or abuse, and this condition leads to negative health outcomes and behaviors in adults. Childhood trauma influences somatic symptoms and neural network development and neuroendocrine system development[55-57]. In a recent study, IBS patients showed enhanced cortisol response to a visceral stressor. The hypothalamic-pituitary-adrenal (HPA) axis hyperresponsiveness to stressor is more related to early adverse life events rather than to the presence of IBS[55].

Early childhood trauma decreases glucocorticoid receptor expression by hypermethylation of glucocorticoid receptor gene[58]. Altered glucocorticoid receptor gene expression, which mediates the negative feedback of the HPA axis, reduces the capability of HPA to deal effectively with stress. In animal model of IBS, animals exposed to perinatal stress had methylation of glucocorticoid receptor promoter, decreased gene expression and prolonged elevation of corticosterone levels[59].

The impact of early adverse life events on developing IBS or other diseases is being explored lately. Gluckman *et al*[60] developed a hypothesis that epigenetic processes, including DNA methylation and histone modification, partially mediate developmental plasticity. Another group searched for a mechanism that link the social environment early in life and long-term epigenetic programming of behavior and responsiveness to stress. They took into account data suggesting that DNA methylation is a dynamic process and postmitotic cells may change methylation pattern responding to different environmental stimuli. This study showed that maternal licking and grooming in the rat triggered activation of 5-HT receptors, activation of the transcription factor nerve growth factor-induced gene A and acetylation of the promoter of the glucocorticoid receptor (mediated by a histone acetyl transferase), leading to differential epigenetic programming of the glucocorticoid receptor[61].

Alterations in acetylation motif change behavior in adult offsprings. Except maternal care, diet may also affect behavioral plasticity[62]. Maternal separation acts as a stressor and helps adult rats to develop intestinal mucosal dysfunction, increased HPA axis responses and anxiety-like behavior[63].

Finally, early life stress increases the levels of proinflammatory cytokines. In IBS patients, levels of IL-6 and IL-8 were high, as a result of epigenetic glucocorticoid alterations[64,65]. Upregulation of proinflammatory cytokines influences tryptophan metabolism, resulting on changes of 5-HT activity[66]. The kynurenine:tryptophan ratio, which shows tryptophan catabolism, is increased in IBS patients with severe symptoms, and they were more likely to have depression or anxiety.

DIET, NUTRIGENOMICS/ NUTRIGENOMICS AND IBS

It is well documented, that the interplay between genes and diet may be reflected in susceptibility to various diseases[67]. Scientific studies have demonstrated the effectiveness of dietary therapies in alleviating the symptoms and even in altering the progression of inflammatory and autoimmune disorders[68,69]. Concerning the IBS, even if many patients recognize the impact of specific diet in symptom occurrence, limited population-based studies have evaluated the importance of diet in IBS and its role remains uncertain[70-72]. Diet may contribute to symptom onset through several mechanisms such as food allergy and intolerance. Additionally, certain food may alter the composition of the luminal milieu, directly or indirectly through effects on bacterial metabolism. Diet is known also to influence the epigenetic modification of genes[73]. Finally, IBS symptoms may develop following exposure to food-born pathogens[72]. Furthermore, an increase probability of developing IBS is associated with the inheritance of a number of contributory genetic polymorphisms, as well as with the altered expression of certain genes[74]. The variant forms of genes often result in an abnormal response to normal gut bacteria that may be changed through inappropriate diet or environment. Shifts in the bacterial makeup of the human gut microbiota have been associated with gut disorders including IBS[75]. In the field of nutritional research, 2 terms have been established: nutrigenomics which aims to study how genotype determines optimal dietary requirements for health on an individual basis, and nutrigenomics which studies the effect on diet on DNA structure and gene expression[76]. However, the most of the nutrigenetic/nutrigenomic work has focused on cardiovascular disease, type Ⅱ diabetes mellitus or inflammatory bowel disease[77,78] and no study has be done on IBS. Low FODMAPs diet, that is elimination of fermentable Oligo-, Di- and Mono-saccharides, and Polyols from diet, is an area of intense investigation for symptoms' alleviation[79,80]. FODMAPs' ingestion could result in the symptomatology of these patients, because they are osmotically active, they are fermented and through bacterial overgrowth can cause bloating, pain and the sequence of symptomatology in IBS. Thus, the application of these approaches in the field of IBS research is open. It is hoped that the nutrigenomics/nutrigenomics implementation will promote the understanding of diet-gene interactions and facilitate a better characterization of individual IBS patients for further identification of nutritional patterns that allow personalized therapies.

CONCLUSION

IBS is a multifactorial disease, whose development and phenotype are related to both genetic and epigenetic

factors. Most factors involve in pathogenesis by causing changes in gene expression. Gene polymorphisms and epigenetic modifications affect the function of brain-gut axis and are responsible for many of the symptoms of the disease. IBS is one of the diseases where the environmental influence is strong. Early life incidents and diet habits play an important role in disease development. The relationship between environmental factors and IBS shows the effect of environment on gene expression alteration by epigenetic modification.

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Is irritable bowel syndrome an organic disorder?

Magdy El-Salhy, Doris Gundersen, Odd Helge Gilja, Jan Gunnar Hatlebakk, Trygve Hausken

Magdy El-Salhy, Section for Gastroenterology, Department of Medicine, Stord Helse-Fonna Hospital, 5409 Stord, Norway
Magdy El-Salhy, Odd Helge Gilja, Jan Gunnar Hatlebakk, Trygve Hausken, Section for Gastroenterology, Department of Clinical Medicine, University of Bergen 5006 Bergen, Norway
Doris Gundersen, Department of Research, Helse-Fonna, 5528 Haugesund, Norway
Author contributions: El-Salhy M collected the literature data and wrote the manuscript; Gundersen D, Gilja OH, Hatlebakk JG and Hausken T contributed equally to discussions about the collected data and commented upon the manuscript; all of the authors approved the submitted version of the manuscript.
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Correspondence to: Magdy El-Salhy, Professor, Consultant Gastroenterologist, Section for Gastroenterology, Department of Medicine, Stord Helse-Fonna Hospital, Karmsundgata 120, Box 4000, 5409 Stord, Norway. magdy.el-salhy@helse-fonna.no
Telephone: +47-5-3491000 Fax: +47-5-3491000
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Abstract

Irritable bowel syndrome (IBS) is a common gastrointestinal disorder that is generally considered to be functional because there appears to be no associated anatomical defect. Stress and psychological factors are thought to play an important role in IBS. The gut neuroendocrine system (NES), which regulates all functions of the gastrointestinal tract, consists of endocrine cells that are scattered among the epithelial cells of the mucosa, and the enteric nervous system. Although it is capable of operating independently from the central nervous system (CNS), the gut NES is connected to and modulated by the CNS. This review presents evidence for the presence of an anatomical defect in IBS patients, namely in the gastrointestinal endocrine cells. These cells have specialized microvilli that project into the lumen and function as sensors for the luminal content and respond to luminal stimuli by releasing hormones into the lamina propria, which starts a chain reaction that progresses throughout the entire NES. The changes in the gastrointestinal endocrine cells observed in IBS patients are highly consistent with the other abnormalities reported in IBS patients, such as visceral hypersensitivity, dysmotility, and abnormal secretion.

Key words: Cholecystokinin; Dysmotility; Endocrine cells; Enteric nervous system; Ghrelin; Peptide YY; Secretion; Secretin; Serotonin; Visceral hypersensitivity

Core tip: This review presents recent observations in irritable bowel syndrome (IBS) patients that point toward the existence of an anatomical defect in the gastrointestinal endocrine cells. It includes also an argument that IBS is an organic disorder and that the abnormalities in the gastrointestinal endocrine cells can explain the visceral hypersensitivity, dysmotility and abnormal secretion reported in these patients.

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INTRODUCTION

Irritable bowel syndrome (IBS) is a common chronic gastrointestinal disorder with a reported prevalence of 5%-20% and an incidence of about 200 per 100000 of the world population[1-29]. Patients with IBS suffer from recurrent abdominal pain/discomfort and altered bowel habit, which vary in both degree and time pattern between patients: from tolerable to severe, and from daily symptoms to intervals of weeks/months, respective-

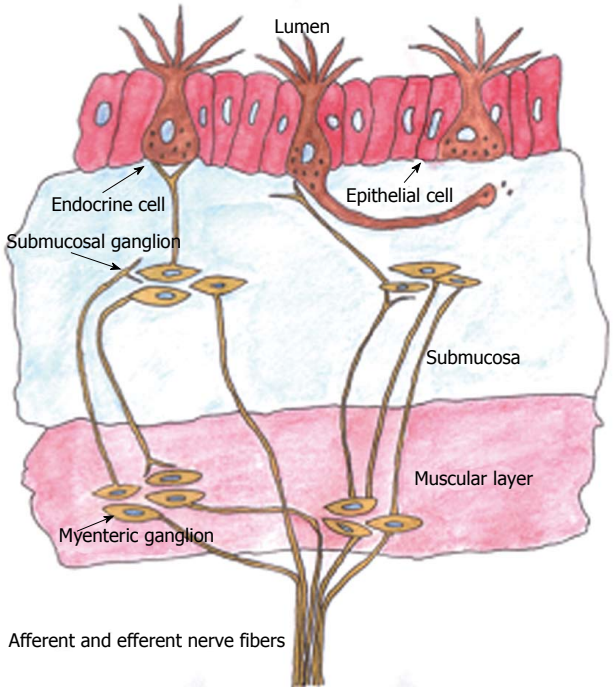

Figure 1 Schematic illustration of the gut neuroendocrine regulatory system. The endocrine cells are scattered among the epithelial cells and have specialized microvilli that project into the lumen and function as sensors of the gut contents, and they respond to luminal stimuli by releasing hormones into the lamina propria, where they exert their action locally on nearby structures. These endocrine cells interact with the enteric nervous system, which is in turn connected to and modulated by the central nervous system through afferent and efferent nerves.

ly[3,11,30-40]. IBS is more common in females than males, and in patients younger than 50 years of age[3,11,14,15,19,21,26,30,31,33-40].

IBS is not associated with the development of serious disease or with an excessive mortality rate[41,42]. However, it considerably reduces the sufferer's quality of life, interfering with their education, working ability and social life. Moreover, IBS represents an economic burden to both patients and society[23,43-49], since IBS patients visit their doctors more frequently, undergo more diagnostic tests, consume more medications, miss more workdays, are less productive at work, and are hospitalized more frequently than those without IBS[28,32,39,50-53].

There are no biomarkers for the diagnosis of IBS[54,55], which is instead based on the assessment of symptoms such as the Rome Ⅲ criteria[56,57]. IBS patients are subgrouped according to differences in the predominant bowel symptoms as IBS-diarrhea (D), IBS-constipation (C), IBS-both diarrhea and constipation (M), and non-subtyped IBS (patients with insufficient abnormality of stool consistency to meet the criteria for IBS-C, -D, or -M)[56,57]. The Rome criteria were introduced to facilitate positive diagnoses, reduce the costs due to unnecessary testing, and guide treatment; however, they fall short on these expectations and are generally neglected in clinical practice by both general practitioners and gastroenterologists[54,58-64].

IBS is considered to be a functional disorder in the absence of a known anatomic defect[65], the pathophysiology of which is incompletely understood. The pathogenesis appears to be multifactorial, with several factors suggested to play a role in the process, such as psychological factors, genetic factors, low-grade chronic intestinal inflammation, an abnormal gut neuroendocrine system (NES) and/or altered signaling in this system, dietary factors, and intestinal flora[66].

IBS patients can be roughly divided into two subsets: sporadic (nonspecific) and postinfectious (PI-IBS)/inflammatory bowel disease (IBD)-associated (IBD-IBS)[66]. Sporadic IBS includes patients who have had symptoms for a long time and without any associated events, in particular gastrointestinal or other infections. PI-IBS is defined as a sudden onset of IBS symptoms following gastroenteritis in individuals who have previously had no gastrointestinal complaints, and IBD-IBS is defined in IBD patients in remission who display IBS symptoms[66]. PI-IBS constitutes about 6%-17% of patients with IBS[67], and IBD-IBS occurs in 33%-46% of ulcerative colitis patients and 42%-60% of those with Crohn's disease[68-72].

This article summarizes the published findings on abnormalities in the gut neuroendocrine cells, discusses them in view of the currently known facts about IBS, and presents an argument for IBS being an organic gastrointestinal disorder.

GUT ENDOCRINE CELLS

The gut contains a large number of endocrine cells that are dispersed among the epithelial cells of the gut mucosa in all of the gut segments except for the esophagus[66,73-78]. These cells constitute the largest endocrine organ in the body and comprise about 1% of all epithelial cells in the gastrointestinal tract, where they are separated from one another by epithelial cells[73,74,79-81]. These cells have specialized microvilli that project into the lumen and function as sensors for the gut contents and respond to luminal stimuli by releasing hormones that, in general, target other parts of the digestive system (Figure 1)[82-94]. There are at least 15 different populations of endocrine cells in the gastrointestinal tract[60,73-76]. Some of them [including somatostatin and peptide YY (PYY) cells] have long slender cytoplasmic processes that project toward neighboring cells, increasing their paracrine effects (Figure 2)[95]. The distribution, functions, and modes of action of the most important endocrine/paracrine cells are given in Table 1[60,75,76,96-108].

Some of the different endocrine cell types are located in specific areas of the gut, while others (primarily somatostatin and serotonin cells) are found throughout the gut[73,74,76]. They secrete one or more signaling substances into the lamina propria, where they exert their action locally on nearby structures (autocrine/paracrine mode) and/or *via* an endocrine mode of action (by circulating in the blood to reach distant targets)[109]. These cells interact in an integrated manner with each other and with the enteric nervous system (ENS) and the afferent and efferent nerve fibers of the central nervous system (CNS), in particular the autonomic nervous system[60,76,99,110]. All of the cell types in the crypt/villus originate from pluripo-

Table 1 Overview of the main endocrine cells in the gastrointestinal tract

Cell content	Localization	Source of release	Actions
Serotonin (5-HT)	EC cells in the stomach, large and small intestines	Noradrenalin; acetylcholine; acidification and intraluminal pressure	Inhibits gastric emptying and stimulates colonic motility; accelerates small- and large-intestine transit activates the submucosal sensory branch of the enteric nervous system that conveys sensation from the gut to the central nervous system
Histamine	EC-like cells in the gastric oxyntic mucosa	Vagus nerve stimulation and inhibited by somatostatin	Stimulates gastric acid secretion
Somatostatin	The stomach, and large and small intestines	Mixed meal and acidification of the stomach	Inhibits intestinal contraction; inhibits gut exocrine and neuroendocrine secretion
Ghrelin	Gastric oxyntic mucosa	Protein and fat ingestion; suppressed by carbohydrate ingestion	Increases appetite and food intake; stimulates gastric and intestinal motility
Gastrin	Gastric antral mucosa	Intraluminal peptides; amino acids; calcium; amines; low pH and prostaglandins. Somatostatin inhibits release	Stimulates gastric acid secretion and histamine release; trophic action on gastric mucosa; stimulates contraction of the LES and antrum
CCK	Small intestine, especially the duodenum	Intraluminal protein and fat and inhibited by somatostatin	Inhibits gastric emptying; stimulates gallbladder contraction and intestinal motility; stimulates pancreatic exocrine secretion and growth; regulates food intake
Secretin	Small intestine, especially the duodenum	Acidification and inhibited by somatostatin	Stimulates pancreatic bicarbonate and fluid secretion; inhibits gastric emptying; inhibits contractile activity of the small and large intestines
GIP	Small intestine, especially the duodenum	Intraluminal glucose; amino acids and fat	Incretin action; inhibits gastric acid secretion
Motilin	Small intestine, especially the jejunum	Protein and fat ingestion	Induces phase-III migrating motor complex; stimulates gastric emptying; stimulates contraction of the LES
Neurotensin	Small intestine	Fat	Stimulates pancreatic section; inhibits gastric secretion; delays gastric emptying; stimulates colon motility
PYY	Terminal ileum and large intestine	Protein-rich meals	Delays gastric emptying; inhibits gastric and pancreatic secretion, stimulates the absorption of water and electrolytes; major mediator of the ileal brake
PP	Terminal ileum and large intestine	Protein-rich meals	Inhibits pancreatic secretion; stimulates gastric acid secretion; relaxes the gallbladder; stimulates motility of the stomach and small intestine
Enteroglucagon (oxyntomodulin)	Terminal ileum and large intestine	Intraluminal carbohydrates and fat	Inhibits gastric and pancreatic secretion; reduces gastric motility; has some incretin effect
Chromogranin	All gastrointestinal tract segments	Ingestion of a meal	Induces formation, sorting, and release of secretory granules of the gut endocrine/paracrine cells; an inflammatory mediator

CCK: Cholecystokinin; EC: Enterochromaffin; GIP: Gastric inhibitory peptide; LES: Lower esophageal sphincter; PP: Pancreatic polypeptide; PYY: Peptide YY.

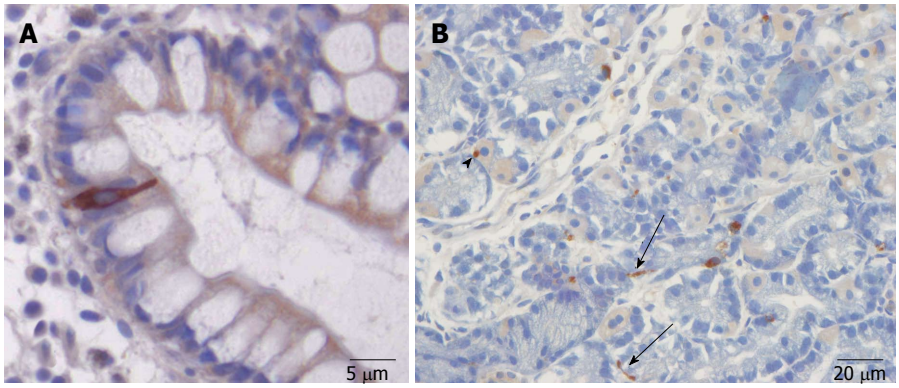

Figure 2 The gut endocrine cells. A: A chromogranin-A-immunoreactive endocrine cell in the ileum. The endocrine cell extends from the basal membrane of the mucosa that project into the gut lumen; B: Somatostatin-immunoreactive cells in the gastric oxyntic mucosa. Note the long cytoplasmic processes (arrows), which can occasionally be seen to end at the base of parietal cells (arrowhead).

tent stem cells of endodermal origin[73,74,111]. Each intestinal crypt contains four to six stem cells that differentiate into all epithelial cell types including enterocytes, goblet cells, Paneth cells, and endocrine cells[112-125]. These cells regulate several functions of the gastrointestinal tract, including sensation, motility, secretion, absorption, local

Table 2 Abnormalities in the densities of gastrointestinal endocrine/paracrine cells in patients with sporadic irritable bowel syndrome

Gastrointestinal segment	Endocrine cell type	IBS-D	IBS-M	IBS-C
Stomach				
Oxyntic mucosa	Ghrelin	High	Normal	Low
	Serotonin	High	Normal	Low
	Somatostatin	Low	Low	High
	Chromogranin A	Normal	Normal	High
Antrum	Serotonin	Normal	Low	High
	Gastrin	High	High	High
	Somatostatin	Low	Low	Low
	Chromogranin A	Normal	Low	High
Small intestine				
Duodenum	Serotonin	Normal	-	Normal
	CCK	Low	-	Normal
	Secretin	Low	-	Normal
	GIP	Low	-	Low
	Somatostatin	Low	-	Low
	Chromogranin A	Low	-	Low
Ileum	Serotonin	Low	Low	Low
	PYY	Normal	Normal	High
	Chromogranin A	Low	Low	Low
Large intestine				
Colon	Serotonin	Low	-	Low
	PYY	Low	-	Low
	Chromogranin A	Low	-	Low
Rectum	Serotonin	Normal	-	Normal
	PYY	Low	-	Low
	Enteroglucagon	Low	-	Low
	Somatostatin	High	-	High
	Chromogranin A	Normal	-	Normal

CCK: Cholecystokinin; PYY: Peptide YY; GIP: Gastric inhibitory peptide; IBS-C: Irritable bowel syndrome (IBS) with constipation as the predominant bowel symptom; IBS-D: IBS with diarrhea as the predominant bowel symptom; IBS-M: IBS with both constipation and diarrhea as the predominant bowel symptoms.

immune defense, and food intake (by affecting the appetite)[60,73,74,76,110].

ABNORMALITIES IN GUT ENDOCRINE CELLS IN IBS PATIENTS

Several abnormalities have been reported in all segments of the gastrointestinal tract of patients with IBS. As mentioned above, the endocrine cells exert their effects in part locally; however for some of them the endocrine mode of action is difficult to elucidate[99]. One example of this is the serotonin cells. The serotonin that they secrete is taken up into the blood and carried by platelets as they circulate through the gut[126-129]. Thus, the circulating serotonin is locked within the dense granules of the platelets, without any possibility of being delivered to distant targets. Therefore, summarizing and discussing abnormalities in the endocrine cells are considered separately herein relative to the various segments of the gastrointestinal tract.

Sporadic IBS

Abnormal endocrine/paracrine cells have been found in the stomach (Figure 3), proximal small intestine (duodenum), distal small intestine (ileum), colon (Figure 4), and rectum of patients with sporadic IBS[130-141]. These abnormalities manifest mostly as changes in the densities of these cells (*i.e.*, an anatomical defect). The abnormalities in the different endocrine cells in the various segments of the gastrointestinal tract of patients with sporadic IBS are summarized in Table 2. In addition to the abnormalities observed in the endocrine cells, there are alterations in the levels of serotonin transporter (SERT), which appear to be increased in the ileum and decreased in the rectum of IBS patients[130,141,142].

PI-IBS and IBD-IBS

Similar to sporadic IBS, abnormal endocrine/paracrine cell densities have been found in both PI-IBS and IBD-IBS. However, the nature of these abnormalities is different from those in sporadic IBS (Table 3)[141,143-150].

POSSIBLE ROLE OF GASTROINTESTINAL ENDOCRINE CELL ABNORMALITIES IN IBS

The mechanisms regulated by gastrointestinal endocrine cells include gut sensation, motility, and secretion. IBS patients exhibit visceral hypersensitivity, disturbed gastrointestinal motility, and abnormal gut secretion[65,107,151-153]. The degree to which the abnormalities in these cells observed in IBS patients contribute to these disturbed functions is discussed to below.

Visceral hypersensitivity

Visceral hypersensitivity has been demonstrated in the colorectal segment of IBS patients[154-161]. Hypersensitivity has also been reported in the esophagus, stomach, and small intestine[162-166]. However, visceral hypersensitivity is not present in all IBS patients, and a large prospective study found that only 20% of IBS patients showed hypersensitivity[167]. Furthermore, visceral hypersensitivity does not seem to be a panintestinal disorder[165]; IBS patients only appear to exhibit rectal hypersensitivity[159]. Whether the severity of abdominal pain is correlated with colorectal hypersensitivity in IBS remains a matter of controversy[154,156,168-172].

As mentioned above, serotonin cells have specialized microvilli that project into the gut lumen and act as sensors for the gut contents, and in particular for increased pressure. Serotonin is released in a regulated and calcium-dependent manner from serotonin cells into the surrounding tissues in response to luminal stimuli[173,174]. It activates the sensory branch of the ENS, which is localized in the submucosal plexus in the submucosa, and the myenteric plexus in between the smooth muscle fibers. These sensory branches convey sensation from the gut to the CNS through the sympathetic and parasympathetic nervous systems (Figure 1)[175-177]. The pain stimuli activate the cerebral cortex through the thalamus and permit the recognition of visceral pain[65,177]. Some studies have found IBS patients to be tolerant of somatic

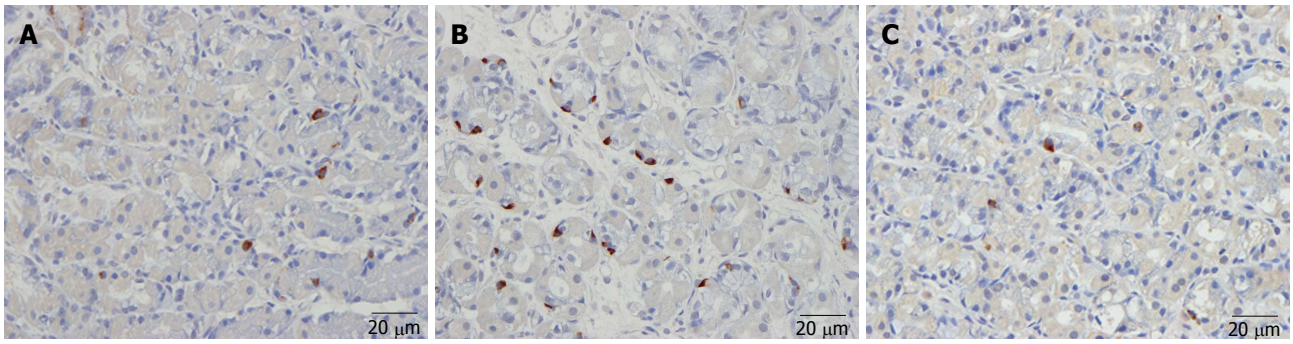

Figure 3 Ghrelin-immunoreactive cells. A: Ghrelin-immunoreactive cells in the gastric oxyntic mucosa of a healthy subject; B: In a patient with irritable bowel syndrome (IBS) with diarrhea as the predominant bowel symptom (IBS-D); C: In a patient with IBS with constipation as the predominant bowel symptom (IBS-C). The density of ghrelin cells is higher in IBS-D and lower in IBS-C than in the healthy control.

Table 3 Abnormalities in the densities of gastrointestinal endocrine/paracrine cells in patients with post infectious irritable bowel syndrome and inflammatory-bowel-disease-associated irritable bowel syndrome

Gastrointestinal segment	Endocrine cell type	PI-IBS	IBD-IBS
Small intestine			
Duodenum	Serotonin	High	-
	CCK	High	-
Large intestine			
	Serotonin	High	High/low
	PYY	High	Low
	PP	-	Low
	Enteroglucagon	-	High

CCK: Cholecystokinin; PYY: Peptide YY; PI-IBS: Post infectious irritable bowel syndrome; IBD-IBS: Inflammatory-bowel-disease-associated irritable bowel syndrome.

pain, and hence the hypersensitivity is confined to the viscera[158,165,178], while other studies found IBS patients to have a lower tolerance to somatic pain than healthy subjects[162,179,180]. Azpiroz *et al*[167] postulated that the exclusive visceral hypersensitivity experienced by some IBS patients could be attributable to abnormalities at the level of the gut, spinal cord, or brain, whereas patients with both visceral and somatic hypersensitivities have a disturbance above the gut level. Those authors also argued that a peripheral mechanism is involved in the visceral hypersensitivity in IBS.

The data presented in Table 2 suggest that none of the abnormalities in the gut endocrine cells could possibly contribute to the development of the visceral hypersensitivity seen in some sporadic IBS patients. However, it has been reported that SERT levels are increased in the ileum and reduced in the rectum of these patients[130,141,142]. The gut mucosa has a high SERT-producing capacity, since all of the epithelial cells lining the luminal surface of the gut express SERT[142,181]. A reduction in SERT results in impaired intracellular uptake and degradation in the gut epithelial cells, consequently increasing the availability of serotonin within the gut mucosa[182,183]. Considering that the serotonin cell density in sporadic IBS does not differ from that of a healthy subject, a decrease in SERT would markedly increase the amount of serotonin available at its receptors[141,142]. An increase in serotonin at the 5-hydroxytryptamine (5-HT)$_3$ receptors of the ENS sensory neurons would activate the sensory nerves, which would then transmit nociceptive information to the nervous system[99]. Conversely, duodenal and rectal serotonin cell densities are high in PI-IBS patients, possibly contributing to the development of visceral hypersensitivity.

Dysmotility

Dysmotility has been reported to occur in all segments of the gastrointestinal tract of patients with IBS, but mostly in the small and large intestines[151,153]. Some studies found lower pressures in the lower esophageal sphincter and abnormal esophageal contractions in IBS patients[184,185]; however, such esophageal motility abnormalities were not confirmed in other studies[186,187]. In addition, some authors have reported abnormal gastric emptying in patients with IBS[153,188-193], while others did not find any such abnormality in these patients[161,194-197]. It therefore seems that abnormalities of gastric emptying do not occur in all IBS patients. Furthermore, while IBS-C patients often exhibit delayed gastric emptying, rapid gastric emptying is found in IBS-D patients[155,189].

The ghrelin cell density in the gastric oxyntic mucosa is low and the serotonin cell density in the antrum of the stomach is high in IBS-C patients, while in IBS-D patients the ghrelin cell density in the gastric oxyntic mucosa is high and the densities of cholecystokinin (CCK) and secretin cells are reduced in the duodenum[132]. Ghrelin is a peptide hormone that was first isolated from the stomach, and originates mostly from endocrine cells in the oxyntic mucosa of the stomach, although small amounts are expressed in the small intestine, large intestine, and the arcuate nucleus of the hypothalamus[198-200]. Ghrelin has several functions, and plays a role in regulating the release of growth hormone from the pituitary gland, which increases appetite and feeding, and also plays a major role in energy metabolism[201-204]. Furthermore, ghrelin has been found to accelerate gastric and small- and large-intestine motility[83,110,205-214]. Serotonin acts on 5-HT$_{1p}$ receptors, which are located on a subset of inhibitory motor neurons of the myenteric plexus, relaxing the stomach *via*

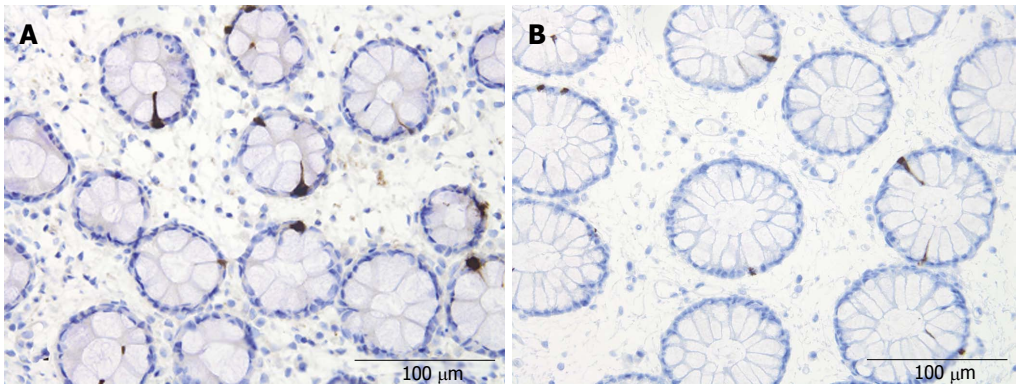

Figure 4 Peptide YY-immunoreactive cells. A: In the colon of a healthy subject; B: In a patient with irritable bowel syndrome (IBS). The density of peptide YY cells in the colon is lower in IBS patients than in healthy controls.

a nitrergic pathway and delaying gastric emptying[98,215-217]. CCK relaxes the proximal stomach in order to increase its reservoir capacity, and inhibits gastric emptying[218-220]. Secretin also inhibits gastric emptying[76,221]. It is therefore conceivable that low gastric ghrelin and high serotonin contribute to the slow gastric emptying in IBS-C, while the high gastric ghrelin and low intestinal CCK and secretin contribute to the rapid gastric emptying in IBS-D.

Several studies have found small-bowel transit to be delayed in IBS-C and accelerated in IBS-D[195,222-226]. However, a study from the Mayo clinic found no overall association between these IBS subgroups[161]. Studies on the motor patterns of the small bowel in IBS yielded contradictory results, which is probably due to marked inter- and intraindividual variations of small-intestine motor patterns[194,227-243]. As mentioned above, ghrelin cell density is low in the gastric oxyntic mucosa and PYY cell density is high in the ileum of IBS-C patients. Since ghrelin stimulates small-intestine motility and PYY stimulates the absorption of water and electrolytes, and is a major regulator of the ileal brake[244-249]. Moreover, it inhibits prostaglandin E2 and vasoactive intestinal polypeptide (which stimulate intestinal fluid secretion)[250-252], it is possible that the abnormalities in gastric ghrelin and ileal PYY contribute to the slow small-intestine transit in IBS-C. Secretin inhibits the contractile activity of the small intestine, and so the high ghrelin cell density and low duodenal secretin cell density may play a role in the rapid small-intestine transit in IBS-D.

It has been reported by some that colorectal transit is delayed in IBS-C and accelerated in IBS-D[153,222,223,253-258]. However, others have found that the colorectal transit time does not differ between IBS patients and controls[225,253,254]. The myoelectric and motor patterns of the large intestine of IBS patients have been investigated by several studies, which have yielded contradictory results[196,254-279]. In IBS-C, ghrelin cell density in the gastric oxyntic mucosa is low and ileal PYY cell density is high. Given that ghrelin stimulates intestinal motility and PYY stimulates the ileal-break, these abnormalities in ghrelin and PYY may promote the delayed colorectal transit observed in some IBS-C patients. CCK and secretin inhibit intestinal motility, and the cell densities of both are low in the duodenum of IBS-D patients. These factors together with a high gastric ghrelin cell density may contribute to the development of the accelerated colorectal transit seen in IBS-D patients.

In PI-IBS, the serotonin cell densities are high both in the small and large intestines, and CCK cell density is high in the small intestine. Serotonin primarily targets the mucosal projections of the intrinsic primary afferent neurons, which initiate peristaltic and secretory reflexes[156-161,175,280-289]. As mentioned above, CCK stimulates intestinal motility; thus, high serotonin and CCK levels could be responsible for the diarrhea seen in PI-IBS.

Abnormal secretion

Few studies have investigated gastrointestinal secretion in IBS patients. Enhanced intestinal secretion in response to bile acid perfusion in the ileum has been reported in these patients[290]. Increased reactivity of the secretory component of the migrating motor complex has been observed in the small intestine of IBS patients, and especially in those with IBS-D[291]. Among the abnormalities in the gut endocrine cells in IBS patients listed in Table 2, the low duodenal CCK and secretin observed in IBS-D, and the high ileal PYY cell density observed in IBS-C are particularly interesting with respect to gut secretions. CCK stimulates the secretion of digestive enzymes from pancreatic exocrine glands, and secretin stimulates pancreatic bicarbonate and fluid secretions[218,219]. The secretion of pancreatic bicarbonate increases the pH of the gut contents, which are highly acidic after leaving the stomach, and PYY stimulates the absorption of water and electrolytes[76]. This change in pH is essential for lipid digestion, since pancreatic lipase is irreversibly inactivated below pH 4.0[218]. It is tempting to speculate that IBS-D patients could suffer from fat maldigestion and a functional pancreatic insufficiency. Indeed, pancreatic enzyme substitution and a low-fat diet have been applied in clinical practice for these patients, with some success[292]. Moreover, an increase in PYY in the ileum of IBS-C patients may result in increased absorption of water from the feces, resulting in hard feces that worsen their to obstipation (Figure 4).

In conclusion, there are sufficient grounds to suspect

that the abnormalities in the gastrointestinal endocrine cells play a role in the development of visceral hypersensitivity, gastrointestinal dysmotility, and abnormal gastrointestinal secretion.

IS IBS AN ORGANIC DISORDER?

It has long been considered that IBS is caused by psychological stress and/or brain dysfunction, and it is overrepresented in patients with psychiatric illness/and or sexually and/or physically abused individuals. During the past decade there has been rapid progress in our understanding of IBS, and there is accumulating evidence of a biological etiology for this condition. Research to establish effective treatments for IBS have been intensified, and societal attitudes toward IBS patients are slowly changing.

This review presents evidence for an anatomic defect in IBS patients, namely the gastrointestinal endocrine cells. However, the data presented on the gastrointestinal endocrine cells in sporadic IBS were obtained by only two research groups. Further studies performed by other researchers involving different patient cohorts are needed before these observations can be confirmed. Conversely, while the data for PI-IBS were reported by several research groups from different countries and related to different patient cohorts, studies on PI-IBS have focused mainly on serotonin and are mostly restricted to the rectum. Further studies of other endocrine cells in different segments of the gastrointestinal tract are needed in PI-IBS. It should be noted that the gastrointestinal endocrine cells interact in an integrated manner with each other and the ENS, and together constitute the so-called neuroendocrine regulatory system of the gut[76,293-295]. It is thus possible that IBS patients have an abnormality in the ENS, in addition to those in the endocrine cells. However, investigating the ENS is very difficult since it would require whole-wall biopsy sampling under laparoscopic control, which represents a risk for both patients and controls. Regardless of the ethical issues this raises, it is unlikely that either patients or healthy subjects would voluntarily submit to laparoscopy and whole-wall biopsy sampling.

The abnormalities in the gut endocrine cells differ between sporadic IBS and PI-IBS/IBD-IBS, and their etiologies also appear to be different. Familial aggregation, twin, and genetic studies provide evidence for a genetic predisposition in sporadic IBS[296-306], and these patients describe their symptoms as commencing in childhood, suggesting the presence of genetically defective gastrointestinal endocrine cells. However, gastrointestinal mucosal cells - including the endocrine cells - have a rapid turnover, and it is also possible that factors related to luminal content such as diet or bacterial flora can provoke an increase or decrease in the endocrine cell population.

The etiology of the gastrointestinal endocrine cell abnormalities in PI-IBS and IBD-IBS appears to differ from that of sporadic IBS. Patients who develop PI-IBS and IBD-IBS likely have a genetic predisposition (host related) as well as other factors, such as infecting-organism-associated risk factors[307-315]. Following infection, these patients develop a low-grade inflammation that manifests as an increased intraepithelial and mucosal infiltration of lymphocytes and mast cells[143-149,316]. There is some evidence that inflammation and immune cells affect the gastrointestinal endocrine cells[317]. The secretion of serotonin by enterochromaffin (EC) cells can be enhanced or attenuated by the secretory products of immune cells, such as $CD4^+$ T, and also modulates the immune response[126,317]. The EC cells are in contact with or very close to $CD3^+$ and $CD20^+$ lymphocytes, and several serotonergic receptors have been identified in lymphocytes, monocytes, macrophages, and dendritic cells[318]. Therefore, it is conceivable that the abnormalities in the gastrointestinal endocrine cells in PI-IBS and IBD-IBS are caused by endocrine/immune interactions (*i.e.*, the endocrine/immune axis), which are in turn caused by low-grade inflammation in predisposed individuals[319,320].

CONCLUSION

The gut NES comprises the gastrointestinal endocrine cells and the ENS[76]. This regulatory system controls all gastrointestinal functions independently from the CNS[76,156]. However, the gut NES and the CNS are connected, and the CNS modulates the gastrointestinal functions through this connection[152]. Thus, a defect in the gut NES should be suspected in patients with IBS[76,294]. The gastrointestinal endocrine cells serve as chemical and mechanical transducers for afferent projections to the ENS, and subsequently to the CNS[294,321]. The present review describes evidence in the literature of an anatomic defect in the NES in IBS, namely defective gastrointestinal endocrine cells. Therefore, in line with some other gastroenterologists, we consider it highly likely that IBS is an organic disorder[107].

The endocrine cells interact in an integrated manner with each other. It is possible that the abnormality in many endocrine cells of the gut seen in IBS is caused by a defect in one or more endocrine cell types, which in turn results in changes in the other endocrine cell types.

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Sex hormones in the modulation of irritable bowel syndrome

Agata Mulak, Yvette Taché, Muriel Larauche

Agata Mulak, Department of Gastroenterology and Hepatology, Wroclaw Medical University, 50-556 Wroclaw, Poland
Yvette Taché, Muriel Larauche, CURE: Digestive Diseases Research Center and Oppenheimer Family Center for Neurobiology of Stress, Digestive Diseases Division, Department of Medicine, University of California Los Angeles, VA Greater Los Angeles Healthcare System, Los Angeles, CA 90073, United States
Author contributions: Mulak A designed and wrote the paper; Taché Y designed, wrote and reviewed the paper; Larauche M wrote and reviewed the paper.
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Correspondence to: Agata Mulak, MD, PhD, Department of Gastroenterology and Hepatology, Wroclaw Medical University, Borowska 213, 50-556 Wroclaw, Poland. agata.mulak@wp.pl
Telephone: +48-71-7332120 Fax: +48-71-7332129
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Abstract

Compelling evidence indicates sex and gender differences in epidemiology, symptomatology, pathophysiology, and treatment outcome in irritable bowel syndrome (IBS). Based on the female predominance as well as the correlation between IBS symptoms and hormonal status, several models have been proposed to examine the role of sex hormones in gastrointestinal (GI) function including differences in GI symptoms expression in distinct phases of the menstrual cycle, in pre- and post-menopausal women, during pregnancy, hormonal treatment or after oophorectomy. Sex hormones may influence peripheral and central regulatory mechanisms of the brain-gut axis involved in the pathophysiology of IBS contributing to the alterations in visceral sensitivity, motility, intestinal barrier function, and immune activation of intestinal mucosa. Sex differences in stress response of the hypothalamic-pituitary-adrenal axis and autonomic nervous system, neuroimmune interactions triggered by stress, as well as estrogen interactions with serotonin and corticotropin-releasing factor signaling systems are being increasingly recognized. A concept of "microgenderome" related to the potential role of sex hormone modulation of the gut microbiota is also emerging. Significant differences between IBS female and male patients regarding symptomatology and comorbidity with other chronic pain syndromes and psychiatric disorders, together with differences in efficacy of serotonergic medications in IBS patients confirm the necessity for more sex-tailored therapeutic approach in this disorder.

Key words: Brain-gut axis; Irritable bowel syndrome; Microbiota; Pain modulation; Sex hormones

Core tip: Recent clinical and experimental findings support the modulatory actions of sex hormones exerted at different levels of the brain-gut-microbiota axis in irritable bowel syndrome (IBS). Sex hormones may influence peripheral and central regulatory mechanisms contributing to the alterations in visceral sensitivity, motility, permeability, and immune activation of intestinal mucosa. A new concept of "microgenderome" is emerging based on the observations that the gender bias present in numerous diseases may be reinforced by the commensal microbiota of the host. Significant sex differences in epidemiology, symptomatology, and treatment outcome in IBS indicate the necessity for sex-tailored therapeutic approach in this disorder.

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INTRODUCTION

Sex hormones, in particular estrogens, play a significant role in the physiology and pathology of the gastrointestinal (GI) tract including regulation of motor and sensory function[1,2]. Irritable bowel syndrome (IBS) is a GI sensory-motor disorder characterized by abdominal pain or discomfort associated with a change in bowel habits[3]. The role of gonadal hormones in symptomatology and pathophysiology of IBS is being increasingly recognized based on the female predominance as well as the correlation between IBS symptoms and hormonal status during menstrual cycle phases, pregnancy or menopause[4]. Sex differences in stress and pain response are considered as crucial factors in the pathogenesis of functional GI disorders[4,5]. Sex hormones influence peripheral and central regulatory mechanisms of the brain-gut axis involved in the pathophysiology of IBS contributing to alterations in visceral sensitivity, motility, permeability, and immune activation of intestinal mucosa[4,6,7]. Among numerous interactions of sex hormones with other neurotransmitters, estrogen interactions with serotonin and corticotropin-releasing factor (CRF) signaling systems play a pivotal role[8,9]. Estrogens can also modulate neuroimmune interactions triggered by stress *via* the brain-gut axis[10]. Recently, the gut microbiota has been also recognized as an important element in the bi-directional communication along the brain-gut axis through neural, immune, and endocrine pathways[11,12]. In the present article we will review recent clinical and experimental findings supporting the modulatory effect of sex hormones, in particular estrogens, on different levels of the brain-gut-microbiota axis in IBS and their clinical implications regarding the symptomatology, pathophysiology and treatment of IBS.

SEX AND GENDER DIFFERENCES IN IBS PREVALENCE AND SYMPTOMATOLOGY

In Western countries the female-to-male ratio among non-patient population of IBS sufferers is 2:1[13]. Within the patient population in primary or tertiary care settings females outnumber male patients by 3:1 to 5:1, respectively[13-15]. However, in many Eastern countries such as India, China and South Korea, the female predominance among IBS patients is not observed[16]. Likewise, results of recent meta-analysis studies in South Asia, South America, or Africa confirmed that IBS prevalence was not significantly higher in women, compared to men[17]. Therefore gender-related and socio-cultural differences in health care-seeking behavior are suggested to also account in IBS symptoms reporting[18]. Further epidemiologic studies from different world regions are needed to elucidate the complex interactions between genetic, environmental, psychological and/or cultural factors that may contribute to sex differences in IBS symptoms[19,20].

Additionally, as the prevalence of IBS subtypes varied according to gender[17], the dominating subtype of IBS in different countries may also affect the male-to-female ratio. Women with IBS, compared to male patients, are more likely to report constipation, bloating, severe abdominal pain, and feeling of incomplete evacuation, while men with IBS more frequently complain of diarrhea-associated symptoms[17,19,21]. In fact, earlier studies indicate that women have slower colonic transit in comparison with men[22,23]. The results of the recent study by Tang *et al*[24] conducted in Chinese population confirmed significant differences between female and male IBS patients in their rating of abdominal pain/discomfort with regard to severity and duration, but not frequency of pain attacks. Interestingly, sex differences in dietary coping with GI symptoms have also been reported[25]. Female IBS patients seem to be more willing to implement nutritional behavior changes alleviating the GI problems than men, although both men and women could benefit similarly from these changes[25].

Cain *et al*[26] reported higher GI symptoms (pain, distension, bloating, intestinal gas) in postmenopausal women than in men, but the greatest differences in the overall symptom reporting between men and women were associated with somatic symptoms such as joint and muscle pain. This gender-related difference was most prominent when postmenopausal women where compared to men. Gender differences were much weaker for psychological and emotional symptoms except for fatigue, sleep disturbances and stress[24,26]. Noteworthy, there is a wide spectrum of chronic pain disorders frequently overlapping with IBS namely fibromyalgia, migraine headache, chronic pelvic pain, interstitial cystitis, and chronic fatigue syndrome. These diseases are also characterized by female predominance with a correlation between their symptoms and hormonal status[27-29]. In addition, women with IBS, more frequently show comorbidity with affective or mood symptoms including anxiety and depression as compared to women without IBS[30]. There are also reports indicating that women with IBS exhibit more anxiety and depressive symptoms compared to men with IBS[24,26]. Sex differences in the prevalence of concomitant somatic symptoms, as well as anxiety and depression may significantly contribute to the greater impairment of quality of life in female patients and affect treatment results[24,31].

Sex-related difference in IBS prevalence emerges around the time of puberty and increases during the early adult years. Women are suffering from IBS most commonly in the late teenage to mid forties, additionally suggesting the role of reproductive hormones in the pathophysiology of the disorder. The incidence of IBS in women steadily declines with age and approaches the rate observed among men by the 7th decade of life[32]. By contrast, the prevalence of IBS among males is fairly constant within the age range of 20-70 years[32].

CORRELATION BETWEEN IBS SYMPTOMS AND HORMONAL STATUS

Menstrual cycle

The menstrual cycle in women is divided into three

Table 1 Correlation between hormonal status and irritable bowel syndrome symptoms expression[36]

Status	Hormone levels	IBS and pain related symptoms expression	Ref.
Late luteal phase (premenses)	Rapid decline in estrogen and progesterone levels	Exacerbation of bowel symptoms Increased bloating	[33,34]
Menstruation (menses)	Lowest levels of estrogen and progesterone	Exacerbation of bowel symptoms Increased abdominal pain/discomfort Lower rectal sensitivity threshold	[34,35,37,38,40]
Dysmenorrhea	Disturbances in hormonal interactions at different regulatory levels (lower progesterone level)	Exacerbation of bowel symptoms	[41]
Pregnancy	Physiological hyperestrogenemia and hyperprogesteronemia	Reduced pain sensitivity and alleviation of many chronic pain syndromes Exacerbation of constipation (prolonged gastrointestinal transit)	[27,51,73]
Menopause	Decline in ovarian hormones	Decrease in IBS incidence High prevalence of constipation and somatic discomfort syndromes	[26,53,54]
Oral contraceptives	Estrogen and progestin administration	Reduced abdominal symptoms at menses	[55]
Hormone replacement therapy	Estrogen (and progesterone) supplementation	Increased prevalence of IBS in postmenopausal women during HRT Prolongation of IBS symptoms to a later age	[58]
Oophorectomy	Ovarian hormone deficiency	Exacerbation or occurrence of gastrointestinal symptoms after gynecological surgery	[60]
Men with IBS	Lower level of luteinizing hormone in middle-aged men Elevated level of sex hormone-binding globulin in young men	Generally more prevalent diarrhea (compared to women with IBS)	[66,70]
Transsexual women (male-to-female subjects)	Estrogen/anti-androgen treatment	Development of chronic pain including visceral pain	[72]

IBS: Irritable bowel syndrome; HRT: Hormone replacement therapy.

phases: the follicular (proliferative) phase, ovulation, and the luteal (secretory) phase. Estrogen levels are increasing during the midfollicular phase and then drop precipitously after ovulation. This is followed by a secondary rise in estrogen levels during the midluteal phase with a decrease before menstruation. The secondary rise in estradiol parallels the rise of serum progesterone and 17-hydroxyprogesterone levels[33].

Dynamic changes in ovarian hormones during menstrual cycle can modulate GI contractility, transit, secretion, visceral sensitivity, and immune function at multiple target sites, including those located in the periphery and the brain[3]. Clinical studies indicate that declining or low ovarian hormone levels in women (such as during menses) may contribute to the occurrence or exacerbation of GI symptoms, including abdominal pain or discomfort, altered bowel habits and bloating that varies across the menstrual cycle phases (Table 1)[34-37]. Rectal sensitivity thresholds have been shown to be significantly lower in IBS patients at menses relative to those at other cycle phases indicating that IBS symptoms experience may be modified by ovarian hormone status[38]. Also in animal studies it has been shown that both visceral and somatic sensitivity vary over the rat estrous cycle and that high levels of ovarian hormones (proestrus/estrus stages) are associated with enhanced sensitivity[39]. Therefore the menstrual cycle provides a natural model to explore the effects of ovarian hormones on the bowel function.

Approximately one third of otherwise asymptomatic women experience GI symptoms at the time of menstruation[34]. About 40% of women with IBS report influence of the menstrual cycle on their symptoms[35]. Whitehead *et al*[37] found that in women with functional bowel disorders (FBDs), including IBS, bowel symptoms seem to be affected by menstruation to a greater degree than in women without FBDs, suggesting that IBS women may respond differently to the fluctuations in the ovarian hormones. Variation in GI symptoms during the menstrual cycle can be related to motor disturbances and/or a change in perception of colonic motor events, as well as alterations in colonic epithelial barrier and mucosal immunity[10,40].

IBS female patients are more likely to report dysmenorrhea and premenstrual distress syndrome than those who do not suffer from IBS[41-43]. Moreover, IBS patients with dysmenorrhea report noticeably more GI symptoms than non-dysmenorrheic women[41]. In a 10-year follow-up-study conducted in Iceland it has been shown that IBS female patients with dysmenorrhea were twice more likely to have increased symptoms compared to IBS patients without dysmenorrhea[43].

A significant connection between IBS and endometriosis has also been reported[43,44]. Additionally, polycystic ovary syndrome (PCOS), the most common female endocrine disorder affecting up to 10% of reproductive-age women characterized by chronic anovulation and hyperandrogenism, is associated with the increased prevalence of IBS[45-47]. Interestingly, IBS coexisting with PCOS was associated with a higher BMI and percent body fat when compared to PCOS alone[45]. The relationships between obesity, hormonal status and IBS require further investigation, particularly in the context

of obesity being linked with increased inflammatory mediators and in the light of recent reports on the GI dysbiosis[46,48].

Pregnancy

Pregnancy is characterized by high ovarian hormones levels as well as an increase in opioid-mediated antinociception[3,49]. Little is known, however, regarding IBS symptoms and pregnancy. Many chronic pain syndromes frequently associated with IBS, like migraine headache for example, are alleviated during the time of pregnancy[27]. Similarly, in rodents high ovarian hormones levels during pregnancy reduce somatic and visceral pain sensitivity[50]. During the time of the physiological hyperestrogenemia and hyperprogesteronemia a prolonged GI transit is also observed[51]. Additionally, numerous psychological variables affecting the autonomic nervous system (ANS) may trigger or modulate symptoms reported in pregnant women[52].

Menopause

Data concerning the impact of the menopause transition on IBS patients remain inconsistent. Although the decline in ovarian hormones may induce or exacerbate GI symptoms, generally, in postmenopausal period, the incidence of IBS decreases significantly[53-55]. However, according to some recent data, IBS symptoms severity may increase after menopause as well[43]. Cain *et al*[26] found that various GI symptoms were reported more frequently by postmenopausal women compared with men, but these differences were not significant when controlled for age. In one study, gas and excessive flatulence were more prevalent in post- than premenopausal healthy women[53].

Hormone supplementation

Premenopausal healthy women taking oral contraceptives (OCs), monophasic or triphasic preparations, report a typical increase in GI symptoms at menses[55]. However, women with IBS taking OCs, which contain both estrogen and progestin, appeared to have reduced levels of abdominal symptoms compared with IBS women not taking OCs[55]. At the same time, the pattern of GI and non-GI symptoms over the menstrual cycle was similar in female patients with IBS, regardless of OCs use or the predominant bowel pattern[55]. Noteworthy, in women with dysmenorrhea that may coexist with IBS, OCs often reduce the symptoms[15]. Recently, Bird *et al*[56] reported an increased risk for development of IBS with drospirenone. Drospirenone is a synthetic progestin approved in combination with ethinyl-estradiol as an OC. Although it was designed as an antimineralocorticoid steroid, it exhibits antiandrogen activity[56]. In another study evaluating the effect of hormone supplementation on IBS symptoms, the therapeutic efficacy of gonadotropin-releasing hormone agonist (leuprolid) in female patients with menstrual cycle-related symptoms has been reported[57]. However, the use of this medication is limited by its side effects[57].

Based on the recent meta-analysis, there are insufficient data to determine the exact effect of hormone supplementation during menopause on IBS symptoms[19]. In postmenopausal women, hormone replacement therapy (HRT) has been reported to be associated with the increased prevalence of IBS. HRT may prolongs IBS symptoms to a later age or even induce changes in GI function in females not previously affected[58]. One of the confounding factors may be related to the fact that women with IBS are more likely to report various pre- and postmenopausal symptoms, and thus may be prescribed HRT to a greater degree. However, Ruigómez *et al*[58] have shown that both current and past users of HRT presented an increased risk of IBS compared to non-users, even after adjusting for comorbidity and consultation pattern. This increased risk was irrespective of treatment duration, regimen or route of administration of HRT[58].

Gynecological surgery

There are few data concerning the prevalence of oophorectomy or hysterectomy in IBS female patients, mostly because these surgical procedures are excluding factors in the studies of IBS patients. However, it has been reported that the rate of hysterectomy is about twice higher in women with IBS compared to controls[59]. It is conceivable that IBS patients, because of the chronic abdominal pain, are more likely to be qualified for various surgical procedures (not only gynecological, but also GI surgery like cholecystectomy and appendectomy)[59]. In fact, in a number of women, GI symptoms emerge for the first time after gynecological surgery[60]. Preclinical studies however remain controversial. There is indeed evidence in mice that ovariectomy generates a slow developing and persistent hyperalgesic state localized to the abdomen, lower limbs and abdominal viscera, which is reversed by estrogen supplementation[61,62]. In contrast, in rats, ovariectomy decreased the magnitude of the visceromotor response to colorectal distension compared with cycling rats[63] and abolished restraint stress-induced visceral hypersensitivity[64]. The sensitivity to colorectal distension and the influence of stress on visceral pain were restored by estrogen replacement at a dose comparable to the proestrus level[65].

Male sex hormones

Most of the explanations of sex-related differences in IBS have focused on the concept that women might be more susceptible, while less attention has been given to the concept that male hormones may be protective against pain disorders including IBS[66]. Androgens, higher in males than females, appear to protect against the development of chronic pain disorders in humans, and testosterone exerts an analgesic effect in experimental pain models, in both men and women[67-69]. Differences in androgen levels, their receptors as well as sites of action may play a role in the sex difference in the risk of developing chronic pain disorders. There are only few reports concerning the role of sex hormones in male

patients with IBS[67,70,71]. Houghton *et al*[66] found that testosterone levels, although similar in the patient and control groups, correlated negatively with perceptual thresholds of rectal distension and overall well-being in IBS patients. In the same study it was found that middle-aged male IBS patients tended to have lower levels of luteinizing hormone compared with male control subjects[66]. Kim *et al*[70] have also reported that the sex hormone status in young male patients is different from that of older male patients and that an elevated sex hormone-binding globulin level might play a key role in the pathophysiology of IBS in young men. Interestingly, a highly significant reduction in male-trait scores in men with IBS has been confirmed[71]. Another unique model to study the relationship between sex hormones and chronic pain was proposed by Aloisi *et al*[72] who evaluated the results of sex-crossed hormone administration in transgender subjects. About half of the female-to-male subjects treated with testosterone reported a significant improvement of the chronic pain (*e.g.*, headache) present before the treatment. Conversely, about one-third of the male-to-female subjects receiving estrogen/anti-androgen treatment developed chronic pain including headaches, breast and musculoskeletal pain, and in some cases visceral pain as well[72]. These findings support experimental and clinical data suggesting that sex steroid hormones play a crucial role in pain perception and modulation.

SEX HORMONE MODULATION OF THE BRAIN-GUT AXIS AT THE CENTRAL NERVOUS SYSTEM LEVEL

Estrogens

The abundant distribution of estrogen receptors (ERs) at all levels of the brain-gut axis, including the central nervous system (CNS), spinal cord, and the enteric nervous system supports the multiplicity of neuronal action[73]. There are two subtypes of ERs: ER-α and ER-β. Estrogens, similarly to progesterone and testosterone, exert their function by binding to either specific intracellular (nuclear) receptors that act as ligand-dependent transcription factors (classical mechanisms) or membrane-bound receptors (mERs) that stimulate several signal transduction pathways (non-classical mechanisms). The family of nuclear receptors mediate rather slow genomic action of estradiol resulting in enhancement or repression of gene transcription and thus protein synthesis alterations. In contrast, mERs are involved in the rapid action of estrogens related to the activation of various protein-kinase cascades and phosphorylation of proteins, but estrogenic rapid signaling can also occur by recruiting intracellular pathways that can act *via* the genome through phosphorylated cyclic adenosine monophosphate (cAMP) response element protein (pCREB) and intermediate early genes[74]. In addition to the well described G protein-coupled receptor (GPR30), multiple mERs have recently been discovered, such as the classical nuclear ER-α and ER-β, ER-αΔ4, ER-X and mER-Gαq[74-76].

ERs are spread throughout the brain, including the amygdala, hypothalamus, pituitary, hippocampus, cerebral cortex, mid-brain, and brain stem, providing neuroanatomical support for potential numerous target sites of estrogen actions on neurocognitive processes[73,77]. Based on the results of brain imaging studies, greater responsiveness of emotional arousal circuits in relation to visceral pain has been implicated as induceing central mechanisms of pain amplification in IBS, with female subjects showing greater response than male subjects[78]. Recent results confirmed sex differences in emotion-related cognitive processes and functioning of brain networks including the prefrontal regions, cingulate, insula, and amygdala in IBS and healthy control subjects[79].

Estrogens may act in the CNS through multiple pathways modulating production and action of neurotransmitters, influencing electrical excitability and synaptic function, and changing the morphological features of neural elements involved in the function[77,80,81]. Estrogens have been documented to exert differential, sometimes opposite effects on pain. Clinical and experimental data indicate that both analgesic and hyperalgesic responses can be induced by estrogens depending upon the experimental conditions[67]. Estrogens were shown to enhance neuronal system activities during development and in adult life, for instance through the hippocampal neuronal circuits involving acetylcholine, glutamate and brain-derived neurotrophic factor[82]. Noteworthy, elevated levels of estrogens in fertile women have been associated with the increased number of μ-opioid receptors in the brain regions related to pain processing[68]. There is accumulating evidence that estrogens have a significant impact on neuronal plasticity-related process and ameliorate recovery after chronic stress (Table 2)[73].

Estrogens may also contribute to the important sex differences in the stress-related hypothalamic-pituitary-adrenal (HPA) axis response that have been documented in a number of clinical and experimental studies[83]. The menstrual cycle phases, menopausal status and pregnancy have been shown to affect the HPA axis as well as ANS functions[5]. Women between puberty and menopause usually show lower HPA axis and autonomic responses to psychological stressors than men of the same age[84]. However, the HPA axis response to psychological stressors is higher in the luteal phase, when post-stress free cortisol level approaches that for men[84]. CRF is a key mediator of the HPA axis and the brain-gut axis response to stress at both central and peripheral levels[9,85,86]. The co-localization of ER-α with CRF receptors in the hypothalamus represents one of the possible neuroendocrine interactions between CRF signaling pathways and estrogens[87]. Importantly, activation of both receptors ER-α and ER-β has been shown to stimulate *CRF* gene expression in the hypothalamic paraventricular nucleus (PVN)[83,88]. Additionally, estrogens induce also an increase in glucocorticoid receptor expression in the amygdala[88]. In the recent functional magnetic resonance imaging study, it was demonstrated that significant sex differences in brain activity in stress

Table 2 Sex hormone modulation of the brain-gut-microbiota axis

Level of the brain-gut-microbiota axis	Estrogen	Progesterone	Testosterone
Central nervous system	Analgesic or hyperalgesic effect[67] Excitatory action on neurons[72] Estrogen-induced increase in the number of μ-opioid receptors[68] Enhancement of serotonergic postsynaptic responsiveness in the brain[8] Central interaction with CRF signaling pathways-modulation of stress responsiveness[87,89]: Stimulation of *CRF* gene expression in PVN[83] Increase in glucocorticoid receptor expression in the amygdala[83] Influence on neuronal plasticity-related processes[73]	Activation of the γ-aminobutyric acid (GABA) receptors, major inhibitory receptors in the brain[77] Neuroprotective action in the hippocampus[80]	Analgesic effect[72] Inhibition of stress-induced ACTH release[103]
Autonomic nervous system	Attenuation of sympathetic responsiveness[108]	Reduced cholinergic responsiveness[5]	Regulation of parasympathetic tone[110]
Enteric nervous system/ Gut immune system	Expression of estrogen receptors in enteric neurons, regulation of neurogenic reflexes[73] Activation of colonic NK1 receptors in stress-induced visceral hypersensitivity[64] Augmentation of mast cells secretion[118] Effects on both pro- and anti-inflammatory pathways[113] Peripheral interaction with CRF signaling pathways, modulation of colonic motor and sensory responses to stress[87] Regulation of 5-HT3 receptor expression in rat colon[120] Regulation of secretory and absorptive function of gastrointestinal epithelial cells[128] Enhanced expression of trans-membrane tight junction protein in non-inflamed colon[124] Decreased production of proinflammatory cytokines in experimental colitis in female rats[125,126]	Inhibition of gastrointestinal motility[130] Inhibition of visceral signaling following colonic inflammation[100] Inhibition of mast cells degranulation[131] Immunosuppressive action related to inhibition of NFκB activation in macrophages[133]	Stimulation of smooth muscle contractions[135] Decreased production of proinflammatory mediators inducing visceral hyperalgesia[69,136] No effect on mast cells degranulation[137] Decreased TLR4 expression of macrophages and monocytes[138]
Gut microbiota	ER-β expression affects the gut microbiota composition[143] Microbial β-glucuronidase activity determines estrogens deconjugation enabling their reabsorbtion *via* enterohepatic circulation[146] Direct effect on bacterial metabolism, growth and expression of virulence factors[132]	Direct effect on bacterial metabolism, growth, and expression of virulence factors[132]	Reversible 17β reduction of androgens may regulate testosterone level[148] Commensal microbiota-dependent testosterone production protects against autoimmune disease in mice[149]
	Bacterial hydroxysteroid dehydrogenase regulates the balance between active and inactive steroids[132]		

ACTH: Adrenocorticotropic hormone; CRF: Corticotropin-releasing factor; NFκB: Nuclear factor κB; PVN: Paraventricular nucleus; TLR4: Toll-like receptor 4.

response circuitry were dependent on women's menstrual cycle phase[89]. In addition, chronic treatment with estrogens modulates brain circuitry responsive to stress[90]. Furthermore, administration of estradiol and progesterone directly to the amygdala in rats increases pain response to visceral stimulation suggesting that an amygdala-dependent mechanism may be responsible, at least in part, for the exacerbation of visceral symptomatology in females[91]. A recent meta-analysis by Tillisch *et al*[92] points to the amygdala, a brain region known to facilitate HPA axis output, as one of the most consistently activated areas following rectal stimulation in IBS patient compared with controls. Of significance, the activation of the amygdala by corticosterone eliminates spontaneously occurring differences in visceral and somatic pain perception in cycling female rats, resulting in visceral hypersensitivity during metestrus/diestrus, and increased somatic sensitivity during both metestrus/diestrus as well as proestrus/estrus[39]. This observation could explain the lowered pain thresholds and higher incidence of somatic pain observed in women with IBS[39].

Childhood trauma (early adverse life event, EAL) is associated with changes in HPA axis responsiveness in IBS[93]. Dysregulation of the HPA axis in IBS patients has been related to blunted adrenocorticotropic hormone (ACTH) levels and enhanced cortisol response to visceral stimulation[94]. However, little is known on sex-differences in EAL-induced visceral pain. Interestingly, sexually dimorphic effects of unpredictable EAL on visceral pain behavior in a rodent model has been demonstrated[95]. Female rats exposed neonatally to different pairings of an odor and shock developed visceral hypersensitivity in adulthood, while in contrast, in male rats, visceral sensitivity was not significantly different after EAL. Visceral sensitivity following unpredictable EAL was reversed by ovariectomy and reestablished by estradiol

replacement. These data suggest estrogen-mediated pivotal mechanisms in maintaining visceral hypersensitivity[95].

The serotonergic system represents another potential contribution to sex differences in pain modulation[3,8]. In the CNS, serotonin (5-HT) generally has been associated with descending pain inhibition, whereas in the periphery, 5-HT is an inflammatory mediator and is generally pronociceptive and prokinetic. Estrogens enhance serotonergic postsynaptic responsiveness in the brain[8]. Additionally, estrogens enhance 5-HT synthesis in most part of the brain by increasing expression of the enzyme tryptophan hydroxylase and decreasing the expression of the serotonin re-uptake transporter[96]. The serotonergic and reproductive endocrine systems are also prominently involved in both the regulation of mood and behavioral states. In addition, interactions between these systems have profound implications for the etiology and treatment of anxiety disorders[97]. A growing body of evidence also indicates sex-dependent differences in serotonin-related genetic polymorphisms in IBS patients[98], particularly with regard to anxiety and depressive disorders more common in women with IBS[99].

Progesterone

The role of progesterone in sex-related differences in pain modulation is less clear. Progesterone activates intracellular receptors to regulate genomic processes, and also affects cell membrane receptors, especially in neurons[100]. Membrane progesterone receptors present in the hippocampus were suggested to contribute the neuroprotective action of the hormone[80]. At the CNS level, progesterone action seems to be dependent on the activation of the γ-aminobutyric acid receptors that are major inhibitory receptors in the brain[101].

Androgens

While estrogens are commonly indicated as CNS stimulant, androgen receptor-mediated actions are often related to CNS inhibition, which may underlie the lower incidence of many forms of chronic pain in men[72]. Androgens participate in the regulation of the HPA axis response to chronic stress and the autonomic circuitry[102]. Optical and electron microscopic immunocytochemical studies in rodents have revealed that the distribution of androgen receptors is overlapping with that of ER-α, ER-β, as well as progesterone receptors in three major autonomic regions in the brain: the rostral ventrolateral medulla, nucleus of the solitary tract and PVN[80]. In male rats, testosterone inhibits the acute restraint stress-induced ACTH release[103] that, ultimately, may impact on other brain stress-related CRF-mediated influence on colonic motility and visceral pain[9].

SEX HORMONE EFFECTS ON THE AUTONOMIC NERVOUS SYSTEM

Estrogens

Estrogens influence also nociceptive pathways at the level of primary afferent nerves and spinal cord projections[104]. A direct involvement of ERs in nociceptive transmission is possible *via* their activation of enkephalin synthesizing cells in the superficial laminae of the spinal cord[105]. Additionally, estrogens modulate the responsiveness of primary vagal afferents neurons to substance P and the activation of glutamate receptors involved in the afferent pain pathways[40]. Spinal estrogen receptors ER-α and ER-β have been also shown to contribute to the facilitation of N-methyl-D-aspartate-dependent colon-to-urethra cross-organ reflex sensitization, which is presumed to underlie pelvic viscero-visceral referred pain[106].

Autonomic dysregulation in response to a visceral stressor is an objective physiologic correlate in IBS[107]. Tillisch *et al*[108] reported gender differences in the ANS reactivity to colorectal distension in IBS patients, with men demonstrating increased sympathetic nervous system activation and decreased parasympathetic activation compared to women. There are also data indicating menstrual cycle-linked differences in the ANS tone that are likely to result from estrogen exposure, and its attenuating influence on sympathetic responsiveness[109]. Furthermore, many other chronic pain syndromes, frequently coexisting with IBS can be also related to autonomic disturbances[28].

Progesterone and androgens

Progesterone has been shown to reduce cholinergic responsiveness[5]. However, little is known about the effect of testosterone on the ANS. Recently, it has been noticed that testosterone deficiency is accompanied by a decrease in basal parasympathetic tone and reduced baroreflex sensitivity in men with heart failure[110].

SEX HORMONE ACTIONS AT THE ENTERIC NERVOUS AND GUT IMMUNE SYSTEMS

Estrogens

Within the enteric neurons of the colon, where both CRF receptor subtype 1 (CRF_1) and ERs are expressed, interactions between CRF signaling pathways and estrogens participate in the stimulation of the colonic motor function[90]. Additionally, a local paracrine/autocrine pro-inflammatory action by CRF_1 receptor activation was reported in several models of intestinal inflammation both *in vitro* and *in vivo*, as well as the up-regulation of CRF and CRF_1 expression in immune cells of the human colonic lamina propria in response to inflammation[86].

There is compelling evidence suggesting an up-regulated gut immune function in patients with IBS, particularly with post-infectious IBS[111]. Gastrointestinal inflammation seems to be strongly modulated by stress, especially in IBS patients being characterized by enhanced stress responsiveness[15]. Important sex-related differences in IBS patients related to neuroimmune inter-

actions have been suggested[71,112]. Female sex is an independent risk factor for developing postinfectious IBS[3]. The following observations support sex differences in immune response: females produce stronger cellular as well as humoral immune reaction, have a greater resistance to bacterial infections, and are more likely to develop autoimmune diseases compared to men, symptoms of which depend on hormonal status[113]. Estrogens may influence both pro- and anti-inflammatory pathways. The effect of estrogens in inflammatory responses has been found extremely complex and dependent on the estrogen level, the cell type, specific inflammatory factors, the type of tissue that is inflamed, the time course of the inflammatory response (*e.g.*, acute *vs* chronic), and the time point at which estrogen exposure occurs[113]. In an experimental model, estrogens contributed also to the colonic neurokin-1 receptor-mediated effects of stress-induced visceral hypersensitivity to colorectal distension[64].

Mast cells represent another crucial link in sex-dependent neuroimmune interactions as they co-express CRF and sex hormone receptors[114-116]. The number of colonic mucosal mast cells was found to be higher in female compared to male IBS patients[10]. Mediators released by activated mast cells, characterized by extensive anatomical and functional communication with intrinsic and extrinsic nervous system of the gut, evoke visceral hypersensitivity and increase mucosal permeability[117]. Notably, mast cells are involved in many other disorders, frequently overlapping with IBS such as fibromyalgia, interstitial cystitis, chronic fatigue syndrome and migraine, all of which occur more often in women, are exacerbated during ovulation and reduced during pregnancy[10]. These sex-related differences in the prevalence and severity of chronic pain disorders could be related to the fact that mast cells express progesterone and estrogen receptors[10]. Estradiol has been shown to augment mast cells secretion, whereas tamoxifen (an estradiol receptor antagonist) inhibits this function[118].

The serotonergic system at the peripheral level may also contribute to sex differences in modulation of GI motility, secretion and sensitivity[3,8]. Fluctuations in estrogen levels during ovarian cycle cause predictable changes in 5-HT system in women[8]. Moreover, 5-HT concentration varies with sex and menstrual status in patients with diarrhea-predominant IBS[119]. Experimental studies indicate that colonic 5-HT_3 receptor gene expression is increased in ovariectomized rats exposed to restraint stress and restored with hormone replacement after ovariectomy[120]. Recently, Galligan *et al*[121] proposed serotonine transporter (*SERT*) gene knockout (KO) rats as a new interesting model for studying interactions between serotonin, sex, and visceral sensation. SERT KO female rats display an increased colonic extracellular serotonin associated with visceral hypersensitivity and hyperexcitability of colon projecting sensory neurons, which is not observed in male SERT KO rats[121]. Gender difference has been also shown in SERT activity and serotonin concentration in platelets of IBS patients[122].

Estrogen-dependent modulation of the intestinal barrier function is another component in sex-related differences in IBS. It has been well established that stress involving the activation of CRF_1 receptors alters intestinal barrier that appears to be a prerequisite for the development of visceral hypersensitivity in both human and rodents[6,123]. In the colon, ERs signaling enhances expression of trans-membrane thigh junction proteins in non-inflamed conditions[124], and decreases production of proinflammatory cytokines in experimental colitis[125,126]. In human, acute experimental stress evokes a differential gender-dependent increase in intestinal macromolecular permeability[127]. A significant increase in albumin permeability in healthy women, but not in men, could explain enhanced female susceptibility to IBS[127].

Additionally, ERs are localized on the epithelial cells throughout the GI mucosa and may affect secretory and absorptive functions[37,128,129]. The fluid retention that occurs in females during the cycle may be associated with the extra-nuclear action of estrogen that can stimulate calcium entry into colonic epithelial cells as well as suppress c-AMP-dependent chloride secretion in the distal colonic epithelium in females only, both in rats and humans[128].

Progesterone and androgens

At the peripheral level progesterone has been suggested to influence both visceral sensitivity and motility *via* prostaglandins[100]. Overexpression of progesterone receptors in colonic muscle in women with slow transit constipation is associated with lower levels of prostaglandin $PGF_{2\alpha}$ and tromboxane A that cause muscle contraction, and higher levels of PGs that cause muscle relaxation (such as PGE_2)[130]. Progesterone may also inhibit estrogen-dependent mast cells degranulation[131]. Progesterone receptors have been identified in epithelial cells, granulocytes, macrophages, and lymphocytes[132]. Progesterone is known to exert an immunosuppressive action as it inhibits the activation of nuclear factor (NF)κB and increases the expression of the suppressor of cytokine signaling protein (COS1) in macrophages[133].

Testosterone and its active metabolite 5α-dihydrotestosterone are potent modulators of colonic motility by stimulating smooth muscle contractions through non-genomic calcium sensitization pathways[134]. In the urethral calculosis model of visceral pain, Aloisi *et al*[135] did not find any significant effect of testosterone on visceral pain. However, there is growing body of evidence that androgens may contribute to the modulation of visceral pain by decreasing pro-inflammatory mediators that participate in the development of hyperalgesia[69,136]. Apart from female sex hormone receptor expression, mast cells also express androgen receptor, however, testosterone treatment had no effect on mast cell degranulation[137]. Testosterone decreases also the expression of macrophage and monocyte Toll-like receptor 4, which is involved in the activation of the innate system response to pathogen challenge[138].

INTERACTIONS BETWEEN SEX HORMONES AND THE GUT MICROBIOTA

The increasing knowledge of the role of microbiota in health and disease state has shed a light on the critical role of the enteric microbiota, both commensal and pathogenic organisms, in regulation the brain-gut axis. This has consequently led to the coining of a new term: the brain-gut-enteric microbiota axis[11]. The bi-directional communication between the gut bacteria and the brain occurs through neural, immune, and endocrine pathways[12,48,139,140] which may be modulated by sex hormones, in particular estrogens. In fact, it has been recently reported that the microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner[141]. Clarke *et al*[141] found that male germ free mice, unlike females, display a significant elevation in the hippocampal concentration of 5-HT and its metabolite, compared with conventionally colonized control animals.

Numerous studies have reported the effects of sex hormones on the dimorphic sex differences in the response to microbial and viral infections[132]. Besides the role of sex hormones in the modulation of the immune system, they have a direct effect over bacterial metabolism, growth, and expression of virulence factors. For instance during pregnancy, the proportion of certain bacteria species associated with plaque microbiota is altered with a noticeable increase in the ratio of anaerobic to facultative bacteria[142]. Of significance, recent studies indicate that steroid nuclear receptor expression including ER-β can determine the intestinal microbiota composition[143].

Moreover, the gut microbiota may also affect estrogens metabolisms and their systemic level[144]. Conjugated estrogens are excreted in the bile and pass into the distal ileum, where they are variably deconjugated and may be reabsorbed from the gut lumen and enter the circulation *via* the portal vein[145]. It has been shown in men and postmenopausal women that the intestinal microbiota richness and function, associated for example with β-glucuronidase activity, influence levels of non-ovarian estrogens *via* enterohepatic circulation[146]. Bacteria are capable of metabolizing sex hormones through the activity of various enzymes such as hydroxysteroid dehydrogenase that regulate the balance between active and inactive steroids[130]. In particular, fecal bacteria can perform hydrolytic, reductive and oxidative reactions of estrogens and androgens[147]. Reversible 17β reduction of androgens carried out by the gut microbiota is suggested to play a role in the regulation of testosterone level[148]. The results of a landmark study published recently by Markle *et al*[149] provide astonishing conclusions indicating that sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. In the study performed in the non-obese diabetic mouse model of type 1 diabetes, they showed that male puberty in mice leads to changes in the gut microbiota that reinforce testosterone production, which is protective against the development of T and B cell functions linked to autoimmune disease[149]. In mice, the properties of the male-associated microbiota can be transferred to younger females and exert testosterone-mediated protection from autoimmune disease upon recipients. The observations that early-life microbial exposures determine sex hormone levels and modify sex-mediated immune regulation may have crucial implications for the pathophysiology of IBS. A new concept of "microgenderome" is emerging based on the recent observations that the gender bias present in numerous diseases is not entirely a host-intrinsic factor, but may be exercised and/or reinforced by the commensal microbiota of the host[150]. Undoubtedly, further studies are needed to elucidate the role of microgenderome in IBS.

THERAPEUTIC IMPLICATIONS

The modulator role of sex hormones on the bi-directional interactions within the brain-gut-microbiota axis may have significant therapeutic implications in IBS. However, although gender differences in responses to treatment modalities exist, the approach to IBS patients in both genders is quite similar so far. Clinical observations confirm that alosetron, a 5-HT_3 receptor antagonist, is more effective in improving urgency and loose stools in IBS-diarrhea predominant women than men[120,151,152]. The basis for this noticeable sex difference in therapeutic efficacy of alosetron could be associated with sex-related differences in 5-HT_3 receptor expression, lower alosetron clearance in women, and/or greater 5-HT synthesis in certain brain regions in IBS male patients compared with female IBS patients[153]. Sex difference in genetic polymorphism of the 5-HT transporter (SERT) promoter region has been also suggested and may induce the different expression of affective symptoms in women compared with men[154]. Additionally, the potential role of interaction between gonadal hormones and the cytochrome P450 pathway may be considered in sex-related differences in drug clearance[155]. Differences in adipose tissue compartment in women compared to men may affect this process as well[40].

Women with IBS are more susceptible to anxiety and depression and other stress-related disorders. However, in the study comparing the efficacy of treatment with paroxetine alone or combined with psychotherapy, no gender effect was reported[156]. Preliminary observations suggesting that IBS female patients may better respond to hypnotherapy[157] is yet to be confirmed. The recent results of randomized controlled trial have shown that gender, age, disease duration and IBS type have no influence on the long-term success of gut-directed hypnotherapy[158].

Regarding the role of sex hormones in the pathogenesis of IBS, therapeutic approaches aiming to suppress ovarian steroidogenesis have been also considered. In fact, gonadotropin-releasing hormone agonist (leuprolide)

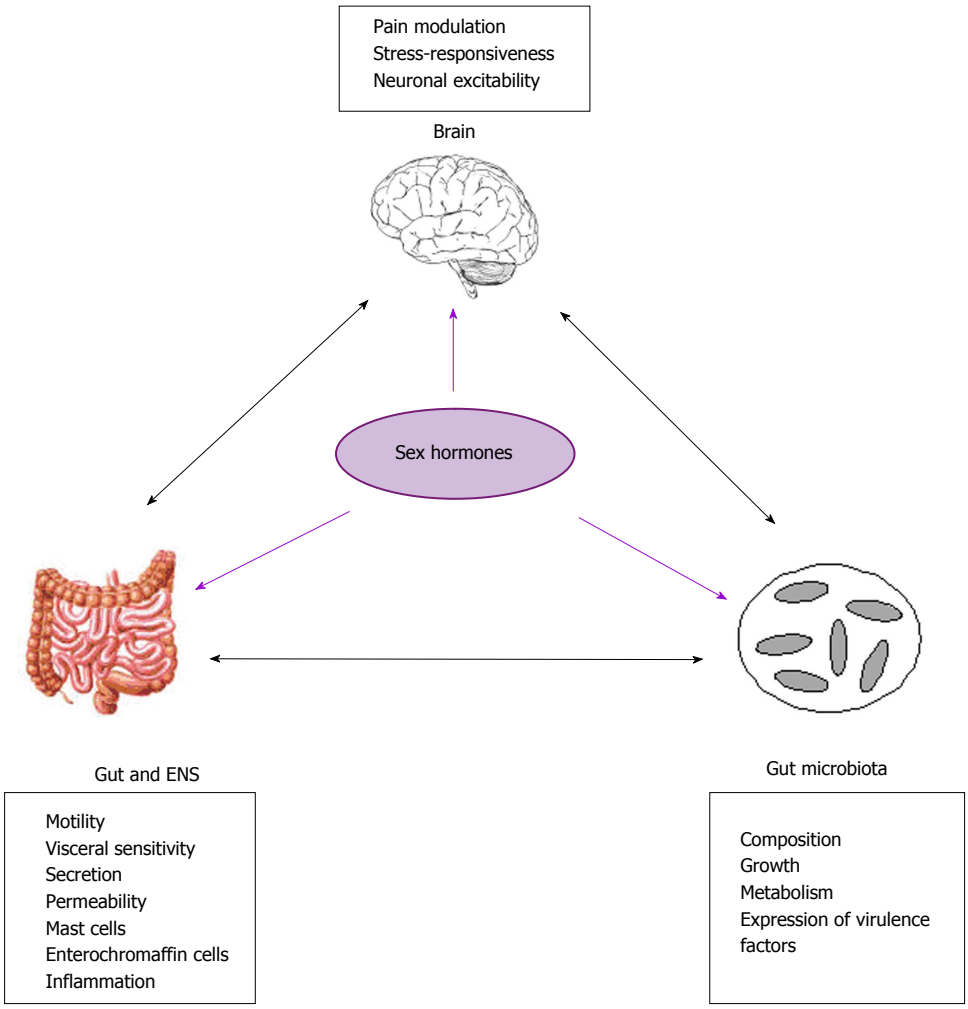

Figure 1 Sex hormones in the mutual brain-gut-microbiota interactions. Sex hormones influence peripheral and central regulatory mechanisms involved in the pathophysiology of irritable bowel syndrome contributing to the alterations in stress response, visceral sensitivity and motility, intestinal barrier function, and immune activation of intestinal mucosa. Sex hormones have also a direct effect on the gut microbiota. ENS: Enteric nervous system.

was reported to be effective in IBS female patients with menstrual cycle-related symptoms[57]. Nevertheless, many unpleasant side effects of leuprolide similar to climacteric-like syndrome significantly limit its application[57].

Noteworthy, interactions between gonadal hormones and pain modulation are bi-directional, as pain therapies in different experimental and clinical conditions have been found to affect the gonads as well[159,160]. For example, morphine treatment increased estrogen receptor, androgen receptor and *TRPV1* genes expression in the ovary, whereas in the testis the opiate reduced ER-α and ER-β mRNA expression not affecting androgen receptor and *TRPV1* expression[160].

A pivotal interdependence between the composition and stability of the gut microbiota and GI function as well as stress-related behavioral changes indicate a great therapeutic potential of probiotics, prebiotics and antibiotics in IBS[161,162]. So far, no gender specificity in probiotics efficacy in IBS patients has been reported[163]. Nevertheless, in the light of the microgenderome concept and sex-dependent differences in the immune regulation driven by gut microbiome[150], gender specificity in microflora manipulation seem to be essential and is expected to be extensively explored in the near future.

CONCLUSION

The results of epidemiological studies and clinical observations confirm significant sex and gender differences in the IBS prevalence and symptomatology. Furthermore, a growing number of clinical and experimental data strongly support a crucial role of sex hormones in the regulatory mechanisms of the brain-gut-microbiota axis involved in the pathophysiology of IBS (Figure 1). Some discrepancies in the results, especially related to the influence of estrogens, may result from different experimental conditions or heterogeneous groups of patients (*e.g.*, different age, menstrual status), but they also reflect the very complex nature of sex hormone actions. Estrogens can induce dual effects, both analgesic or hyperalgesic, as well as pro- or anti-inflammatory. Noteworthy, alterations in estrogen-induced visceral sensitivity seem to depend not only on the gonadal hormones levels, but more so on sudden changes in their levels, their sus-

tained genomic effects, and complex interactions with other neurotransmitters. Concomitant alterations in the number (up- or down-regulation) and sensitivity of ERs may play a crucial role in these processes as well. Thus, the physiological fluctuation in sex hormones may evoke for example different responses in IBS female patients compared to healthy women. Furthermore, a growing body of evidence indicates a protective role of androgens in pain modulation and anti-inflammatory properties of testosterone that may inhibit the development of visceral hyperalgesia. That could contribute to the higher susceptibility of women to IBS. A better understanding of the role of sex hormones in the modulation of the brain-gut-microbiota axis should enable a more effective and sex-tailored therapeutic approach in IBS.

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Overgrowth of the indigenous gut microbiome and irritable bowel syndrome

William Bye, Naveed Ishaq, Terry D Bolin, Vic M Duncombe, Stephen M Riordan

William Bye, Naveed Ishaq, Terry D Bolin, Vic M Duncombe, Stephen M Riordan, Gastrointestinal and Liver Unit, Prince of Wales Hospital, Randwick 2031, New South Wales, Australia
Stephen M Riordan, Prince of Wales Clinical School, Randwick NSW 2031, Australia
Author contributions: Riordan SM conceived and drafted the manuscript; all authors reviewed the literature and were involved in editing the manuscript.
Correspondence to: Stephen M Riordan, Professor, Gastrointestinal and Liver Unit Prince of Wales Hospital, Barker Street, Randwick 2031, New South Wales, Australia. sriordan@ozemail.com.au
Telephone: +61-2-93823100 Fax: +61-2-93822727
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Abstract

Culture-independent molecular techniques have demonstrated that the majority of the gut microbiota is uncultivable. Application of these molecular techniques to more accurately identify the indigenous gut microbiome has moved with great pace over recent years, leading to a substantial increase in understanding of gut microbial communities in both health and a number of disorders, including irritable bowel syndrome (IBS). Use of culture-independent molecular techniques already employed to characterise faecal and, to a lesser extent, colonic mucosal microbial populations in IBS, without reliance on insensitive, traditional microbiological culture techniques, has the potential to more accurately determine microbial composition in the small intestine of patients with this disorder, at least that occurring proximally and within reach of sampling. Current data concerning culture-based and culture-independent analyses of the small intestinal microbiome in IBS are considered here.

Key words: Gut microbiome; Small intestinal bacterial overgrowth; Irritable bowel syndrome

Core tip: The majority of the gut microbiota is uncultivable. Use of culture-independent molecular methods, without reliance on traditional microbiological culture techniques, has the potential to determine microbial composition in the small intestine of patients with irritable bowel syndrome. Current data concerning culture-based and culture-independent analyses of the small intestinal microbiome in patients with irritable bowel syndrome are considered here.

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INTRODUCTION

Culture-independent molecular techniques have demonstrated that the majority of the gut microbiota is uncultivable[1,2]. Application of these molecular techniques to more accurately identify the indigenous gut microbiome has moved with great pace over recent years, leading to a substantial increase in understanding of gut microbial communities in both health and a number of disorders, including irritable bowel syndrome (IBS). Most studies of the gut microbiome in this highly prevalent disorder, characterised by abdominal pain, abdominal distension and altered bowel habit, have to date focussed on analyses of faecal samples and have demonstrated disturbances in a range of bacterial populations in both adults and children with IBS[2-10]. In adults, disturbances in faecal *Clostridium cocleatum*, *Clostridium coccoides*, *Clostridium*

thermosuccinogenes, *Collinsella aerofaciens*, *Coprococcus eutactus*, *Staphylococcus aureus* (*S. aureus*), *Bifidobacterium catenulatum* (*B. catenulatum*), *Ruminococcus torques*, *Ruminococcus bromii*-like, bifidobacteria, Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, *Lactobacillus* spp and *Veillonella* spp have been demonstrated in IBS[2,3,5-10]. In children, a faecal microbiome characterised by a significantly increased percentage of Gammaproteobacteria, including *Haemophilus parainfluenzae*, a novel *Ruminococcus*-like microbe and an increased number of several bacterial taxa from the genus *Alistipes* has been reported in the IBS setting[4]. Analyses of colonic mucosa-associated microbial populations, as determined from mucosal biopsies, suggest compositional differences compared to faecal microbiota may occur in IBS[11] and it has been hypothesised that disturbances at this mucosa-associated level may be more important than those occurring luminally in the pathogenesis of IBS symptoms[12]. In further support of the notion that the gut microbiome participates in the pathogenesis of IBS are the findings of systematic reviews and a meta-analysis, which suggest that probiotics may be of therapeutic value, although results of individual studies are inconsistent and trial designs variable, such that it remains uncertain as to which bacterial species or strains may be of most benefit for which particular symptom component of the IBS complex[13-15].

As opposed to faecal and colonic mucosa-based analyses, possible disturbances in the microbial ecology of the small intestine in patients with IBS have been less well studied. In particular, the prevalence of small intestinal bacterial overgrowth (SIBO) has long remained a matter of conjecture, with concern over the accuracy of diagnostic tests for SIBO one factor clouding this issue. Notably, reported prevalence rates of SIBO in patients with IBS are lower when the diagnosis of SIBO has been based on culture of proximal small intestinal luminal secretions compared to when based on indirect breath hydrogen tests, performed following the ingestion of a fermentable substrate such as lactulose[16]. False-negative culture results have been hypothesised as an explanation for this discrepancy, as a result of SIBO possibly occurring distal to the region of sampling[17]. Conversely, a high false-positive rate of the lactulose breath hydrogen test (LBHT) for SIBO is recognised, based on an initial study performed to investigate the diagnostic accuracy of the LBHT in patients with predisposition to SIBO in which breath testing was combined with scintigraphy[18], recently replicated in the IBS setting[19], that demonstrated that a "positive" result for SIBO may, in fact, result from the test substrate being metabolised by colonic, rather than small intestinal, microbial flora. Sensitivity for culture-proven SIBO has also been shown to be lacking, even with combined scintigraphic assessment[18], such that the LBHT has fallen out of favour as a diagnostic test for SIBO, including in patients with IBS[20].

Another possibility is that disturbances of the small intestinal microbial ecology - either overgrowth or reduced levels of various bacterial species - may, indeed, be present in the region of sampling in patients with IBS but simply not be represented by standard bacteriological culture results, due to the inherent inability to properly demonstrate the gut microbiota in this way. Use of culture-independent molecular techniques already employed to characterise faecal and, to a lesser extent, colonic mucosa-associated microbial populations in IBS, without reliance on insensitive, traditional microbiological culture techniques, has the potential to more accurately determine microbial composition in the small intestine of patients with this disorder, at least that occurring proximally and within reach of sampling. Current data concerning culture-based and culture-independent analyses of the small intestinal microbiome in IBS are considered here.

PROXIMAL SMALL INTESTINAL MICROBIOTA IN IBS

A total of six published studies have investigated the proximal small intestinal microbiota in well-categorised cohorts of IBS patients and reported findings in relation to IBS-status[16-21]. Four of these studies analysed microbiota in luminal secretions, using standard culture techniques, with one also employing culture-independent molecular methods[21-24]. An additional two studies analysed mucosa-associated microbiota, with both of these using culture-independent molecular techniques[25,26]. Whether there exist compositional differences between small intestinal luminal and mucosa-associated microbial populations in IBS is currently unknown, as no study performed to date has contemporaneously analysed luminal and mucosa-associated microbiota in the same cohort of IBS patients.

Assessments of luminal secretions

Posserud *et al*[21] prospectively investigated 162 consecutive patients in Sweden with a clinical diagnosis of IBS based on Rome II criteria, including 49 (30%) with diarrhoea-predominant IBS (IBS-D), 37 (23%) with constipation-predominant IBS (IBS-C) and 76 (47%) with alternating-type IBS (IBS-A), with culture of a jejunal aspirate obtained *via* the central lumen of a water-perfused manometry catheter after a meal. The mean age of IBS patients was 38 years. Twenty-six healthy subjects (mean age 40 years) served as controls. No subject had been treated with antibiotics within 2 wk prior to the study or had received medications that might affect the gastrointestinal tract within 48 h of assessment. SIBO, defined by viable counts of colonic-type bacteria $\geqslant 10^5$ colony forming units/mL (CFU/mL), was found in 7 patients (4%) (mean age 49 years), including 2/49 (4%) with IBS-D, 3/37 (8%) with IBD-C and 2/76 (3%) with IBS-A. Bacterial isolates in IBS subjects with SIBO variously included *Escherichia coli*, *Enterococcus* species, *Clostridium* species, *Enterobacter* species, *S. aureus* and *Klebsiella* species. The prevalence of SIBO in patients with IBS was comparable to that in asymptomatic controls (1/26;

Table 1 Studies investigating the prevalence of small intestinal bacterial overgrowth in patients with irritable bowel syndrome, using culture-based assessments of proximal small intestinal luminal secretions

Country	IBS patients	Controls	Mean age (yr)	Aspirate details	Definition and prevalence of SIBO in patients and controls		
Sweden[21]	n = 162 IBD-D, n = 49 IBD-C, n = 37 IBD-A, n = 76	n = 26, healthy	IBS: 38 Controls: 40	Non-fasting; *via* water-perfused manometry catheter from jejunum	$\geqslant 10^5$ CFU/mL colonic-type bacteria:	IBS patients IBS-D IBS-C IBS-A Controls	7/162 (4%) 2/49 (4%) 3/37 (8%) 2/76 (3%) 1/26 (4%)
					$\geqslant 5 \times 10^3$ CFU/mL colonic-type bacteria:	IBS patients Controls	17/162 (11%) 1/26 (4%)
					$\geqslant 5 \times 10^3$ CFU/mL any bacteria:	IBS patients Controls	70/162 (43%)[1] 3/26 (12%)
The Netherlands[22]	n = 8 (out of a cohort of 12 symptomatic patients)	n = 9, healthy	Symptomatic: 39 Control: 26	Fasting; *via* weighted catheter from jejunum	$> 10^5$ CFU/mL colonic-type bacteria:	Symptomatic patients Controls	1/12 (8%) 0/9 (0%)
					Colonic-type bacteria: *Enterobacteriaceae* $\geqslant 10^3$ CFU/mL, *Bacteroides* species $\geqslant 10^2$ CFU/mL or *Clostridium* species $\geqslant 10^2$ CFU/mL	Symptomatic patients Controls	1/12 (8%) 0/9 (0%)
United States[23]	n = 148	n = 527, symptomatic	Overall: 53	Fasting; *via* endoscopy from duodenum	$\geqslant 10^5$ CFU/mL colonic-type aerobic bacteria OR $\geqslant 10^4$ CFU/mL anaerobic bacteria:	IBS patients: Controls	2% 10%
Greece[24]	n = 112 IBD-D, n = 35 IBD-C, n = 19 IBD-A, n = 58	n = 208, symptomatic	SIBO: 63.6 No SIBO: 69.5	Fasting; *via* endoscopy from duodenum	$> 10^5$ CFU/mL colonic-type aerobic bacteria:	IBS patients Controls	24/112 (21%)[2] 11/208 (5%)
					$> 10^3$ CFU/mL colonic-type aerobic bacteria:	IBS patients IBS-D IBS-C IBS-A Controls	42/112 (38%)[2] 21/35 (60%)[2,5] 6/19 (32%)[3] 15/58 (26%)[4] 20/208 (10%)

[1]P = 0.002 compared to controls; [2]P < 0.0005 compared to controls; [3]P = 0.012 compared to controls; [4]P = 0.003 compared to controls; [5]P = 0.001 compared to controls. IBS-D: Diarrhoea predominant-type irritable bowel syndrome; IBS-C: Constipation predominant-type irritable bowel syndrome; IBS-A: Alternating-type irritable bowel syndrome; CFU: Colony forming units; SIBO: Small intestinal bacterial overgrowth; IBS: Irritable bowel syndrome.

4%). Neither did prevalences of SIBO differ significantly between IBS patients and controls when alternative definitions of SIBO were employed (viable counts of any bacteria $\geqslant 10^5$ CFU/mL, 6% and 4%, respectively; viable counts of colonic-type bacteria $\geqslant 5 \times 10^3$ CFU/mL, 11% and 4%, respectively). Conversely, viable counts of any bacteria $\geqslant 5 \times 10^3$ CFU/mL were significantly more common in the IBS cohort than in healthy controls (43% *vs* 12%). While water perfusion through the manometry catheter may have diluted the absolute values of viable bacterial counts obtained, and the ingestion of a test meal prior to sampling for bacteriological analysis may, alternatively, have increased these values compared to those that may have been recovered under fasting conditions, any differences between IBS patients and controls were unlikely explained on these bases, as subjects were studied under identical conditions (Table 1).

As expected since small intestinal dysmotility typically promotes SIBO with colonic-type bacteria[27], this increased prevalence of mildly elevated non-colonic-type bacterial counts in IBS patients reported by Posserud *et al*[21] could not reliably be accounted for by small intestinal dysmotility, as assessed by manometry. Notably, the use of proton pump inhibitors (PPIs) and other drugs that reduce gastric acidity was not controlled for prior to 48 h of study and, given that IBS patients are often treated with such drugs, it is possible that the mildly elevated, non-colonic-type viable bacterial counts found in the IBS cohort may have occurred as a consequence of treatment of symptoms rather than as an initial cause of symptoms. Such a possibility could not be assessed by this study design.

Kerckhoffs *et al*[22] in The Netherlands subsequently reported on 12 symptomatic patients, including 8 with IBS, and 9 healthy subjects, from whom a fasting jejunal aspirate could be obtained using a weighted catheter after infusion of 10 mL of normal saline. Studied IBS patients within the symptomatic group and controls came from initial cohorts of 10 IBS patients (mean age 39 years) and 11 controls (mean age 26 years), respectively, with two IBS patients and two controls ultimately excluded as a jejunal aspirate could not be obtained. Aspirates were subjected to both standard culture and molecular-based analyses, the latter following deoxynucleic acid (DNA) extraction and quantitative polymerase chain reaction (PCR) amplification. No antibiotics were permitted in the two weeks prior to study, although PPI's were permitted until the day before study. With regard to culture results and notwithstanding the possibility of dilution by the saline infusion, SIBO, as defined by a viable colonic-type bacterial count > 10^5 CFU/mL, was present in 1/12 (8%) of the symptomatic group (the 8 IBS patients within the

symptomatic group were not separately reported) and none of the 9 controls. Using an alternative definition still based on colonic-type bacteria (*Enterobacteriaceae* $\geqslant 10^3$ CFU/mL or *Bacteroides* species $\geqslant 10^2$ CFU/mL or *Clostridium* species $\geqslant 10^2$ CFU/mL), the prevalence of SIBO remained 1/12 (8%) in symptomatic patients and 0/9 (0%) in controls. Moreover, no significant difference in median total viable bacterial counts between symptomatic patients and healthy controls was apparent. Similarly, no significant difference in the total bacterial DNA count between symptomatic patients and healthy controls was evident, while PCR analysis demonstrated that levels of the colonic-type flora, *Enterobacteriaceae*, *Faecalibacterium prausnitzii*, *Bacteroides fragilis* and *Clostridium coccoides*, were also comparable in the symptomatic and healthy groups. Sub-analyses in relation to IBS-D, IBD-C and IBD-A status were not included.

In another analysis, Choung *et al*[23] undertook a retrospective assessment of 675 symptomatic patients in the United States who had undergone culture of a duodenal aspirate, obtained endoscopically under fasting conditions, to assess for possible SIBO, including 148 (22%) patients with a clinical diagnosis of IBS. By comparison to the studies from Sweden and The Netherlands[13,14], the mean age of study subjects in this analysis was older (53 years) and no asymptomatic controls were included. The IBS patients included did not represent a consecutive cohort, but rather a select group attending an academic institution whose physicians deemed symptoms troublesome enough to warrant microbiological assessment. SIBO, defined by a viable colonic-type aerobic bacterial count $\geqslant 10^5$ CFU/mL or an anaerobic viable count $\geqslant 10^4$ CFU/mL, was present in only 2% of the IBS group. The species of the overgrowth bacteria isolated from patients with SIBO were not reported. Placed in context, a diagnosis of IBS was associated with an odds ratio for an abnormal aspirate result in keeping of SIBO of only 0.2 (95%CI: 0.1-0.7) compared to the likelihood of SIBO in patients with non-IBS diagnoses, including inflammatory bowel disease, pancreatitis and small intestinal diverticula, which were associated with three-fold, nearly five-fold and over seven-fold increases in odds for SIBO, respectively. Overall, the likelihood of SIBO was significantly related to older age, with the mean age of those with SIBO found to be 66 years. A substantial number of studied IBS patients were taking a PPI at the time of assessment and the proportion of this subgroup that was found to have SIBO remained low (2%). Conversely, the proportion of IBS patients with detectable viable bacterial counts in duodenal secretions, although not sufficient to fulfil criteria for SIBO, was five-fold higher in the setting of PPI use (15%) than in the absence of PPI use (3%), in keeping with the concept that proximal small intestinal microbial ecology may be disturbed by such therapy, even if not to a degree to constitute SIBO as commonly defined. Data in relation to IBS-D, IBD-C and IBD-A sub-categories of IBS were not provided.

A fourth culture-based study investigated the prevalence of SIBO in a consecutive cohort of 320 symptomatic patients undergoing outpatient upper gastrointestinal endoscopy in Greece, including 112 (35%) with a diagnosis of IBS according to Rome Ⅱ criteria (IBS-D: n = 35, 31.2%; IBD-C: n = 19, 16.9%; IBD-A: n = 58, 51.8%)[24]. Most common indications for endoscopy in IBS patients were dyspepsia (n = 75, 66.9%), anaemia (n = 24, 21.4%) and change in bowel habit (n = 9, 8.0%). Aspirates for microbiological assessment were obtained endoscopically under fasting conditions from the third part of duodenum. The prevalence of SIBO, defined by $> 10^3$ CFU/mL of colonic-type aerobic bacteria, was significantly higher in IBS patients than non-IBS patients (42/112, 38% *vs* 20/208, 10%). Among the IBS cohort, SIBO was present in 21/35 (60%) with IBS-D, 6/19 (32%) with IBS-C and 15/58 (26%) with IBS-A. *Escherichia coli* was the colonic-type bacterial species most commonly isolated in IBS patients with SIBO. Using a more restrictive definition of SIBO of $> 10^5$ CFU/mL of colonic-type aerobic bacteria, the prevalence of SIBO remained significantly higher in patients with IBS (24/112, 21%) than in those without IBS symptoms (11/208, 5%). The mean age of patients enrolled in this study was higher than that in the other three above-mentioned reports, with values of 63.6 years and 69.5 years in the SIBO and non-SIBO groups, respectively. That the highest prevalence of SIBO in IBS patients in the four studies discussed here should be found in the oldest of the four study cohorts, especially in those older patients with IBS-D, is in keeping with a previous report demonstrating a high prevalence of SIBO with colonic-type bacteria, including *Escherichia coli*, in the symptomatic elderly, including those with otherwise unexplained chronic diarrhoea[28]. Notably, distinct age-related disturbances in faecal microbiota, including increased levels of *Escherichia coli*, have also recently been demonstrated in the elderly[29].

Assessments of mucosa-associated microbiota

Kerckhoffs *et al*[25] investigated 41 patients with IBS fulfilling Rome Ⅱ criteria, including 14 (34%) with IBS-D, 11 (27%) with IBS-C and 16 (39%) with IBS-A, and 26 healthy controls. The mean age of IBS subjects was significantly older than that of controls (42 years and 31 years, respectively). Duodenal brushings were obtained and samples were subjected to DNA extraction and PCR amplification. Based on detection of significantly lower levels of *B. catenulatum* in faecal samples of the IBS cohort, the authors focussed on whether contemporaneous duodenal mucosal levels of *Bifidobacterium* species were similarly disturbed. A significant reduction in duodenal mucosa-associated *B. catenulatum* levels as a percentage of total duodenal mucosa-associated bifidobacterial loads was found in the IBS group (4.85% ± 0.5%) compared to healthy controls (17.04% ± 2.3%), with this relationship consistent across all three IBS subgroups. By contrast, levels of *B. adolescentis*, *B. bifidum* and *B. longum* did not dif-

fer significantly between healthy subjects, IBS patients or IBS subgroups.

In a subsequent case-control analysis, the same authors collected duodenal mucosal brush and faecal samples from 37 IBS patients (mean age 42 ± 2.3 years), including 13 (35%) IBS-D, 11 (29%) IBS-C and 13 IBS-A (35%), and 20 healthy controls (mean age 32 ± 2.6 years)[26]. Bacterial 16S rRNA gene was amplified and analysed using PCR denaturing gradient gel electrophoresis (DGGE). Pooled average DGGE profiles were generated and fingerprints compared. DGGE band fragments confined to healthy or IBS patient groups were further characterised by sequence analysis. Significantly higher levels of *Pseudomonas aeruginosa* were evident in duodenal brushings of the IBS patients than in healthy subjects (8.3% ± 0.95% of clones *vs* 0.1% ± 0.069% of clones, respectively), a trend replicated in paired faecal samples and across all IBS-subtypes. While antibiotic pre-treatment has been shown to increase the colonisation potential of *Pseudomonas* species[30], it is notable that no antibiotic therapy was permitted within one month of study in this analysis. Nonetheless, it remains to be determined whether the elevated levels of *Pseudomonas aeruginosa* reported by the authors are of pathophysiological relevance or merely epiphenomenal, perhaps related to the reduced expression of *B. catenulatum* previously reported or other factors yet to be defined.

Effect of antibiotic therapy on small intestinal microbiota and symptoms in IBS

Randomised trials of the orally administered antibiotics, neomycin and rifaximin, have separately demonstrated a reduction in IBS symptoms in non-IBS-C patients following antibiotic treatment[31-34]. Nonetheless, whether or not treated patients had SIBO and whether antibiotic use was associated with a reduction in viable small intestinal bacterial counts or microbial compositional change that correlated with symptom improvement was not assessed.

To date, only one study has investigated the impact on antibiotic therapy on SIBO and symptom improvement in patients with IBS[21]. In that analysis, seven patients with culture proven SIBO in jejunal secretions (mean age 49 years) were treated with oral ciprofloxacin, 500 mg twice daily for 10 d. Follow-up cultures following antibiotic treatment showed decreased viable bacterial counts in five patients (71%), although four (57%) still fulfilled criteria for SIBO. Three patients (43%) reported at least a 25% improvement in IBS symptoms following the course of ciprofloxacin, but IBS symptom responder status was not consistently related to reduction in small intestinal luminal viable bacterial counts. Whether symptom responder status may have correlated more closely with any antibiotic-related changes in faecal or colonic microbiota was not assessed.

No data are currently available with regard to the possible impact of antibiotic therapy on duodenal mucosa-associated composition and whether any antibiotic-related compositional change in the duodenal mucosa-associated microbial community correlates with symptom improvement in patients with IBS.

Efficacy of probiotic regimens that include microbiota shown by molecular techniques to be deficient in IBS

The health benefits of *B. catenulatum* for the host, if any, are currently unknown. However, members of the bifidobacteria group are often included in probiotic regimens used for the treatment of IBS[35]. Trials of probiotics that specifically include *B. catenulatum* and any other small intestinal mucosa-associated bacterial species that may be shown in future to be reduced in patients with IBS will be of considerable interest, from both therapeutic and disease mechanism perspectives.

CONCLUSION

Current microbial data, although relatively limited and based predominantly on culture-based assessments of luminal secretions, suggest that only a minority of patients with IBS have luminal SIBO, irrespective of the definition employed, with the exception of older subjects with the diarrhoea-predominant form. Available data obtained from a relatively young cohort demonstrating that symptom improvement following antibiotic therapy in IBS patients with SIBO does not necessarily depend upon reversal of the SIBO, as assessed in luminal secretions, cast doubt as to the importance of luminal SIBO in the pathophysiology of IBS symptoms, at least in younger subjects. Comparable studies have not been performed in elderly IBS patients with luminal SIBO. Similarly, the pathophysiological relevance of any disturbances of duodenal mucosa-associated microbiota in patients with IBS, including the reduced levels of *B. catenulatum* and increased levels of *Pseudomonas aeruginosa* levels so far demonstrated by culture-independent means, remains to be determined.

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Irritable bowel syndrome: Emerging paradigm in pathophysiology

Yoo Jin Lee, Kyung Sik Park

Yoo Jin Lee, Kyung Sik Park, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Keimyung University School of Medicine, Daegu 700-712, South Korea
Author contributions: Lee YJ reviewed the literature and drafted the manuscript; Park KS conceived the topic and produced the final manuscript.
Correspondence to: Kyung Sik Park, Professor, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Keimyung University School of Medicine, 56 Dalseong-ro, Jung-gu, Daegu 700-712,
South Korea. seenae99@dsmc.or.kr
Telephone: +82-53-2507088 Fax: +82-53-2507442
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Abstract

Irritable bowel syndrome (IBS) is one of the most common gastrointestinal disorders, characterized by abdominal pain, bloating, and changes in bowel habits. These symptoms cannot be explained by structural abnormalities and there is no specific laboratory test or biomarker for IBS. Therefore, IBS is classified as a functional disorder with diagnosis dependent on the history taking about manifested symptoms and careful physical examination. Although a great deal of research has been carried out in this area, the pathophysiology of IBS is complex and not completely understood. Multiple factors are thought to contribute to the symptoms in IBS patients; altered gastrointestinal motility, visceral hypersensitivity, and the brain-gut interaction are important classical concepts in IBS pathophysiology. New areas of research in this arena include inflammation, postinfectious low-grade inflammation, genetic and immunologic factors, an altered microbiota, dietary factors, and enteroendocrine cells. These emerging studies have not shown consistent results, provoking controversy in the IBS field. However, certain lines of evidence suggest that these mechanisms are important at least a subset of IBS patients, confirming that IBS symptoms cannot be explained by a single etiological mechanism. Therefore, it is important to keep in mind that IBS requires a more holistic approach to determining effective treatment and understanding the underlying mechanisms.

Key words: Pathophysiology; Irritable bowel syndrome; Inflammation; Immunologic; Genetics; Microbiota; Diet; Enteroendocrine cell

Core tip: In recent years, several novel mechanisms of irritable bowel syndrome (IBS) that likely relate to previously established IBS theories have been identified. Inflammation and postinfectious low-grade inflammation are emerging areas requiring clarification with regard to IBS pathophysiology. Immunological and genetic predisposition along with altered microbiota are critical in IBS development, while several dietary factors and enteroendocrine cells may also play roles in this syndrome. However, none of these accounts for the full repertoire of IBS symptoms, and the pathophysiology of this condition is not fully understood.

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INTRODUCTION

Irritable bowel syndrome is a functional gastrointestinal disorder that manifests symptoms of recurrent abdominal pain associated with changes in bowel habit without

organic abnormalities[1], and its prevalence ranges from 5% to 15%[2]. According to the Rome Ⅲ Diagnostic Criteria, irritable bowel syndrome (IBS) is defined as a syndrome with recurrent abdominal pain or discomfort occurring at least 3 d per month over a 3-mo span. It is associated with two or more of the following characteristics: (1) improvement with defecation; (2) change in stool frequency with onset; and (3) change in stool form with onset[3]. Many studies in IBS pathophysiology over the past decades have focused on colonic dysmotility, visceral hypersensitivity, and the brain-gut interaction. Recently, however, other mechanisms have been actively studied, including inflammation[4], post-infectious low-grade inflammation[5], immunologic factors[6], altered microbiota[7], dietary factors[8] and enteroendocrine cells[9]. However, evidence regarding their roles in IBS remains controversial. Recently, the definition of IBS has been challenged by growing evidence of organic abnormalities in patients who satisfy the Rome criteria for IBS[10,11]. Due to these new paradigms, IBS may no longer classify as an absolute functional disorder. In this article, we briefly summarize the classical concepts and follow with a discussion of the recent research pertaining to the new models of IBS pathophysiology. Better understanding of these emerging paradigms will aid the diagnosis and management of IBS.

CLASSICAL CONCEPTS IN THE PATHOPHYSIOLOGY OF IBS

Gastrointestinal dysmotility

Gastrointestinal dysmotility is recognized as one of the primary pathophysiological mechanisms in IBS, but it does not fully correlate with symptomatic bowel disturbances. Colonic motor activity in healthy subjects mainly consists of non-propagating and sporadic contractions and progression of intestinal contents by propagating movements termed high-amplitude propagated contractions (HAPCs)[12-14]. The frequent occurrence of HAPCs in IBS patients may explain the frequent bowel movements that cause diarrhea in diarrhea-predominant IBS (D-IBS)[15,16], whereas HAPCs are rarer in patients with constipation-predominant IBS (C-IBS)[17]. Colonic transit is generally accelerated in D-IBS and delayed in C-IBS according to several studies; however, reports on the relationship between colonic motility and IBS subtypes are inconsistent[18]. In one survey, 70% of C-IBS and 50% of D-IBS patients noted the feeling of incomplete evacuation[19]. In contrast, more recent data provided evidence that pelvic floor dyssynergia (PFD) causes symptoms characteristic of non-diarrhea predominant IBS (non-D IBS), including straining, incomplete evacuation, blockage, digitation, and anal pain, suggesting that anorectal function tests should be considered in patients with non-D IBS and PFD symptoms[20].

Visceral hypersensitivity

According to the classical concepts, IBS is caused by visceral hypersensitivity resulting in abdominal pain or discomfort and gastrointestinal motor disorder, which lead to alterations in defecation patterns; *i.e.*, diarrhea or constipation. Numerous studies have demonstrated the link between IBS and increased intestinal sensitivity[21]. Rectal hypersensitivity was proposed as a marker for IBS, and rectal sensory thresholds measured by rectal barostat testing were lower in IBS patients compared to healthy controls after rectal distention[22]. Most research so far has focused on colonic sensitivity[23,24] but hypersensitivity has also been observed in the esophagus[25], stomach[26] and small intestine[27] with IBS. Many studies have shown visceral sensitivity in IBS to correlate with stress[28] and food intake[29]. Colorectal sensitivity is attenuated in IBS patients after intake of a meal[30,31], and the visceral stimulus is significantly higher during stress in IBS patients than in healthy controls[32,33]. Therefore, visceral hypersensitivity is considered to be the conglomeration of peripheral and central processes[34], and its determinants are considered to be a combination of intrinsic and environmental factors.

Brain-gut interaction

Alterations in the brain-gut axis are a new concept in IBS pathophysiology. Environmental, cognitive, and emotional states can affect intestinal sensory perception[35,36]. Corticotropin-releasing hormone (CRH) is a major mediator of stress responses in the brain-gut axis, affecting the functions of both the brain and the gut[37,38]. Intravenous administration of CRH exacerbated colonic motility[39], while peripheral administration of a CRH antagonist blocked the stress-induced increase in colonic motility, visceral perception, and negative mood[40]. Several studies have demonstrated brain-gut interactions using brain imaging. For example, Hamaguchi *et al*[41] showed that distention of the descending colon activated portions of the brain that are highly related to pain recognition and emotion. Mayer *et al*[42] reported that IBS patients exhibit increased activation of brain regions that potentially correspond to the perception of rectal distension. Finally, Mertz *et al*[43] showed differences in activation of brain regions in response to a painful rectal stimulus in IBS patients compared to controls.

INFLAMMATION

Recent evidence supports a role for inflammation in IBS pathophysiology and generation of IBS symptoms in a subset of patients. Chadwick *et al*[44] performed studies of colonoscopic biopsy specimens from patients meeting the Rome criteria for clinical diagnosis of IBS. Immunohistological assessment showed an increased number of activated immunocompetent cells, including T-lymphocytes, neutrophils, and mast cells in the intestinal mucosa, suggesting a role for the mucosal immune system in pathogenesis. Subsequent studies demonstrated an increased frequency of several surrogate markers for inflammation in IBS patients, the most con-

sistent finding being an increased number of mast cells in the gastrointestinal (GI) tracts of IBS patients[4,45-47]. Mast cells are associated with wound healing, defense against pathogens, and hypersensitivity in GI mucosa. They degranulate to release inflammatory and immune mediators, which cause the recruitment of other inflammatory cells into the GI mucosa. Several studies have indicated that increased mast cells in IBS patients may correlate with certain symptoms of IBS, such as bloating and abdominal pain[46,48]. Another finding is the presence of activated T-lymphocytes in mucosal biopsy specimens from IBS patients[4,46,49]. Several studies have demonstrated an increase in the infiltration of lymphocytes in the myenteric plexus of patients compared to healthy controls[46,47,50]. Furthermore, patients with IBS have more activated T-cells in their colonic biopsies and blood samples[51]. T lymphocytes are involved in adaptive immunity and have multiple functions, such as the activation of B lymphocytes and macrophages and the destruction of infected host cells[52]. In addition, enhanced expression of proinflammatory cytokines in peripheral blood mononuclear cells[53] and serum[54] may confer a predisposition to immune activation in patients with IBS. In the following section, we will review the data supporting the role of inflammatory and proinflammatory cytokines in IBS.

IBS-like symptoms seen in ulcerative colitis (UC) patients during the remission phase appear to involve inflammation[55-57]. It is assumed that chronic inflammation in the colon during the remission phase, associated with altered sensory and motor functioning, can lead to IBS-like symptoms[58,59]. Fecal calprotectin was significantly higher in IBD patients displaying IBS-like symptoms than those lacking IBS-like symptoms, indicating the presence of occult inflammation in the former[55]. One group reported elevated levels of beta-defensin 2 peptides (HBD-2) in fecal fluid derived from IBS patients[60]. HBD-2 is an antimicrobial peptide recently implicated in the pathogenesis of inflammatory bowel disease[61]. These results suggest an activation of the mucosal innate defense system toward a proinflammatory response in IBS patients without macroscopic signs of inflammation.

There is also evidence of microscopic inflammation in IBS. In our previous study, conducted in 42 IBS patients diagnosed by the Rome Ⅱ criteria, the microscopic findings of mucosal hyperplasia, lymphocyte aggregation, and increased eosinophil counts were more frequently observed in the IBS group than the control group. Microscopic colitis does not appear to be associated with IBS symptoms[62]. A study in Malaysia also identified microscopic inflammations in D-IBS subjects that did not meet the criteria for classical microscopic colitis. In this study, the most common pathological findings were mixed chronic and acute inflammatory cells, lymphocytes, plasma cells and neutrophils[63]. IBS onset following an episode of gastroenteritis [post-infectious IBS (PI-IBS) is indicative of a role for inflammation in the pathogenesis of IBS (discussed below)]. Although large amount of research focusing on inflammation in the pathophysiology of IBS, as discussed in this section, this concept should be studied further to develop a potential future therapy for IBS.

POST-INFECTIOUS LOW-GRADE INFLAMMATION

Recently, numerous studies indicated that bacteriologically confirmed gastroenteritis is critical in the pathogenesis of IBS[5,64,65]. Also called post-infectious IBS (PI-IBS), first proposed by Stewart[66] in 1950, this is a case where IBS symptoms emerge in a patient - who has not previously met the Rome criteria for IBS - following an infectious illness characterized by two or more of the following: fever, vomiting, diarrhea, or a positive bacterial stool culture[67]. Most patients with infectious gastroenteritis recover in a few days, but approximately 10% of patients experience persistent symptoms (*e.g.*, abdominal pain or diarrhea) that progress to IBS[64]. In the meta-analysis by Thabane *et al*[65], the odds of developing IBS increased six- to seven-fold in patients with an episode of acute gastroenteritis. The mechanisms of PI-IBS are still not clear, yet studies have indicated that inflammation[68], genetic polymorphisms in genes associated with immune responses to infectious pathogens[69], and immune functioning[70] may contribute to the occurrence of PI-IBS.

Low-grade inflammation is recognized as the main pathophysiology of PI-IBS. El-Salhy *et al*[71] reported that rectal biopsy specimens taken from patient after *Campylobacter* gastroenteritis showed increases in leucocytes, lymphocytes, mast cells and endocrine cells. Another study reported that 3 mo post-gastroenteritis, patients who had PI-IBS continued to increase their chronic inflammatory cell counts, while those in healthy controls returned to normal levels[72]. Furthermore, several studies demonstrated that intestinal mast cell infiltration and activation following an infection often resulted in mucosal inflammation and the development of PI-IBS[5,73]. Such findings support a relationship between mucosal inflammation and PI-IBS. Development of IBS following non-GI infection has also been reported[74], and other recent study found that viral and bacterial enteritis outbreaks can lead to PI-IBS in a considerable proportion of patients (13%)[75].

Several lines of evidence indicate that inflammation and immune cells play roles in the intestinal neuroendocrine system, which controls GI sensory-motor function[76]. Dunlop *et al*[77] identified an association between PI-IBS and the persistence of mucosal abnormalities, enterochromaffin cell (EC) hyperplasia, and increased mucosal permeability, including intestinal inflammation. Increased permeability facilitates transfer of antigens through the intestinal mucosa, which leads to inflammatory cascades characterized by increased immune cell numbers. Serotonin secretion from EC cells, which regulates the gut immune system, can be attenuated by the secretory products of immune cells[78,79].

There are reports of increased levels of the proinflammatory cytokines in plasma levels of PI-IBS patient[54] and significantly greater IL-1β mRNA expression in the rectal mucosa of patients with IBS symptoms following acute gastroenteritis, but not in asymptomatic control subjects[73,80]. Flagellin antibodies were observed more frequently in patients with PI-IBS, indicating that immune activation in response to luminal triggers plays a role in the development of IBS[81,82]. Flagellins are primary triggers of innate and adaptive immunity, thus driving pathogen-induced acute inflammation[83]. These observations suggest that inflammatory responses to infection, rather than the infective pathogen itself, are an important predisposition to the occurrence of PI-IBS.

IMMUNOLOGIC AND GENETIC FACTORS

More recent data indicate an influence of genetics on the development of IBS. A survey of twins in Norway showed that the concordance for IBS in monozygotic twins was significantly higher than in dizygotic twins, providing robust evidence for the involvement of genetic factors in the etiology of IBS[84]. To date over 60 candidate genes have been reported as positively associated with IBS[85]. It should be noted that many of these studies had conflicting results; nevertheless, similar surrogate markers are being examined. Discrepancies may be due to differences in IBS subtypes of the study subjects, or in the processes by which the studies recruited their control groups, or in the laboratory methodologies used. However, it is noteworthy that many of these cases demonstrated genetics as a potential etiological factor. The representative genetic factors for IBS pathophysiology associate with inflammation, neurotransmitters, and bile acid synthesis.

Inflammation

Transient mucosal inflammation is crucial for the manifestation of IBS, despite the original definition of this syndrome that implies the lack of signs of active inflammation[86]. According to the evidence, subsets of IBS patients share genetic susceptibility loci for inflammation. The relatively well-studied IBS gene is *TNFSF15*, which has been confirmed in genome-wide association studies to mediate mucosal inflammation in IBD[87]. In Crohn's disease, TNFSF15 is up-regulated with intestinal inflammation and functions in nuclear factor κB activation, potentiation of IL-2 signaling, and secretion of interferon gamma by T lymphocytes[88]. Three cohort studies performed in the United Kingdom[69], Sweden and the United States[89], and England[90] identified a significant association between TNFSF15 and IBS. Belmonte *et al*[91] provided further evidence for altered intestinal immune activation. Increased toll-like receptor (TLR) expression has previously been observed in IBD[92]. In this study, the expression of TLR2 and TLR4 differed significantly among the IBS subtypes. The increased TLR expression in mixed-type IBS patients provoked intracellular signaling pathways that resulted in increased expression of the mucosal proinflammatory cytokines IL-1 and IL-8[91]. Villani *et al*[93] suggested genetic risk factors for the development of PI-IBS based on a 2300-patient cohort in Walkerton, Ontario. They found that TLR9, IL-6, and CDH1 variants persisted as independent risk factors for PI-IBS. Similarly, Brint *et al*[94] reported elevated levels of TLR4 and TLR5 level in PI-IBS patients, supporting the involvement of the innate immune system leading to an inflammatory response.

Several studies identified specific genetic polymorphisms in proinflammatory cytokines, which have an influence on GI functions, motility, epithelial permeability, and visceral sensation[95-97]. TNF-alpha is produced by monocyte-derived activated macrophages, and this cytokine plays on important role in chronic inflammatory states such as IBD[98]. According to a study in the Netherlands, increased TNF-alpha levels were significantly more prevalent in IBS patients compared to healthy controls, while no such association was found for polymorphisms in the *IL-10* gene[6], an anti-inflammatory cytokine involved in the regulation of immune and inflammatory responses. Several studies identified that certain IBS patients may be genetically predisposed to decreased production of IL-10 and subsequent development of low-grade inflammatory manifestations of IBS[99]. In a study done in Mexico, the high IL-10 producer genotype is less prevalent in IBS patients than healthy controls[86]. However, in the abovementioned Netherlands study, *IL-10* genotypes were similarly distributed among patients with IBD compared to healthy controls[6]. In contrast, in Japanese subjects, the frequency of the IL-10 genotype was significantly higher in IBS-D and UC than that in controls[100]. Although IL-10 might be associated with susceptibility to IBS development, many important questions remain regarding this relationship.

Neurotransmitters and cytokines

Among the single genetic polymorphisms associated with IBS, the role of the serotonin transporter (*SERT*) gene polymorphism (*SLC6A4*) has been relatively well explored in IBS. This polymorphism varys according to geographical region and ethnic population. In a meta-analysis, a genetic polymorphism in the gene region responsible for *SERT* activity was not associated with IBS[101]. However, subsequent studies reported inconsistent results. Kumar *et al*[102] did show that a *SLC6A4* polymorphism was significantly associated with IBS, and Wang *et al*[103] found that different *SERT* genotypes could influence *SLC6A4* promoter efficiency and *SERT* mRNA and protein expression in the colonic mucosa.

G proteins are expressed in all human cells and play a crucial role in signal transduction, particularly ligand-receptor interactions. The G protein is encoded by the *GNbeta3* gene. Although, *GNbeta3* polymorphisms have been linked to functional dyspepsia, such association was not observed with IBS[104,105]. However, Saito *et al*[106] reported a significant interaction between the *GNbeta3*

polymorphism and infection during IBS development, suggesting that IBS is a complex genetic disorder with both a genetic and environmental component for expression of symptoms.

Neuropeptide S (NPS) is a bioactive 20 amino acid peptide that selectively binds and activates the neuropeptide S receptor (NPSR1). NPSR1 induces the production of several neuropeptides, including cholecystokinin, vasoactive intestinal peptide, peptide YY, and somatostatin. NPSR1 variants are associated with gastrointestinal motor and sensory functions that are relevant to IBS[107].

The endocannabinoid system, involved in motility[108], sensation[109], secretion[110,111] and inflammatory[112,113] functions in the gastrointestinal tract, has been proposed as a mechanism in the development of IBS. The endocannabinoid anandamide is inactivated by the fatty acid amide hydrolase (FAAH), and single nucleotide polymorphisms (SNPs) in the *FAAH* gene (*C385A*) have been associated with accelerated colonic transit time in D-IBS[108].

Genetic variation in bile acid synthesis

Genetic variation in the genes controlling bile acid synthesis may contribute to abnormal bowel pattern and symptoms in IBS. Bile acid malabsorption stimulates colonic motility and secretion and has been associated with D-IBS[114]. Hepatic bile acid synthesis is partially controlled by feedback inhibition *via* the fibroblast growth factor 19 (FGF19); FGF19 binds to the FGF receptor 4 and the co-receptor Klotho-beta (KLB), leading to suppression of the rate-limiting enzyme in bile acid synthesis[115]. Wong *et al*[116] reported that a SNP in the *KLB* gene (rs17618244), is associated with accelerated colonic transit in IBS-D. A previous study suggested that the G protein-coupled bile acid receptor 1 (GpBAR1/TGR5) is expressed in myenteric, cholinergic, nitrergic neurons in the colon and in the proximal small intestine, indicating that bile acids may alter intestinal and colonic motility[117]. Camilleri *et al*[118] demonstrated that variations in TGR5 might contribute to altered SBT and colonic transit in D-IBS patients.

ALTERED INTESTINAL MICROBIOTA

The intestinal microbiota has recently been assumed to be an important predisposition factor for IBS. The most convincing evidence is that IBS can develop in predisposed persons who have experienced gastroenteritis. Other evidence indicates that bacteria may contribute to the pathophysiology of IBS, since luminal- and mucosa-associated microbiota can influence their host *via* immunomicrobial interactions[119]. In addition, small intestinal bacterial overgrowth (SIBO) has been implicated in a subset of IBS patients.

Earlier studies found that the intestinal microbiota in IBS patients differs from that in healthy individuals, with a decrease in the *Bifidobacterium* spp. population and an increase in the *Enterobacter* population being the most consistent findings[120,121]. In a study using real-time PCR assays, results included significantly lower counts of *Lactobacilli* in D-IBS than C-IBS specimens, lower counts of *Bifidobacterium* spp. in D-IBS than the other groups, and significantly higher counts of *Veillonella* spp. counts in the C-IBS group than healthy controls[122]. High-throughput analysis of 16S ribosomal RNA gene cloning and sequencing identified that the fecal microbiota is considerably altered in IBS, as IBS patients have lower *Lactobacillus* and *Bifidobacterium* spp. counts than healthy subjects[123]. Subsequent molecular studies confirmed that IBS patients have fecal microbiota differing from normal subjects[124-126]. Results regarding the intestinal microbiota in IBS are difficult to interpret due to the heterogeneity of the conditions and the observation that alterations of the intestinal microbiota may not be consistent across each subtype of IBS. Furthermore, the precise role of the luminal *vs* the mucosal-associated microbiota in IBS remains uncertain. Nevertheless, previous evidence consistently showed differences in the bacterial composition of feces between IBS and normal controls. Changes in the intestinal flora might result in the proliferation of species that produce more gas[127,128] during the development of IBS symptoms that bring about gas-induced distension. The direct effects of bacterial production on colonic contractility[129], intestinal myoelectrical activity[130], and pain response[131,132] have been identified in several *in vitro* studies. Also, a role for the microbiota in the induction of IBS symptoms is supported by the findings that probiotics improve flatulence and abdominal distension[133,134] and that rifaximin provides significant improvements in IBS symptoms, including bloating, abdominal pain, and loose or watery stools[135].

A growing body of research implicates SIBO in the symptoms of IBS, but this issue remains under debate. SIBO proved to be more prevalent in patients with IBS patients[136-138], and its eradication with antibiotics relieved the symptoms of IBS[139-142]. The presence of SIBO might be associated with abnormalities in small intestinal motor function. Pimentel *et al*[143] found that patients with IBS and SIBO experience few, if any, phase Ⅲ events during short-term manometric measurements compared to controls. In contrast, Posserud *et al*[144] performed intestinal manometry and culturing of intestinal aspirates taken from IBS and control groups, found that IBS subjects have fewer Major Migrating Complex phase Ⅲ events compared to patients without SIBO. However, there were no differences in other motility parameters, and no correlation between bacterial numbers and the pattern of IBS symptoms was detected. SIBO is typically diagnosed *via* indirect methods, such as positive early glucose or lactulose breath tests, and the accuracy of these methods is arguable. These diagnostic limitations have resulted in wide range of reports for SIBO prevalence (10% to 84%) in patients with IBS[145,146]. Regardless, slightly elevated intestinal bacterial numbers are inarguably more prevalent in IBS patients, and so further studies of this area are required.

DIETARY FACTORS

Although the "response to food" is not included in the diagnostic criteria for IBS, most patients claim their symptoms are triggered by certain foods, which are then avoided to alleviate symptoms[147,148]. Many researchers have focused on the role of diet in IBS in recent years. Also, guidance on diet management for patients with IBS has been revealed as improving their quality of life and symptoms[149,150]. The sensory component of the gastro-colonic reflex following nutrient intake is exaggerated in IBS patients[30], and IBS patients with intraluminal lipids exhibited impaired intestinal gas clearance because of an upregulated reflex inhibition in small bowel transit[151]. One study demonstrated that postprandial GI disorders in IBS patients might be associated with cellular immune function along the neuroendocrine-immune axis[152]. Furthermore, altered autonomic responses after a meal might cause exacerbated postprandial symptoms in IBS patients[153].

Food allergy and intolerance

Many IBS patients report that their symptoms are associated with specific foods; thus, the possibility of food allergies causing IBS symptoms has been proposed. Food allergy/hypersensitivity is defined as an allergic response in susceptible individuals following ingestion of a specific food (*e.g.*, cow's milk, peanuts, soybeans)[154,155]. However, there is little evidence that food allergies play a role in IBS. Several studies have reported that fructose-sorbitol malabsorption frequently occurs in IBS patients, but the results were similar in healthy volunteers; further, the response to a low lactose diet was disappointingly low in IBS patients experiencing lactose malabsorption, indicating a lack of obvious association between food allergy and IBS[156,157]. Several lines of evidence indicate that an altered immune response and inflammation may be involved in food hypersensitivity in IBS patients. There are reports of IgG-mediated food hypersensitivity and improved IBS symptoms when patients are placed on elimination diets[158-160]. Carroccio *et al*[161,162] demonstrated in IBS patients with food hypersensitivity an activation of serum basophils after stimulation with food antigens and increased levels of fecal eosinophil cationic proteins and tryptases. However, further investigations are necessary to validate the accuracy of the methods used in these studies before any claims can be made.

Food intolerances are defined as non-toxic and non-immune-mediated adverse reactions to food or to the presence of pharmacological agents within food, including histamines, sulfates, monosodium glutamate, serotonin, norepinephrine and tyramine[163]. Food intolerance is a possible factor underlying the pathogenesis of IBS, according to the finding that symptoms improved with an elimination diet[164]. However, subsequent studies showed little benefit from these diets[165,166]. Although specific food intolerances in IBS have been explored through patient questionnaires[167,168], the role of food intolerance in IBS remains questionable due to the lack of a reliable methodology and well-designed trials. Well-designed studies with standardized protocols are thus necessary.

Poorly absorbed nutrients

A recently proposed mechanism by which dietary factors might contribute to IBS symptoms suggests that poor absorption of nutrients influence GI function and sensation through osmotic actions and colonic fermentation[163]. Short-chain carbohydrates, such as fructose and dietary starch, are poorly absorbed, causing a number of ingested carbohydrates to enter the distal small bowel and colon. Consequently, these provide substrates for short-chain fatty acid (SCFA) generation by bacterial fermentation and increase the osmotic pressure[169]. The short-chain carbohydrates called Fermentable Oligo-saccharides, Di-saccharides, Mono-saccharides And Polyols (FODMAPs) contribute to IBS; however, IBS symptoms in such cases are triggered by luminal distension that induces abdominal pain, bloating, flatus, and altered bowel habits[170]. A number of studies suggesting effects of dietary manipulation, particularly elimination of FODMAPs, further support the importance of poor nutrient absorption in the development of IBS symptoms[171,172]. In addition, fecal SCFA were increased in D-IBS[173]. SCFA stimulate colonic transit and motility *via* intraluminal release of 5-hydroxytryptamine (5-HT)[174] and high-amplitude propagated colonic contractions[175], according to *in vivo* studies.

Limited data also suggest that changes in intestinal microbiota may be relevant for fermentation of non-absorbable nutrients[169]. Tana *et al*[176] showed that an altered GI microbiota contributes to the higher levels of SCFA and abdominal symptoms in IBS. In addition, many studies have explored the effect of dietary fiber on IBS symptoms. Although dietary fiber has commonly been a standard recommendation for patients with IBS[177], some evidence suggests that it may aggravate symptoms of IBS, such as flatulence, bloating and abdominal pain[76,127,128]. In a recent meta-analysis, patients administered fiber in their diets had persistent or unimproved symptoms compared to control groups that ingested placeboes or lower fiber diets[177]. Some investigators suggest that insoluble fiber intake does not significantly improve IBS symptoms, whereas soluble fiber intake can effectively improve overall IBS symptoms[178,179]. These results suggest that not all types of fiber are equally influential on IBS.

Gluten intolerance

Patients with celiac disease (CD) often experience IBS-like symptoms[180,181]. Therefore, it has been proposed that IBS patients should be routinely examined for CD[163,181]. Certain evidence suggests that dietary gluten intolerance also occurs in patients with IBS, and those whose symptoms improve with such diets may have a genetic susceptibility to gluten[182,183]. However, two groups of investigators recently published contrasting

results. Vazquez-Roque *et al*[184] conducted randomized controlled trials in D-IBS patients consuming gluten-free diets *vs* gluten-containing diets. The group consuming gluten showed increased stool frequency, small intestinal permeability, and reduced mRNA expression of tight-junction proteins in bowel mucosa compared to the patients consuming the gluten-free diet. However, Biesiekierski *et al*[185] reported that gluten might be not be a specific trigger of GI symptoms in IBS patients, as most patients' symptoms were not exacerbated with gluten exposure; there was no evidence of specific or dose-dependent effects of gluten in the expression of serum and fecal markers of intestinal inflammation/injury and immune activation. Further studies are being conducted to determine the role of gluten intolerance in IBS.

ENTEROENDOCRINE CELLS

Abnormalities in neuroendocrine peptides and amines derived from enteroendocrine cells can cause disturbances in digestion, GI motility and sensation in IBS patients[76,186]. These abnormalities are stimulated by gut luminal content, contributing to the development of symptoms in IBS[187]. Enteroendocrine cells release various bioactive substances, including gastrin, secretin, stomatostatin, cholecystokinin, chromogranins and serotonin[79]. Enterochromaffin cells, which are scattered throughout the GI mucosa, are the dominant type of enteroendocrine cells; they synthesize, store, and release serotonin in response to luminal stimuli[187]. Serotonin affects motility, sensation, and secretion in the gut through the activation of receptors present on enteric nerves and sensory afferents[188]. Studies demonstrated an increase in the release of serotonin in patients with D-IBS[189,190] and PI-IBS[191], while impaired release was found in patients with C-IBS[191,192]. The increased release of 5-HT triggered by luminal stimuli activates immune cells supporting the role of 5-HT in gut inflammation[79].

In addition to serotonin, enteroendocrine cells release chromogranin and secretogranin, which can influence several GI functions, such as immune modulation and inflammation[79]. El-Salhy *et al*[193-195] showed that decreased density of chromogranin A (CgA)-containing cells was found in duodenum, terminal ileum and colonic mucosa of IBS patients. Whereas, other studies demonstrated that serum CgA levels increase in IBS patients[196,197]. Recently, Ohman *et al*[9] showed that IBS patients with rapid colonic transit have higher levels of fecal CgA, secretogranin (Sg) Ⅱ, and SgⅢ, but lower levels of chromogranin B, compared to healthy subjects. Based on the data, granins could serve as useful biomarkers of IBS; however, the role of granins in IBS has not been revealed.

CONCLUSION

There is abundant evidence supporting the claim that IBS should no longer be considered an absolute idiopathic functional disease. In recent years, attention has been directed towards the role of inflammation, gut microbiota, immunity, genetics, dietary factors, and enteroendocrine cells. As a result, IBS is regarded as a multifactorial condition that affects individuals differentially. Understanding these mechanisms will be useful for the development of a more specific, individualized treatment strategy and for the clinical management of IBD patients.

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Unraveling the ties between irritable bowel syndrome and intestinal microbiota

Sung Noh Hong, Poong-Lyul Rhee

Sung Noh Hong, Poong-Lyul Rhee, Division of Gastroenterology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul 135-710, South Korea

Author contributions: Hong SN and Rhee PL participated in conception and design of the paper, and analysis of the literature data; Hong SN provided the first draft of the manuscript; Rhee PL was involved in critical discussion and revision of the manuscript for the intellectual content.

Correspondence to: Poong-Lyul Rhee, MD, PhD, Professor, Chairman, Division of Gastroenterology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, 50 Irwon-dong, Gangnam-gu, Seoul 135-710, South Korea. plrhee@skku.edu
Telephone: +82-2-34103409 Fax: +82-2-34106983

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Abstract

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

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

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

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INTRODUCTION

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

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

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

HUMAN INTESTINAL MICROBIOTA

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

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

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

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

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

INTESTINAL MICROBIOTA COMPOSITION OF IBS PATIENTS

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

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

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

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

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

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

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

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

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

PATHOGENIC ROLE OF INTESTINAL DYSBIOSIS IN IBS

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

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

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

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

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

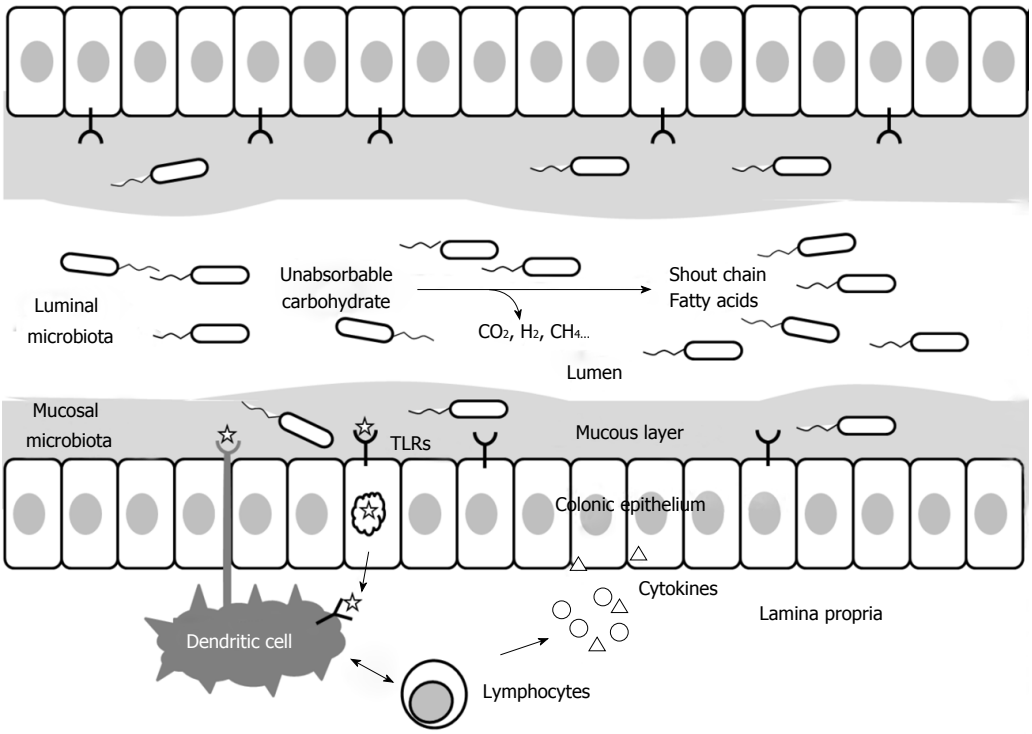

Figure 1 Luminal and mucosal intestinal microbiota and roles in gut homeostasis.

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

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

SMALL INTESTINAL BACTERIAL OVERGROWTH AND ANTIBIOTICS

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

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

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

EVIDENCE OF THE ROLE OF POTENTIALLY PROBIOTIC BACTERIA IN IBS

An improved understanding of host-microbiota interac-

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

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

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

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

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

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

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

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

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

CONCLUSION

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

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Irritable bowel syndrome and small intestinal bacterial overgrowth: Meaningful association or unnecessary hype

Uday C Ghoshal, Deepakshi Srivastava

Uday C Ghoshal, Deepakshi Srivastava, Department of Gastroenterology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow 226104, India
Author contributions: Ghoshal UC conceived, reviewed the data and wrote the manuscript; Srivastava D contributed to review of the data and writing of the manuscript.
Correspondence to: Uday C Ghoshal, MD, DNB, DM, FACG, RFF, Professor, Department of Gastroenterology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Raebareli Road, Lucknow 226014, India. udayghoshal@gmail.com
Telephone: +91-522-249-4405 Fax: +91-522-2668017
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Abstract

Irritable bowel syndrome (IBS) is a common condition characterized by abdominal pain or discomfort, bloating, and altered stool form and passage. Small intestinal bacterial overgrowth (SIBO) is a condition in which there is overgrowth of bacteria in small bowel in excess of 10^5 colony forming units per milliliter on culture of the upper gut aspirate. Frequency of SIBO varied from 4%-78% among patients with IBS and from 1%-40% among controls. Higher frequency in some studies might be due to fallacious criteria [post-lactulose breath-hydrogen rise 20 PPM above basal within 90 min (early-peak)]. Glucose hydrogen breath test (GHBT) has a low sensitivity to diagnose SIBO. Hence, studies based on GHBT might have under-estimated frequency of SIBO. Therefore, it is important to analyze these studies carefully to evaluate whether the reported association between IBS and SIBO is over or under-projected. This review evaluates studies on association between SIBO and IBS, discordance between different studies, their strength and weakness including methodological issues and evidence on therapeutic manipulation of gut flora on symptoms of IBS.

Key words: Glucose hydrogen breath test; Lactulose hydrogen breath test; Functional bowel disease; Dysbiosis; Gut flora

Core tip: Irritable bowel syndrome (IBS) has been conventionally thought to be a disorder without an organic basis. However, recently data are emerging to show that it may have organic basis at least in a subset of patients. Though several studies reported an association between small intestinal bacterial overgrowth (SIBO) and IBS, the frequency of SIBO reported to vary between 4% and 78%. The current review suggests that the association between SIBO and IBS is definite, but the studies reporting high prevalence of SIBO in IBS over-estimated its frequency due to use of fallacious diagnostic methods. Better test to diagnose SIBO in patients with IBS is highly needed.

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INTRODUCTION

Irritable bowel syndrome (IBS) is a common condition characterized by abdominal pain or discomfort, bloating, associated with altered stool form (such as diarrhea, constipation) and passage. World-wide, 4%-30% of subjects suffer from IBS[1-7]. Small intestinal bacterial overgrowth (SIBO), which has been conventionally described in patients with anatomical abnormalities in the gut such

Table 1 Summary of prevalence of small intestinal bacterial overgrowth in irritable bowel syndrome by different diagnostic methods *n* (%)

Ref.	Type of the study	Frequency of SIBO in cases	Frequency of SIBO in controls	Methane producers in cases	Methane producers in controls	Method for diagnosis
Park *et al*[86]	Prospective (Case-control)	34/76 (44.7)	16/40 (40)	19/76 (25)	10/40 (25)	LHBT
Scarpellini *et al*[88]	Prospective (Case-control)	28/43 (65)	4/56 (7)	4/43 (9.3)	0	LHBT
Carrara *et al*[14]	Prospective	55/127 (43)	NCG	ND	ND	LHBT
Mann and Limoges-Gonzales[87]	Prospective	89/258 (34.5)	NCG	ND	ND	LHBT
Nucera *et al*[89]	Prospective	64/98 (65)	NCG	ND	ND	LHBT
Pimentel *et al*[17]	Prospective	157/202 (78)	NCG	ND	ND	LHBT
Sachdeva *et al*[18]	Prospective (Case-control)	14/59 (23.7)	1/37 (2.7)	5/59 (8.5)	9/37 (24.3)	GHBT
Reddymasu *et al*[90]	Prospective	35/98 (36)	NCG	Data NA	ND	GHBT
Lombardo *et al*[91]	Prospective (Case-control)	49/200 (24.5)	3/50 (6)	ND	ND	GHBT
Ford *et al*[74]	Meta-analysis	595/1921 (31)	NCG	ND	ND	GHBT
Parodi *et al*[92]	Prospective (Case-control)	21/130 (16.1)	3/70 (4.2)	34/130 (26)	Data NA	GHBT
Rana *et al*[93]	Prospective (Case-control)	25/225 (11.1)	1/100 (1)	ND	ND	GHBT
Majewski *et al*[94]	Prospective (Case-control)	93/204 (46)	NCG	27/204 (13.2)	ND	GHBT
Cuoco and Salvangnini[75]	Retrospective	44/96 (45.8)	NCG	ND	ND	GHBT
Lupascu *et al*[95]	Prospective (Case-control)	20/65 (31)	4/102 (4)	ND	ND	GHBT
Ghoshal *et al*[16]	Prospective (Case-control)	11/129 (8.5)	1/51(2)	ND	ND	GHBT
Posserud *et al*[12]	Prospective (Case-control)	6/162 (4)	1/26 (4)	ND	ND	Hydrogen breath test and culture of small bowel aspirate

SIBO: Small intestinal bacterial overgrowth; IBS: Irritable bowel syndrome; LHBT: Lactulose hydrogen breath test; GHBT: Glucose hydrogen breath test; NCG: No control group; ND: Not done; NA: Not available; *n*: Number of cases and controls.

as ileo-transverse anastomosis, stricture, fistula, slow motility and reduced gut defence, is also characterized by abdominal pain or discomfort, bloating, flatulence and loose motion[8-10]. Recently, it has been realized that SIBO may occur in the absence of apparent anatomical abnormalities[11]. These patients, however, may be wrongly diagnosed as IBS.

Small intestinal bacterial overgrowth is currently defined as presence of bacteria in excess of 10^5 colony forming units per milliliter on culture of the upper gut aspirate[12,13]. Since this is an invasive test, several non-invasive methods including lactulose and glucose hydrogen breath tests (LHBT and GHBT) have been popularly used to diagnose SIBO[14-18]. This condition is being increasingly recognized among patients with IBS. In different studies, frequency of SIBO among patients presenting with IBS varied from 4% to 78% (Table 1), more so among patients with diarrhea-predominant IBS[12,14,18,19]. Not only quantitative increase (SIBO) but qualitative change in the gut bacteria (dysbiosis) has been reported among patients with IBS[20]. These studies led to a paradigm shift in understanding pathogenesis of IBS and have led to increasing debate on therapeutic manipulation of gut microbiota to treat this enigmatic chronic disorder using antibiotics, probiotics and lately fecal transplantation[21-23]. However, it is important to recognize the wide-variability in frequency of SIBO among patients with IBS in different studies; such wide-variability in frequency may suggest that it is important to analyze these studies carefully to evaluate whether the reported association between IBS and SIBO is over or under-projected in some of the earlier studies?

We hereby review the studies on association between SIBO and IBS, discordance between different studies, their strength and weakness including methodological issues and evidence on therapeutic manipulation of gut flora on symptoms of IBS.

REVIEW OF STUDIES ON SIBO IN IBS

Table 1 summarizes the results from studies on SIBO among patients with IBS. As can be noted from this table, the frequency of SIBO among patients with IBS varied from 4% to 78% and that among controls from 1% and 40%. In most studies, frequency of SIBO among patients with IBS was higher than that among controls. Therefore, it can be concluded that SIBO is associated with IBS. But it is important to critically evaluate the reasons for such a wide variability in frequency of SIBO in different studies.

CRITICAL EVALUATION OF STUDIES ON SIBO IN IBS: REASONS FOR DISCORDANCE

Could IBS phenotype determine frequency of SIBO?

IBS is a heterogeneous condition. The sub-types may be diarrhea or constipation-predominant or may be alternating. Patients with diarrhea-predominant IBS more often have organic cause including SIBO than other sub-types of IBS. In a study on 129 patients with non-diarrheal IBS, 73 with chronic non-specific diarrhea including diarrhea-predominant IBS, and 51 healthy controls, frequency of SIBO using GHBT was 11 (8.5%), 16 (22%) and 1 (2%), respectively[16]. Similar findings were reported in other studies as well[18,24]. Diarrheal IBS, therefore, should be particularly evaluated for SIBO than other types of IBS. Moreover, studies which included larger proportion of patients with diarrhea-predominant

IBS are likely to show higher frequency of SIBO.

Bloating is a frequent symptom reported by patients with IBS. Frequency of bloating has been reported to vary from 26% to 83% in studies on IBS from Asia[3,25]. The pathogenesis of bloating may be related to increased amount of gas in the gut, its abnormal distribution and increased visceral perception in response to distension of the gut[26,27]. Patients with SIBO may have increased amount of gas inside the gut, hence, it is logical to believe that IBS patients with marked bloating are expected to have SIBO[16]. However, there are limited data on this issue. Several studies showed that both fasting as well as post-substrate (*e.g.*, glucose, lactulose) breath hydrogen is higher among patients with IBS than controls[16,28]. Probiotics and antibiotics, which are known to reduce gas inside the gut, are known to relieve abdominal bloating among patients with IBS[17,29,30]. It has been reported that successful treatment with antibiotics causes the hydrogen breath test to revert to normal[17]. Hence, patients with IBS with marked bloating and flatulence should be particularly evaluated for SIBO. More studies, however, are needed on this issue.

Could technique used to diagnose SIBO determine its frequency?

Several techniques have been used to diagnose SIBO; these include LHBT, GHBT, ^{14}C xylose breath test, and quantitative culture of jejunal aspirate[12,15,31,32]. Principle of hydrogen breath tests is summarized in Figure 1. Dietary carbohydrates, unabsorbed in the small intestine, produce hydrogen in the large intestine by bacterial fermentation[33]. In patients with SIBO, the bacteria ferment these carbohydrates in the small bowel itself producing hydrogen, which gets absorbed and is exhaled in the breath[33].

Hydrogen breath test involves giving patients a load of carbohydrate (usually in the form of glucose and lactulose) and measuring expired hydrogen concentrations over a period of time. Diagnosis of SIBO using hydrogen breath test is based on the physiological principle that in patients with SIBO, glucose would be fermented by bacteria in the small bowel resulting in production of hydrogen gas that is absorbed and exhaled in expired air (Figure 1, A1)[31,33]. In contrast, lactulose, which is a non-absorbable disaccharide, will produce an early peak due to fermentation in small bowel (typically within 90-min) or double peak (first due to small bowel fermentation and second from colon), if SIBO is present (Figure 1, B2 and B3)[33]. There are several limitations in hydrogen breath test for diagnosis of SIBO. There may be similarities in the pattern of gas production in patients with SIBO and subjects with rapid intestinal transit, thus making distinction difficult[34]. An early peak is often false positive in people with fast gut transit time. For example, in a study from India, median oro-cecal transit time was 65 min (range 40-110 min) in healthy subjects[35]. In another study from Taiwan, mean oro-cecal transit time was 85 min[36]. This has also been substantiated in Western population recently by simultaneously using LHBT and radio-nuclide method to assess gut transit[37-39]. Double peak criteria for diagnosis of SIBO using LHBT is quite insensitive[15,33]. Sensitivity of GHBT to diagnose SIBO is 44% considering quantitative culture of upper gut aspirate as gold standard[15]. Hence, it is expected that the authors who used an early peak criteria in LHBT would get a high frequency of SIBO among patients with IBS as well as controls. In contrast, those who would use either GHBT or double peak criterion in LHBT would get a low frequency of SIBO both in patients with IBS and controls. It is worth noting from the Table 1 that the frequency of SIBO among patients with IBS and controls on LHBT (early peak criteria) varied from 34.5% to 78% and 7%-40%, respectively; in contrast, the frequency on GHBT varied from 8.5%-46% and 2%-18%, respectively.

Fifteen percent of people may have methanogenic flora in the gut[34,40]. *Methanobrevibacter smithii*, *Methanobrevibacter stadmanae* and possibly some of the coliform bacteria are methanogens[41]. In these subjects, only hydrogen breath tests may not diagnose SIBO, estimation of methane is also needed (Figure 1). Table 1 shows that 8.5%-26% of IBS patients and 0%-25% of controls exhaled methane in their breath. Therefore, SIBO could not have been diagnosed if methane was not estimated in them. As summarized in Table 1, in a large proportion of studies, methane was not estimated, which could have resulted in underestimation of frequency of SIBO in these studies. Excessive methane production is associated with constipation[42]. Hence, methane estimation in breath, which is not available in several commercially available hydrogen breath test machines, is particularly important in patients with constipation-predominant IBS. Some individuals may have slow transit through the small intestine making prolonged testing up to five hours necessary and many individuals may not like to undergo such prolonged testing[43,44]. However, a shorter period of testing for them may miss the diagnosis of SIBO.

The jejunal aspirate culture has traditionally been used as the gold standard to diagnose SIBO (Figure 2)[15,45]. However, the limitations of this test include invasiveness and the challenges posed by attempting to culture all strains and species[46]. In fact, use of air during endoscopy can lead to a false negative result as anaerobes do not grow once these are exposed to oxygen present in the air[13]. Also, a large proportion of bacteria are not cultured[47,48]. In contrast, single lumen catheter passed through the nose or through the biopsy channel of endoscope, may lead to contamination by oropharyngeal flora giving false positive result[13]. Therefore, we designed a double-lumen catheter to prevent such oro-pharyngeal contamination (Figure 2)[13]. Studies on SIBO among patients with IBS using quantitative culture of small bowel aspirate are scanty (Table 1). A study by Posserud *et al*[12] reported a frequency of SIBO of 4% among patients with IBS. Considering the result of other studies using GHBT, which has sensitivity of 44%

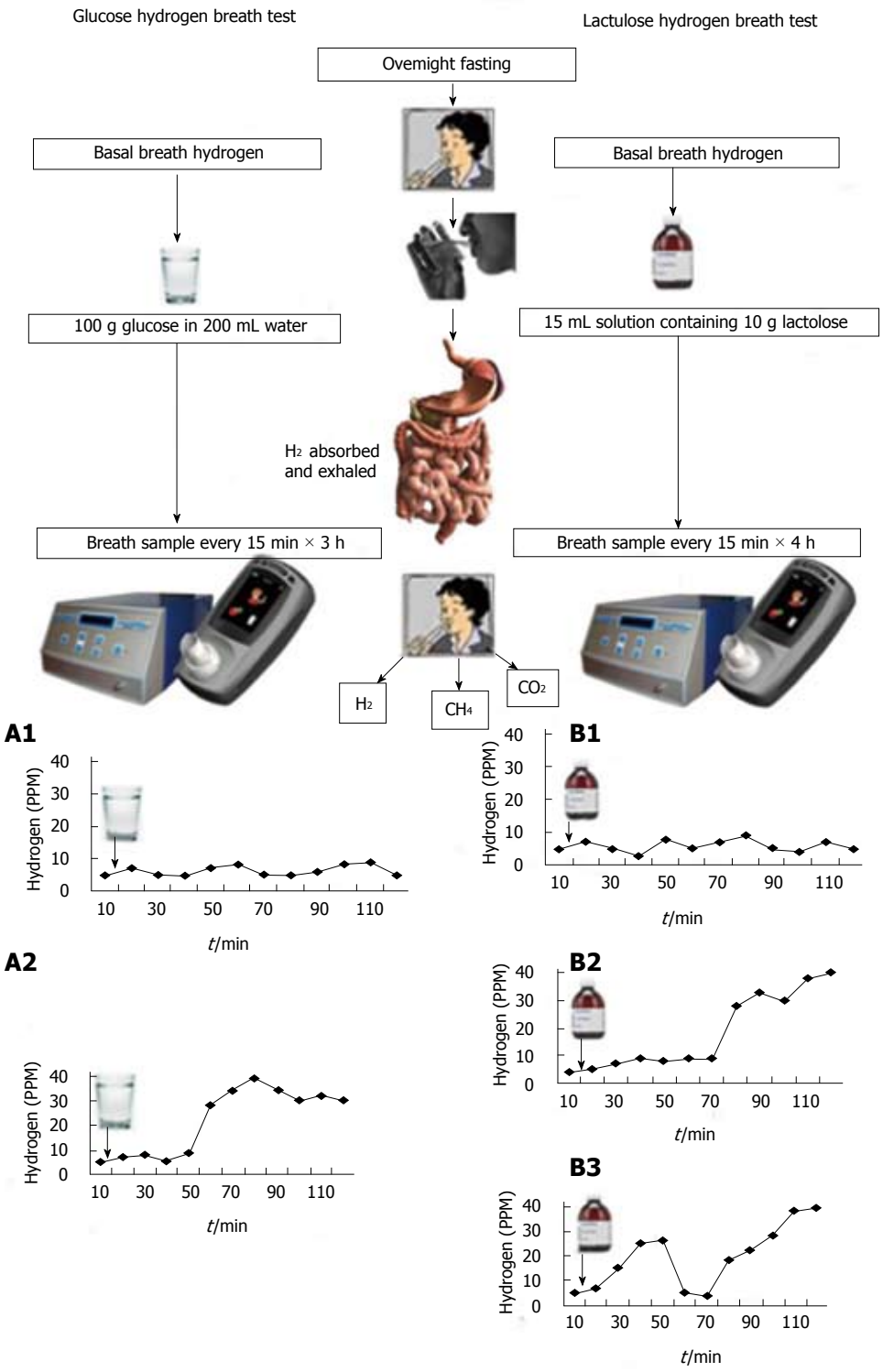

Figure 1 Outline of principle of method and interpretation of glucose and lactulose hydrogen breath tests. On the left panel, method and result of glucose hydrogen breath test (GHBT) is shown. A1: GHBT is negative for SIBO as there is no peak in hydrogen production; A2: GHBT shows presence of SIBO. On the right panel, method and result of lactulose hydrogen breath test (LHBT) is shown. B1: LHBT is negative for SIBO as there is no peak in hydrogen production; B2: LHBT shows an early peak (within 90 min of lactulose ingestion); B3: LHBT shows double peaks in hydrogen, the earlier one from small bowel due to SIBO and the later one from the colon. Please note that Quintron machine of the left gives values of hydrogen, methane and CO_2 (for correction) and the Bedfont machine of right side estimates hydrogen only. It is also important to note that in the graphs hydrogen breath test do not show methane levels. SIBO: Small intestinal bacterial overgrowth; GHBT: Glucose hydrogen breath test; LHBT: Lactulose hydrogen breath Test; PPM: Parts per million.

to diagnose SIBO considering upper gut aspirate as gold standard, the former study appears to have falsely underestimated the frequency of SIBO among patients with IBS[15]. More studies are needed on this issue.

^{13}C and ^{14}C based tests have also been developed based on the bacterial metabolism of D-xylose (Figure

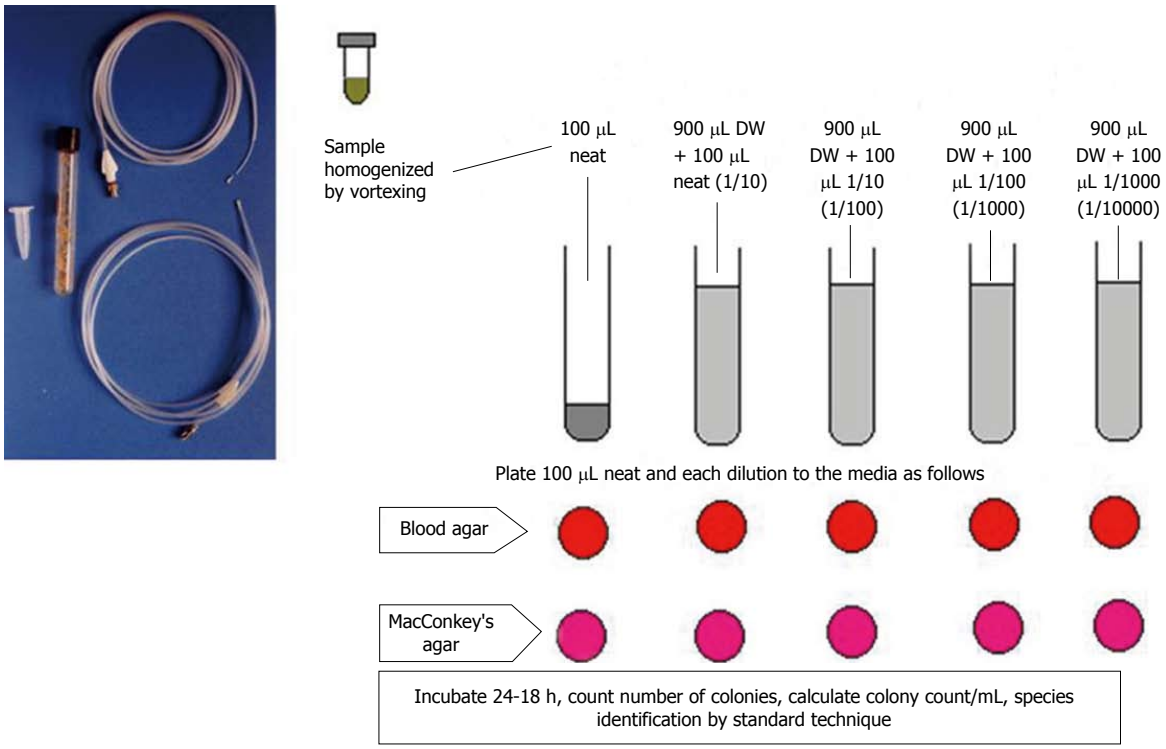

Figure 2 Outline of method of culturing bacteria from the upper gut aspirate and counting the colonies by serial dilution technique. DW: Distilled water.

3)[32]. Bacterial de-conjugation of bile acids containing ^{13}C and ^{14}C can also be used to diagnose SIBO[49]. The glycocholic acid breath test involves the administration of the bile acid ^{14}C glycocholic acid, and the detection of $^{14}CO_2$, which would be elevated in SIBO (Figure 3)[50,51].

MECHANISM OF IBS SYMPTOMS IN PATIENTS WITH SIBO

Bacteria in gut lumen play important role in modulating multiple intestinal functions. Quantitative change in luminal bacteria in the small intestine (SIBO) disrupts digestion and absorption. Gut bacteria are also important for immune activation[52]. Immune mediated cytokines could have multiple actions including altered epithelial secretion, exaggerated nociceptive signalling and abnormal motility[52,53]. Together, these changes may lead to IBS like symptoms. It has also been proposed that this mechanism could account for overlap syndromes, such as fibromyalgia[54,55]. There has been renewed interest in gut flora recently, as there are recent developments on relationship between gut flora and intestinal function, pathogenesis of various diseases and potential value of probiotics, and other means of modifying gut flora as therapeutic modalities.

In patients with SIBO, bacteria ferment ingested carbohydrates in the small intestine causing increased gas production[15]. Accumulation of this gas in the intestine results in bloating and flatulence[56,57]. Excessive luminal distension may even cause abdominal pain or discomfort[57]. 15% of the population produces methane instead of hydrogen gas[13,34]. Evidence suggest that excessive methane produced by overgrowth of methanogenic flora causes constipation[42]. Reduction in breath methane by therapeutic manipulation of gut flora improves constipation[58].

Bacteria in the intestine may produce toxic by-products after fermentation, which may damage the inner lining of the small intestine and colon[59]. A study on small bowel biopsies in patients with SIBO revealed thinning of the mucosa and crypts and increased intra-epithelial lymphocytes[60]. This may cause osmotic load in the intestine resulting into diarrhea. Studies have revealed that patients with IBS having SIBO more often have diarrhea-predominant disease[11,12,14,18,19]. Bacteria also de-conjugates bile salts present in the intestine. These de-conjugated bile salts can stimulate colonic water secretion causing diarrhea. Thereafter, free bile acids, which are toxic to the intestinal mucosa may cause mucosal inflammation and release of pro-inflammatory cytokines[61,62]. SIBO is known to be associated with increased IL-8 levels (pro-inflammatory cytokine)[63].

Pathophysiology of IBS includes altered motility, visceral hypersensitivity and abnormal brain-gut interaction. Bacteria affect the sensori-motor functions of the gut[64]. Bacterial chemotactic peptides, such as formyl-methionyl-leucyl-phenylalanine, stimulate the enteric nervous system and afferent nerves, while endotoxins (lipopolysaccharides) may affect gut motility[65]. Bacteria in the small intestine in patients with SIBO produce short-chain fatty acids (SCFA) such as butyrate, acetate, and propionate. Colonic motility is increased due to acidification by SCFA[66,67]. In contrast, SCFA causes reduction in motil-

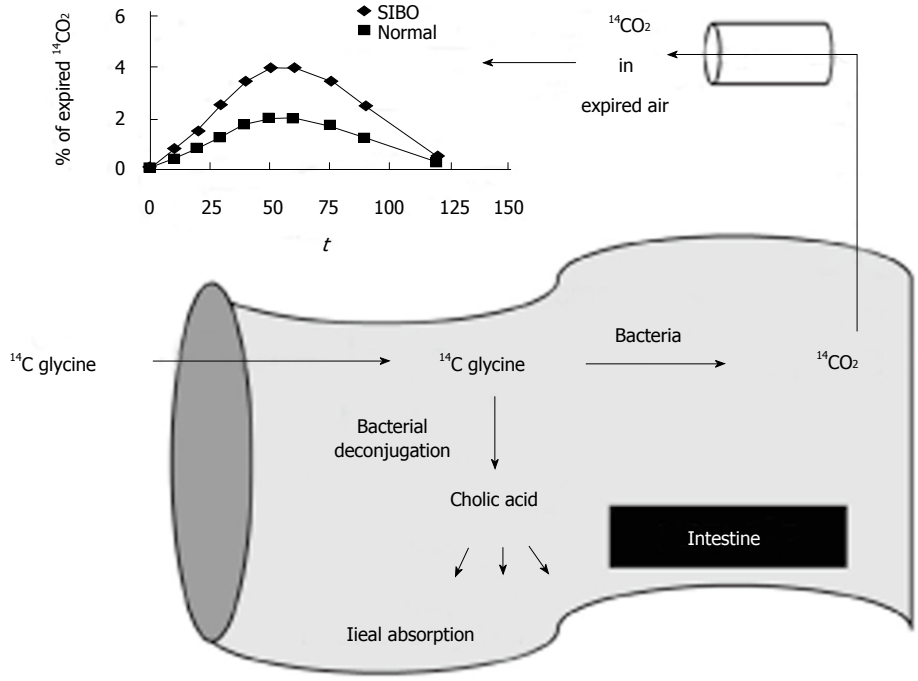

Figure 3 Bile acid breath test involves the administration of the bile acid ^{14}C glycocholic acid, and the detection of $^{14}CO_2$, which would be elevated in small intestinal bacterial overgrowth.

ity of proximal intestine due to release of peptide YY, neurotensin and glucagon-like peptide-1 in the ileum[68]. Therefore, alteration in gut flora may cause altered motility and predispose to IBS like symptoms.

EVIDENCE FROM STUDIES ON THERAPEUTIC MANIPULATION OF GUT FLORA IN IBS

Gut flora of IBS patients is different from that of healthy subjects, resulting in more gas production due to bacterial fermentation[69-71]. Evidence suggest that therapeutic manipulation of gut flora either with antibiotics or probiotics may lead to relief in symptoms of IBS[72,73]. Several meta-analysis have reported the presence of SIBO in a subset of IBS patients[14,17,74]. Recent studies have revealed that treatment of SIBO relieves symptoms of IBS[17,75]. In a study, eradication of SIBO by open label antibiotic treatment resolved symptoms of IBS to the extent of Rome I criteria turning negative in 48% of patients[17]. SIBO is more often found in diarrheal IBS than other subtypes[16]. Treatment of patients with diarrhea-predominant IBS with antibiotics, which is the primary treatment of SIBO, may lead to relief in symptoms including bloating, abdominal pain and loose stools. Rifaximin, a non-absorbable broad spectrum antibiotic, has been widely used for treatment of SIBO[76]. In a phase 3, double-blind, placebo-controlled trial on IBS patients without constipation, treatment with rifaximin provided significant relief in bloating, abdominal pain, and loose or watery stools as compared to placebo[77].

Methane produced by methanogenic flora is shown to cause slow transit constipation, which is associated with IBS[78]. Recently, rifaximin is found to reduce methane gas and improve gut transit[58]. A combination of rifaximin and neomycin is more effective in treating methane-producing IBS patients as compared to treatment with neomycin and rifaximin as single agents (87% *vs* 33% and 28%, respectively)[79].

Some studies support the use of probiotics to be as effective as antibiotics in relieving IBS related symptoms[80,81]. A study showed that treatment with probiotics was effective in reducing symptoms of abdominal pain, bowel frequency, urgency and distension in patients with chronic diarrhea[82]. Lactobacilli are less gas producing than other bacteria[70]. Therefore, administration of Lactobacilli in patients with IBS was associated with reduced gas-related symptoms[83]. In a single blinded randomized control trial, IBS patients randomized to receive *Lactobacillus acidophilus*, *Lactobacillus helveticus*, and Bifidobacterium showed significant improvement in pain and bloating as compared to those who received placebo[84]. Another study showed *Bacillus subtilis* and *Streptococcus faecium* to be effective in reducing abdominal pain as compared to placebo in patients with diarrhea or alternating type of IBS[85]. However, more studies are needed to know the best strains of probiotic bacteria, their dose and duration for treatment of patient with IBS.

CONCLUSION

Association between IBS and SIBO is definite. In fact, controversy exists whether patients presenting with IBS but found to have SIBO on further testing should be diagnosed as IBS or should be considered as SIBO as

symptoms of IBS and SIBO are similar. However, in the current diagnostic algorithm of Rome Foundation, IBS is diagnosed by symptom-based criteria. Exclusion of SIBO by appropriate testing is not essential before diagnosing IBS. In future iteration on IBS diagnostic algorithm by Rome Foundation, it may be important that consideration is given to exclude SIBO before a diagnosis of IBS is made, at least in a subset of patients with higher probability of SIBO. However, as evident from the review of the existing data, we conclude that whereas in some studies, the frequency of SIBO was over-estimated, in others it was under-estimated. Studies that used an early-peak criteria on LHBT showed higher frequency of SIBO than those that used other methods to diagnose. In the context of recent studies that showed that early-peak criteria on LHBT if often false positive, it is likely that all these studies over-estimated frequency of SIBO and therefore, created an un-necessary hype[14,17,86,87]. GHBT has a low sensitivity to diagnose SIBO[15]. Therefore, studies based on GHBT might have under-estimated frequency of SIBO. Though jejunal aspirate culture is considered as gold standard for diagnosis of SIBO, this has limitations. Most importantly, this is invasive and hence, not acceptable to the patients. Therefore, there is urgent need to know the clinical predictors for considering diagnosis of SIBO in patients presenting as IBS and better diagnostic techniques to confirm this.

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Melatonin for the treatment of irritable bowel syndrome

Kewin Tien Ho Siah, Reuben Kong Min Wong, Khek Yu Ho

Kewin Tien Ho Siah, Reuben Kong Min Wong, Khek Yu Ho, Division of Gastroenterology and Hepatology, University Medicine Cluster, National University Hospital, Singapore 119228, Singapore
Kewin Tien Ho Siah, Reuben Kong Min Wong, Khek Yu Ho, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 119228, Singapore
Author contributions: Siah KTH, Wong RKM and Ho KY contributed equally to this work.
Correspondence to: Khek Yu Ho, Professor of Medicine, Chief, University Medicine Cluster, National University of Singapore Tower Block, Level 10, 1E Kent Ridge Road, Singapore 119228, Singapore. khek_yu_ho@nuhs.edu.sg
Telephone: +65-67795555 Fax: +65-67794112
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Abstract

Irritable bowel syndrome (IBS) is a common disorder characterized by recurrent abdominal pain or discomfort, in combination with disturbed bowel habits in the absence of identifiable organic cause. Melatonin (N-acetyl-5-methoxytryptamine) is a hormone produced by the pineal gland and also large number by enterochromaffin cells of the digestive mucosa. Melatonin plays an important part in gastrointestinal physiology which includes regulation of gastrointestinal motility, local anti-inflammatory reaction as well as moderation of visceral sensation. Melatonin is commonly given orally. It is categorized by the United States Food and Drug Administration as a dietary supplement. Melatonin treatment has an extremely wide margin of safety though it may cause minor adverse effects, such as headache, rash and nightmares. Melatonin was touted as a potential effective candidate for IBS treatment. Putative role of melatonin in IBS treatment include analgesic effects, regulator of gastrointestinal motility and sensation to sleep promoter. Placebo-controlled studies in melatonin suffered from heterogeneity in methodology. Most studies utilized 3 mg at bedtime as the standard dose of trial. However, all studies had consistently showed improvement in abdominal pain, some showed improvement in quality of life of IBS patients. Melatonin is a relatively safe drug that possesses potential in treating IBS. Future studies should focus on melatonin effect on gut mobility as well as its central nervous system effect to elucidate its role in IBS patients.

Key words: Melatonin; Irritable bowel syndrome; Pain; Sleep; Analgesia

Core tip: Irritable bowel syndrome (IBS) is a common disorder associated with significant disability and high social cost. This is partly due to lack of effective treatment with low side effects. Melatonin is a drug that was postulated to be a potential useful arsenal in battling IBS. Its role in analgesia has been recognized in several other fields of medicine. Several well-designed placebo-controlled trials in IBS patients had consistently showed improvement of abdominal pain when taking 3 mg of melatonin with no serious side effect. Future studies should examine the long term effect of Melatonin as well as its effect on central nervous system and gut motility.

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INTRODUCTION

Irritable bowel syndrome (IBS) is a common disorder characterized by recurrent abdominal pain or discomfort, in combination with disturbed bowel habits in the absence of identifiable organic cause. It is associated with significant disability and health care costs. In the West,

the prevalence of IBS in the community is reported to be between 10%-20%[1-3]. In Asia, we have also seen a steady rise of IBS in the community. In Singapore, prevalence of IBS was reported to be 2.3%[4] in 1998, by the Manning criteria, and 8.6% in 2004 (as defined by the Rome Ⅱ criteria)[5]. In addition, a recent study has shown that the disease burden extends beyond the patient and has significant impact on the spouse or family members as well, with burden proportionally increasing with IBS severity[6], underscoring the need for effective treatment of the patient's symptoms.

Traditionally, IBS is treated with a combination of treatment modality, from antispasmodic, psychopharmacological treatment like tricyclic antidepressant to mindfulness therapy like hypnotherapy. Newer drugs such as linaclotide, prucalopride, tegaserod and lubiprostone[7] have given hope to clinicians treating the many disabling symptoms of IBS. However, worry about potential side effects, the need for long-term medication and high drug costs have been a deterrent for many IBS patients. Melatonin is one of the drug that was identified as potentially useful in IBS especially for pain symptom as well as bowel motility in constipation predominant IBS.

Melatonin (N-acetyl-5-methoxytryptamine), a hormone produced by the pineal gland, has been studied as a potential treatment of circadian rhythm sleep disorders, cancer, immune disorders, cardiovascular diseases and insomnia[8,9]. The melatonin signal chemically regulates the sleep-wake cycle by causing drowsiness and lowering body temperature[10]. The gastrointestinal tract is another large source of endogenous melatonin.

MELATONIN IN GASTROINTESTINAL TRACT

Melatonin is produced by enterochromaffin cells of the digestive mucosa. There is higher concentration of melatonin in the gastrointestinal tract than the blood or the pineal gland. The finding that the concentration of melatonin in the gastrointestinal tissues surpasses that in the blood by 10-100 times[11] suggests that melatonin may play an important role in the digestive system. Circadian variation of gastrointestinal (GI) melatonin does not appear to be controlled by photoperiodicity (like the pineal gland), but by eating and food composition. A sharp increase in the content of melatonin in GI tract tissue and circulation in response to food intake was reported in volunteers[12,13]. Melatonin played several pivotal local intestinal functions: (1) Regulation of GI motility: Melatonin exerts both excitatory and inhibitory effects on gut smooth muscles. The precise mechanism through which melatonin regulates gastrointestinal motility is still not very clear. Small doses of melatonin accelerated the intestinal transit in rats, while high doses reversed this effect[14]. In one study focusing on gastric emptying, melatonin partially inhibited gastric motility by activating sympathetic neurons. In the stomach, melatonin also reduces nitrergic myenteric innervation[15]; (2) Anti-Inflammatory Reaction: It increases natural killer cell activity and Th2 cell mediated immune responses[16]. Melatonin was shown to reduce the severity of intestinal inflammatory pathologies such as colitis in animal models[17]. Melatonin had also been shown to scavenge reactive oxygen species and inhibit macrophage by suppressing proinflammatory gents including inducible nitric oxide synthase and cyclooxygenase-2[18-20]; and (3) Moderation of Visceral Sensation: Melatonin may also be involved in mediating gut visceral sensation because patients with functional abdominal pain are reported to have a lower urinary excretion of 6-sulphatoxy melatonin and to exhibit a circadian rhythm of lower amplitude compared with healthy controls[21].

Melatonin might be a candidate for IBS treatment based on the following considerations: (1) melatonin has analgesic effects which may help to alleviate abdominal pain and influence the sensation of abdominal distention in IBS patients; (2) melatonin has regulatory effects on gastrointestinal tract motility and sensation which may improve the bowel habits and alleviate abdominal pain or distention in IBS patients; (3) melatonin could have a sleep promoting effect which may useful to treat the sleep disturbance of IBS patients; and (4) melatonin has mood regulation and anti-stress effects which could help alleviate the abnormal psychological parameters observed in IBS patients. Thus, we believe that melatonin might serve the several aspects of IBS treatment strategy because it targets not only the psychological component, *i.e.*, stress, anxiety, depression and sleep disorder but also the peripheral elements of abnormal bowel sensation and motility. Below we examine the possible mechanisms of melatonin in the treatment of IBS.

PHARMACOLOGY OF MELATONIN

Melatonin is commonly given orally though it also can be given *via* intravenous, intranasal or transbuccal routes. Melatonin is readily absorbed when it is administered *via* any route. It crosses all morphophysiological barriers, *e.g.*, blood-brain barrier and placenta, with ease.

The absorption and bioavailability of melatonin varies widely. When given by mouth, peak melatonin concentration occurs within an hour and serum half-life is approximately 35-50 min[22]. Because of its fast clearance, regular melatonin formulations can produce physiological levels for only 2-4 h[23]. The typical dose range in studies of melatonin's effects on sleep disturbance has been between 0.3-5 mg, with 2-3 mg commonly being used. Ingested melatonin that did not undergo first-pass metabolism in the liver is eventually metabolized, mainly in the liver. After conjugation with sulfuric or glucuronic acid, it is excreted by the kidneys. A single night-time dose is cleared by the following morning. Legal availability of melatonin varies in different countries from over the counter in United States to prescription only in other countries. It is categorized by the United States Food and Drug Administration as a dietary supplement. Melatonin treatment has an extremely wide margin of safety though

it may cause minor adverse effects, such as headache, rash and nightmares. Studies of human subjects given varying doses of melatonin (1-6.6 g/d) for 30-45 d did not reveal abnormalities at the end of the test period except drowsiness[24,25]. However, in a placebo-controlled trial using 3-6 mg of melatonin for eight weeks on IBS women, drowsiness only happened in a minority of participants and there was no difference between the groups[26,27]. Lu *et al*[26] also showed that baseline saliva melatonin levels were lower in IBS compare to normal control and oral melatonin supplement was able to increase the level of melatonin in the saliva.

WHAT ARE THE PUTATIVE SITES OF ACTION OF MELATONIN IN IBS?

Sleep promoter

Besides the bowel symptoms, sleep disturbance is commonly observed in patients with IBS, it being reported to occur in 26%-55% of IBS patients[28-30]. Although the cause and effect association is not clear, there is some evidence supporting the "bad bowels cause bad dream" hypothesis[31-36] including the finding that IBS patients have more frequent rapid eye movement (REM) sleep - a sleep phase that is characterized by arousal - than non-REM sleep[37,38]. IBS patients were also found to have higher rapid eye movement latency[34,39]. IBS patients with sleep dysfunction were also found to have abnormal physiological threshold of pelvic muscles. IBS patients had a significantly lower threshold volume for urge and anal sphincter pressure for maximal squeeze as compared with those without sleep dysfunction[40].

It has been suggested that melatonin has a sleep promoting effects by cueing circadian rhythms and thus indirectly promoting sleep[41]. In addition, melatonin was also suggested to have a role in direct promotion of sleep[42]. Currently, there is a general agreement that melatonin is probably not a direct soporific or hypnotic compound[43]. Rather, the most commonly proposed mechanism for melatonin to induce sleepiness relates to its effects on the circadian clock, *i.e.*, it "opens the sleep gate"[44] and also it slightly reduces body temperature which promotes sleep[45]. Clinical trials in healthy volunteers have shown that exogenous melatonin accelerates sleepiness probably *via* thermoregulatory mechanisms[46]. Melatonin has these effects over a wide range of doses, ranging from physiological (250 μg) to pharmacological (1-10 mg) levels[40]. Besides the above effects of melatonin on sleep in healthy subject or animals, many clinical trials and reviews have shown that melatonin may exert sleep promoting effect in a number of circadian rhythm sleep disorders[20,47-49].

Brain-gut interaction: mood enhancer

Patients with IBS often complain of a wide variety of symptoms apart from GI symptoms, which may not necessarily originate from the GIT but from central abnormal psychological conditions such as stress, anxiety and depression. Psychological distress and major life events are frequently present in IBS. The most common comorbid psychiatric disorders seen in IBS patients include anxiety disorders (panic and generalized anxiety disorder), depression, somatoform disorders and phobic disorders[50-52]. Compared with healthy controls, patients with IBS are observed to have higher scores for anxiety, depression, hostile feelings, sadness and interpersonal sensitivity[53-55]. In United States, Whitehead *et al*[56] reported a prevalence of 30.5% for depression and 15.5% for anxiety state in IBS patients. IBS symptoms are often exacerbated by psychological stress. In Hong Kong, Generalized Anxiety Disorder was five times more common among IBS patients than non-IBS control[57].

Melatonin is documented to have a possible role in regulation of mood disorders, such as anxiety and depression, both of which are often caused by certain acute or chronic stress events[52,58]. Many studies reported decreases in nocturnal melatonin concentrations in depressed patients, compared with controls[19,59]. Antidepressant therapy has been reported to restore the circadian melatonin rhythm in depressive patients[60]. It was observed that most melatonin treated women reported a general improvement in mood and a significant mitigation of depression[61]. Reduction of nocturnal melatonin peak has been observed in depressed patients in most studies and an increase in nocturnal melatonin levels has been found in patients during treatment with desipramine[52,62].

Clinical studies in IBS patients with melatonin had mixed result when it comes to depression and anxiety. Two studies in Singapore using 3 mg of melatonin showed that there was no difference in depression and anxiety score in subjects taking melatonin compared to placebo[23,63]. Another study in India showed improvement of psychological well-being and mood in the treatment group taking 3 mg of melatonin for 2 wk, however, the details of psychological parameters were not provided[24].

Antinociceptive action of melatonin

The clinical finding that patients suffering less from pain during the night when melatonin level is higher led to the suggestion that melatonin has a possible analgesic effect. This suspicion was supported by the finding that pinealectomy abolished such dark phase analgesia[64] and that it could be restored using melatonin replacement[65]. However, the mechanism of the analgesic effects of melatonin is still not clear at present. It may include complex interactions among melatonin, opioidergic system and melatonin receptors. Met-enkephalin and beta-endorphin are two endogenous opioids involved in the regulation of pain sensitivity in hypothalamus. The levels and circadian rhythmicity of these two opioids changed in rats that received pinealectomy[66,67]. This may imply that the change in the brain concentration of these endogenous opioids could be a mechanism for the mediation of the melatonin induced modulation of pain sensitivity. However, a recent study found that melatonin exerts its analgesic actions not by binding to opioid receptor subtypes but by

Table 1 Placebo-controlled studies: treatment effect of melatonin in irritable bowel syndrome patients

Ref.	Subjects (total, age, IBS Criteria)	IBS subtypes	Treatment *vs* control	Pain	Bloating/ distention	Motility	Sleep	Psycho-logical	Overall IBS score	Outcome
Song *et al*[63], 2005, Singapore	40, 20-64 years old, ROME Ⅱ IBS (with sleep disturbance)	14 IBS-C, 18 IBS-D, 8 IBS-A	20 (3 mg, bedtime, 2 wk) *vs* 20 Placebo	Yes[4]	No	No	No	No	N/A	Decreased abdominal pain and increased pain threshold
Lu *et al*[26], 2005, Singapore	17, 41+/-14 years old woman, ROME Ⅱ IBS	N/A	12 (3 mg bedtime for 8 wk) *vs* 12 Placebo	Yes[5]	Yes[5]	No[1]	No	No	N/A	Effective in improving bowel symptoms in female IBS patients
Saha *et al*[27], 2007, India	18, 18-65 years old, ROME Ⅱ IBS	N/A	9 (3 mg bedtime for 8 wk) *vs* 9 Placebo	Yes	Yes	Yes	N/A	Yes	Yes[2]	Improved overall IBS score, extracolonic score as well as QOL
Chojnacki *et al*[72], 2013, Poland	80, 48-65 years old woman, ROME Ⅲ IBS	40 IBS-C, 40 IBS-D	40 (3 mg morning, 5 mg bedtime for 6 mo) *vs* 40 placebo	N/A	N/A	Yes[3]	N/A	N/A	Yes[3]	Improved visceral pain and abdominal bloating for IBS-C patients

[1]CTT significantly prolonged in control subjects. Only a trend of prolonging CTT in IBS patients; [2]Improved overall IBS score (45% *vs* 16.66%, $P < 0.05$); [3]Significant result only for IBS-C, the intensity of visceral pain and abdominal bloating had decreased in 70% of patients ($P < 0.01$) and constipation in 50% of patients ($P < 0.05$); [4]melatonin taken for two weeks significantly decreased mean abdominal pain score (2.35 *vs* 0.70; $P < 0.001$) and increased mean rectal pain threshold (8.9 *vs* 21.2 mmHg; $P < 0.001$); [5]The improvement in mean ± SD. IBS symptom score was significantly greater after treatment with melatonin (3.9 ± 2.6) than with placebo therapy (1.3 ± 4.0, $P = 0.037$ The beneficial effects of melatonin were most marked in symptoms such as abdominal plain, abdominal distension and abnormal sensation of defecation. CTT: Colonic transit time; N/A: Not available; IBS: Irritable bowel syndrome; QOL: Quality of life.

Table 2 Placebo-controlled studies: side effects of melatonin in irritable bowel syndrome patients

Ref.	Subjects (total, age, IBS criteria)	Dosage, frequency, duration	Sleepiness	GI side effect	Others
Lu *et al*[26], 2005, Singapore	17, 41+/-14 years old woman, ROME Ⅱ IBS	3 mg bedtime for 8 wk	1 × Daytime sleepiness (both treatment and placebo group)	Nil	Nil
Saha *et al*[27], 2007, India	18, 18-65 years old, ROME Ⅱ IBS	3 mg bedtime for 8 wk	1 × Drowsiness (both groups),	Nil	1 decreased libido
Chojnacki *et al*[72], 2013, Poland	80, 48-65 years old woman, ROME Ⅲ IBS	3 mg morning, 5 mg bedtime for 6 mo	Nil	Nil	2 fatigue, 1 vertigo

IBS: Irritable bowel syndrome; GI: Gastrointestinal.

binding to its own receptors and increasing the release of beta-endorphin[68]. Another study also showed that of the three other subtypes of melatonin receptors identified, *i.e.*, Mel1, Mel2 and Mel3, only Mel2 receptor is involved in the analgesic activity of melatonin[69]. Importantly, this anti-nociceptive effect may be unrelated to and independent of the sleep-inducing effects of melatonin, as was demonstrated in the study by Song *et al*[63], where melatonin was found to increase rectal pain thresholds but had no significant effect on sleep. Human studies have shown that melatonin is a hormone with potential therapeutic use for treatment of diseases with pain. Melatonin was documented to be effective in treating many types of headache, such as chronic cluster headache and migraines[70,71].

All these evidence support the belief that melatonin is involved in the modulation of pain and has analgesic effects. However, its potential as a therapeutic agent for treatment of diseases with pain still needs to be further investigated.

Outcomes in placebo-controlled studies

Placebo-controlled studies in melatonin suffered from heterogeneity in methodology (Table 1). Most studies utilized 3 mg at bedtime as the standard dose of trial. The duration of investigation also differ from 2 wk to 6 mo. Chojnacki *et al*[72] used a twice a day dosing in their study with 3 mg in the morning and 5 mg at night for 6 mo. However, there was no increased sleepiness or gastrointestinal side effects reported (Table 2). There was a variety of outcome measures from overall IBS score to quality of life. Lu *et al*[73] examined the effect of melatonin on colonic transit time (CTT) and found that melatonin increased CTT in both control and IBS patients, but only the result in control subjects was significant statistically. In other hand, Chojnacki *et al*[72] showed that with 6 mo treatment with melatonin, 50% of IBS-C patients showed improvement of constipation. However, all studies had consistently showed improvement in abdominal pain for IBS patients. Song *et al*[63] also reported increase of rectal pain threshold after 2 wk of melatonin treatment. Finally, Saha *et al*[27] showed that the overall improvement in quality of life score was 43.63% in melatonin group and 14.64% in placebo group.

CONCLUSION AND THE FUTURE FOR MELATONIN IN IBS

It is still unclear how melatonin may be useful and its mode of action in IBS patients. Current available evi-

dence showed that it is likely to be useful in battling the pain and increasing pain threshold in IBS patients. Different dosing as well as treatment period of melatonin should be studied. Melatonin is a relatively safe drug that possesses potential in treating IBS. Its attractiveness also stem from its relative low cost to the patients. Future studies should focus on melatonin effect on gut mobility especially in IBS-C patients as well as its true central nervous system effect in view of high placebo rate often observed in IBS patients.

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Irritable bowel syndrome: The evolution of multi-dimensional looking and multidisciplinary treatments

Full-Young Chang

Full-Young Chang, Environmental Health and Safety Office and Division of Gastroenterology, Taipei Veterans General Hospital, and National Yang-Ming University School of Medicine, Taipei 11217, Taiwan
Author contributions: Chang FY contributed to the manuscript.
Correspondence to: Full-Young Chang, MD, Professor, Environmental Health and Safety Office and Division of Gastroenterology, Taipei Veterans General Hospital, and National Yang-Ming University School of Medicine, 201 Shih-Pai Road, Section 2, Taipei 11217, Taiwan. changfy@vghtpe.gov.tw
Telephone: +886-2-28757308 Fax:+886-2-28757310
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Abstract

Irritable bowel syndrome (IBS) is common in the society. Among the putative pathogeneses, gut dysmotility results in pain and disturbed defecation. The latter is probably caused by the effect of abnormal gut water secretion. The interaction between abnormal gas accumulation, abdominal pain and bloating remains controversial. Visceral hypersensitivity and its modification along with the central transmission are the characteristics of IBS patients. The identification of biologic markers based on genetic polymorphisms is undetermined. Imbalanced gut microbiota may alter epithelial permeability to activate nociceptive sensory pathways which in turn lead to IBS. Certain food constituents may exacerbate bowel symptoms. The impact of adult and childhood abuses on IBS is underestimated. Using the concept of biopsychosocial dysfunction can integrate multidimensional pathogeneses. Antispasmodics plus stool consistency modifiers to treat the major symptoms and defecation are the first-line drug treatment. New drugs targeting receptors governing bowel motility, sensation and secretion can be considered, but clinicians must be aware of their potential serious side effects. Psychiatric drugs and modalities may be the final options for treating intractable subjects. Probiotics of multi-species preparations are safe and worth to be considered for the treatment. Antibiotics are promising but their long-term safety and effectiveness are unknown. Diet therapy including exclusion of certain food constituents is an economic measure. Using relatively safe complementary and alternative medicines (CAMs) may be optional to those patients who failed classical treatment. In conclusion, IBS is a heterogeneous disorder with multidimensional pathogeneses. Personalized medicines with multidisciplinary approaches using different classes of drugs, psychiatric measures, probiotics and antibiotics, dietary therapy, and finally CAMs, can be considered.

Key words: Antispasmodics; Biopsychosocial dysfunction; Comorbidity; Genetics; Irritable bowel syndrome; Microbiota; Probiotics; Visceral hyperalgesia

Core tip: Irritable bowel syndrome (IBS) is common in the society. Patients with this disorder have a poor quality of life with severe impact on their social and economic burdens. Its pathogenesis remains evolutional, involving biological, psychiatric and social factors. Therefore, the biopsychosocial dysfunctional model has attempted to integrate all the above mentioned mechanisms in order to understand how IBS can develop under such complex interaction. Since the etiology of IBS is heterogeneous, the currently recommended treatments are multidisciplinary and also individualized, *e.g.*, using different classes of drugs, psychiatric measures, probiotics and antibiotics, dietary therapy, and finally complementary and alternative medicines.

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INTRODUCTION

Irritable bowel syndrome (IBS) is an essential member of the functional gastrointestinal disorder (FGID) family. According to the globally accepted Rome Ⅲ definition, it is characterized by chronic and recurrent abdominal pain/discomfort associated with disturbed defecation[1,2]. As a functional disorder, IBS definition remains evolutional over recent decades. For example, the Manning criteria released in 1978 are just to identify IBS. The later released Rome Ⅰ-Ⅲ criteria are broadly to diagnose all FGIDs including IBS. Now the Rome Ⅳ criteria are undergoing preparation but not formally announced[3]. Regarding various criteria, a review indicated that the Manning criteria are the most valid and accurate, whereas the Rome Ⅲ criteria are not valid and are poorly adopted, especially for clinical trials[4]. It is also controversial whether abdomen pain is virtually required to diagnose IBS. For example, constipation-predominant IBS (IBS-C) and functional constipation are two exactly distinct FGIDs because the latter lacks obvious abdominal pain, but a study indicated that their discrepancy was not easily to detect since marked overlapping was observed between the two conditions[1,5]. Accordingly, an expert meeting recommended that current criteria to diagnose IBS need further revision, particularly the significance of abdominal bloating should be included and the pain component is best to de-emphasize[6]. Overall, IBS is common in the society with worldwide prevalence ranging from 5% to 15%[3,7-10]. The reported IBS prevalence is determined by a number of factors including subject gender, used criteria, questionnaires, study methods, locations, geographical characters, cultural and social backgrounds, and ethnicity[3,8,9,11]. Clinically, IBS is not only confined to the colon but may also extend to other organs and systems since IBS individuals usually have multiple comorbidities such as dyspepsia, gastro-esophageal reflux disease, interstitial cystitis, fibromyalgia, chronic fatigue, insomnia, headache/migraine and psychiatric disturbances[12-18]. Owing to the commonly associated somatic comorbidities and high level of psychiatric disturbances, IBS subjects often have absenteeism, reduced quality of life (QoL) and multiple healthcare seeking behaviors, which lead to great social and economic burdens[13,16,19-21]. Because IBS is a functional disorder with multi-dimensional looking, current IBS management is towards multidisciplinary approaches[1,7,22-24]. The purpose of this review attempts to introduce what are the updated pathogeneses and managements of IBS based on the multi-dimensional looking and multidisciplinary approaches.

PATHOGENESIS OF IBS

Biopsychosocial model

Current mechanisms to address IBS pathogenesis consist of the defects involving biological, chemical, physical, environmental, economic, cultural, moral, and spiritual events, particularly these defects may interact with each other and lead to IBS. Overall, these mechanisms can be simply categorized into three major issues in terms of biological defects, psychological disturbances and social impacts[1,25]. In order to illustrate and understand why a disease or disorder will develop under the complex interaction involving many mechanisms, the framework of a biopsychosocial model has been introduced to unify biological, psychological and social issues together to indicate their final interaction[25]. Figure 1 briefly depicts that the original existence of any defects among three categories during the early life and adolescent period may initiate biopsychosocial interaction and the following IBS symptoms.

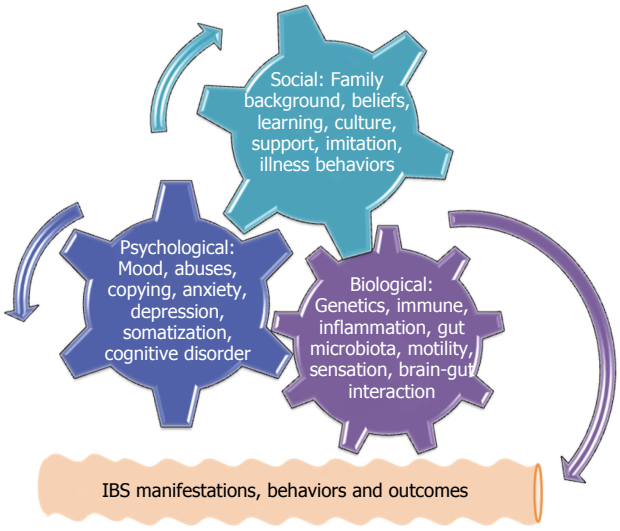

Figure 1 Three-axis cogwheel system to illustrate how biopsychosocial dysfuntion can integrate many putative pathogeneses leading to irritable bowel syndrome. The irritable bowel syndrome (IBS) clinical manifestations, disease behaviors and future outcomes are also under the impact of this dysfunction.

Alternatively, genetics- or environment-determined biological defects at any level of neural control and modulation of gut motility, digestion, sensation, endocrine, secretion and immune functions may result in IBS symptoms, while the psychological disturbances which are closely related to a number of social impacts such as early life abuses, stresses, social learning, and copying patterns are able to trigger neuroimmune reactions *via* the brain-gut axis and lead to exacerbated IBS symptoms[25]. Most importantly, the biopsychosocial model is characterized by bi-directional causality and feedback. Accordingly, any adolescent modification coming from the biological, psychological and social impacts manifests different levels of symptoms, behaviors and outcomes of IBS in adulthood, while their symptomatic manifestations are in turn to modify the existent psychological and social events[1,25-27]. This is why many associated comorbidities are reported among IBS subjects. Interestingly, the biopsychosocial model is not only confined to the IBS but

also adopted in many pain related disorders such as migraine, tension headache, chronic fatigue syndrome, and fibromyalgia[28,29]. Thereafter, a concept of central sensitivity syndromes is proposed to unite these comorbidities that apparently share the same biopsychosocial dysfunction[30,31]. Although the unified biopsychosocial model can help easily to understand how IBS will develop under this interaction, the individual pathogeneses are worthy of being introduced before their final integration.

Motility disorders

Based on the predominant defecation pattern, various IBS subtypes are traditionally defined[1]. Accordingly, it is reasonable to speculate that bowel dysmotility may result in IBS, particularly the disordered defecation. For instance, abnormal small intestinal motility was indicated to lead to IBS in some subjects[32]. In addition, rapid small intestinal transit among the diarrhea-predominant IBS (IBS-D) subjects and delayed transit in IBS-C subjects were reported[33,34]. Using ingested radiopaque markers to count scattering index representing small intestinal transit, another study pointed out the same transit among three categories in terms of IBS-C, IBS-D and control subjects[35]. Accordingly, observed small intestinal dymotility is likely to exist in certain IBS subjects, but the intra- and inter-individual variations in motility measurements limit their interpretation of small intestinal dysmotility in clinical usefulness[36].

Defecation is a complex event involving the coordination of colon transit, high amplitude propagated contractions (HAPC) and pelvic floor synergia while the integrated central (CNS), autonomic and enteric nervous systems (ENS) are virtually required to mediate their correct process[37-39]. Abnormal colon motilities have been observed among IBS subjects. For example, the total colonic transit time in IBS-D patients measured by ingested radiopaque markers was prolonged after pinaverlum bromide treatment. The effectiveness of this agent to treat IBS-D appears *via* correction of abnormal colon transit[40]. Similarly, a radiopaque study confirmed again that Japanese IBS-D subjects had accelerated colon transit compared to controls, whereas those in IBS-C subjects and controls were the same[35]. Left colonic segmentation pressure waves and HAPC were altered among some non-IBS-C patients[41]. Besides, certain IBS-C patients had delayed total colon and right segmental transit[42]. Like small intestinal transit, it is concluded that abnormal colon transit probably exists in some, but not all IBS subjects, because IBS is heterogeneous in its pathophysiology.

Regarding the autonomic nervous activity, IBS-D subjects manifested an enhanced adrenergic sympathetic dominance compared with controls and IBS-C subjects, while this dominance was likely the effect of vagal withdrawal rather than true enhancement[43]. As one of mechanisms leading to functional constipation, pelvic floor dyssynergia was also observed in some non-IBS-D patients[34,44]. Since pelvic floor dyssynergia obviously overlaps with the spectrum of functional anorectal disorder defined by the Rome Ⅲ criteria[25], it is debatable what is the demarcation between IBS and functional anorectal disorder. Overall, colonic dysmotility probably exists in certain, but not all IBS patients. Using various colon motility measurements to diagnose IBS may be unreliable.

Gut water secretion

Gut water component has been a main factor to determine hard or loose stool. IBS subtypes are traditionally classified by the predominant stool pattern. Alternatively, it means that the gut water secretion in IBS subjects may be different. Unlike other mechanisms that are extensively evaluated, only a few studies have addressed this issue. For example, a rat IBS model study pointed out that the fecal water content was lower in IBS-C rats, whereas an excessive secretion existed in the IBS-D group[45]. The densities of some peptides mediating gut motility, secretion and sensation, *e.g.*, serotonin, peptide YY, pancreatic polypeptide, enteroglucagon, somatostatin, *etc.* were obviously reduced in human IBS colon. It looks to mean that the abnormal gut water secretion is one of components leading to IBS[46]. In addition, using lubiprostone with the ability to increase gut water secretion in softening stool for IBS-C subjects appears to support the role of gut water secretion in IBS[47]. Overall, the abnormal gut water secretion should not be forgotten as a candidate of IBS pathogeneses.

Bowel gas

Both abdominal bloating and fullness are common among IBS subjects. Therefore, abnormal bowel gas accumulation may account for these annoying symptoms[6]. Unfortunately, bowel gas studies report conflicting results. An earlier study did not find abnormal bowel gas accumulation among the very limited IBS like subjects[48]. In contrast, IBS patients had impaired transit and tolerance to the loading of intestinal gas[49]. A Japanese study pointed out the excessive bowel gas volume among IBS subjects. However, neither symptoms nor subtypes correlated well with abnormal bowel gas accumulation[50]. This means that other factors apart from bowel gas may be responsible for the bloating symptom. Alternatively, bloating symptom is additionally associated with visceral hypersensitivity and delayed transit, and the impaired gas handling may be observed in some, but not all IBS subjects[51].

Visceral hypersensitivity

Abdominal pain has been a key component of IBS. It is expected that visceral hypersensitivity may account for IBS. Studies using rectal balloon distension repeatedly confirmed that IBS subjects have diminished threshold and exaggerated painful severity to balloon distension[41]. Accordingly, visceral hypersensitivity appears a candidate of biological hallmark to diagnose IBS[52]. In fact, hypersensitivity among IBS subjects is not only confined to the colon but also extends upward to CNS[53-55]. For example,

abnormal activation of certain brain regions following painful rectal stimulation confirmed the altered processing of afferent signals along the brain-gut axis[54]. Visceral hypersensitivity is additionally modified by the gender, peptide, immune and emotional factors[14,25,56,57]. The central projection and modulation of visceral pain are complex, and many transmitted tracts have not been clearly revealed. It is believed that prefrontal lobe may modulate the neural activities coming from limbic and paralimbic regions, anterior cingulate cortex, and hypothalamus, which in turn down modifies the activities of descending inhibitory and facilitatory pathways through the periaqueductal gray and pontomedullary nuclei. The neuronal activities among these cortico-limbic pontine networks can coordinate the final perception of cognitional and emotional impacts on the visceral pain and discomfort in IBS subjects[56,58].

Based on the neuroimage technique, IBS subjects were observed to have long-term micro-structural brain changes, particularly the regions integrating sensory information and cortico-thalamic modulation[59]. These observed brain structural changes among IBS patients appear to challenge the concept of IBS as a functional disorder without existing structural abnormality. The altered functional connectivity between brainstem pain-modulating circuits and cortical-limbic centers suggests a bi-directional interaction between pain and mood. Interestingly, this dysfunctional pain network not only exists in IBS but also is observed among other comorbidities, *e.g.*, migraine, fibromyalgia, anxiety disorders, *etc.*[60]. Allodynia is a pain condition originated from a stimulus, which does not normally provoke pain. Alternatively, it is a central hypersensitivity phenomenon with diminished threshold to triggers[61]. Apart from visceral hypersensitivity, IBS subjects also had cutaneous allodynia following a number of repetitive nociceptive thermal stimuli[62,63]. Overall, the broadly existing somatic, visceral and central hypersensitivities support why IBS patients always have multiple somatic and psychiatric comorbidities.

Genetics

Twins are an ideal model to resolve whether genetics or environmental factor is essential to determine IBS in a family. Unfortunately, the results of twin studies are conflicting. Concordance for IBS was significant among monozygotic (17.2%) twins compared to dizygotic (8.4%) twins[64,65]. In contrast, similar prevalences were reported between monozygotic (17%) and dizygotic (16%) twins[66]. A meta-analysis based on twin studies further indicated that heritability is more significant among migraineurs (50%) compared to IBS subjects (25%)[67]. It means that both environmental factors and learning behaviors, rather than the heredity only, are the necessary determinants leading to IBS. This viewpoint confirms again that IBS is most likely the final result of biopsychosocial dysfunction involving the interaction of genetically determined biological and psychological factors and exposed environmental factors coming from biological, psychological and social events. Of mitochondrial dysfunctions and associated DNA sequence variants of maternal inheritance, 60% were related to bowel dysfunction, whereas 16% were probable non-maternal inheritance. This suggests that defective mitochondrial energy metabolism among matrilineal relatives probably leads to FGIDs including IBS[68]. Overall, genetics may be a factor leading to IBS, but environmental and learning factors are also involved.

There are numerous peptides/substances and their corresponding receptors that are involved in IBS pathogenesis. Their roles are mainly to mediate gut motility, sensation, permeability, secretion and immune response. The most frequently addressed peptides include 5-hydroxytyptamine (5-HT), cholecystokinin, glucagon-like peptide, somatostatin, neuropeptide Y, endocannabinoid, vasoactive intestinal polypeptide, corticotropin releasing hormone (CRH), *etc.*[1,7,23,41,46,69-72]. For example, the fact that 5-HT related agonists and antagonists have been developed effectively to treat either IBS-C or IBS-D patients strongly suggests that certain peptide dysfunction is one of important mechanisms leading to IBS[1,7,23]. Second, human IBS colon was observed to have low densities of gut peptides including serotonin, peptide YY, pancreatic polypeptide, enteroglucagon, somatostatin, *etc.*[46]. Third, CRH has been a main mediator of stress response in the brain-gut axis, while IBS is believed a dysfunctional brain-gut link which can be exaggerated *via* CRH related stress[71].

Peptide abnormalities among IBS subjects are sometimes genetically determined. Accordingly, variation of genotypes or polymorphisms among those genes governing peptide synthesis and metabolism, mucosal ion channel functions, reuptake of neurotransmitters and their optimal functioning in receptors, and inflammation susceptibility may account for the IBS phenotypes and symptomatic severity[73]. Some genetic polymorphisms have been identified in relation to IBS even with impacts on the therapeutic response, *e.g.*, *CRH-R1* gene polymorphism of TT genotype of rs7209436 and rs242924 among Japanese IBS patients, SS genotype of serotonin reuptake transporter polymorphism among Indian C-IBS subjects, mitochondrial adenosine triphosphate 6 and 8 polymorphisms among Chinese IBS-D patients, and serotonin transporter promoter genetic polymorphisms influencing response to alosetron therapy among American IBS-D patients[74-77]. Current IBS candidate genes consist of serotonin transporter (*SLC6A4*), norepinephrine transporter (*NET*), alpha-2A-adrenergic receptors (*ADRA2A*), interleukin-10 (*IL-10*), G protein β3 subunit (*GNβ3*), sodium channel (*SCN5A*), *etc.*[78]. Regarding genes controlling inflammation, a meta-analysis indicated that high producer IL-10 (-1082 G/G) polymorphism diminishes the IBS risk in the European IBS population, whereas tumor necrotic factor (TNF) (-308 G/G) polymorphism increases IBS susceptibility and TNF (-308 G/A) polymorphism decreases IBS susceptibility in the Asian IBS population[79]. Overall, IBS genetic

polymorphism studies are criticized with drawbacks of very limited case number, inconsistent results, lack of reproducibility, heterogeneous nature of IBS, *etc.*, while no single gene is globally confirmed to be responsible to IBS[80]. Nevertheless, genetic polymorphisms or pharmacogenetics open a door using an optimal substance to treat appropriate subjects *via* proper genetic mapping in the future.

Gut microbiota and immunity

Human fetus is initially sterile before birth and begins to be infected by many microorganisms since birth through the contact with external environment, while the human immune system is gradually maturing to adapt and tolerate the challenge of exposed microorganisms. Among organs with microorganism residence, the colon owns the most number of resided microorganisms[81]. In fact, the colon microbiota provides numerous physiologic events, namely, supplying energy, nutrient accessibility including short-chain fatty acids, enhancing immune and normal homeostasis, influencing organ development such as morphogenesis of the bone and visceral organs, and even the host metabolism[81,82]. Regarding their clinical impact, inflammatory bowel disease has been the consequence of uncontrolled and imbalanced gut microbiota with altered defense system, permeability and immune response[83]. Similarly, dysfunctional gut microbiota may activate mucosal innate immune responses, which in turn increase epithelial permeability, activate nociceptive sensory pathways, dysregulate the ENS, and finally lead to various FGIDs including IBS. For example, a 16S rRNA-based microbiota profiling study demonstrated both quantitative and qualitative changes of mucosal and fecal gut microbiota among IBS subjects[84]. Second, Japanese IBS subjects had much higher counts of *Veillonella* and *Lactobacillus* than controls, while the products of microbiota such as acetic acid, propionic acid and total organic acids were also significantly higher among these subjects[85]. Third, the methanogenic flora in North Indian IBS patients measured *via* lactose hydrogen breath test was lower compared to controls and this observation was suggested to be the nature of flatulence among them[86].

Apart from the suggested alteration in brain gut axis functions, colonic immunological changes such as chronic and low-grade immune activation are reported among IBS patients. The mediators released by these immune responses may have an impact on the functions of gut mucosal permeability and nerves, leading to the further closed interaction between the immune system and the brain gut axis and finally the observed IBS symptoms[87,88]. For example, post-infectious IBS is to address a phenomenon that previous enteritis may be followed with IBS symptoms, particularly the IBS-D seen months later[89]. Briefly, these patients have excessive numbers and increased activation of mucosal immune cells including mast cells and lymphocytes. In addition, releasing factors such as proteases, histamine, and prostanoids attenuate permeability and activate abnormal neural response, leading to abdominal pain and changed bowel habits, which correlate well with IBS symptoms[88,89]. In addition, psychological stress and activation of Toll-like receptors are also involved in the neuroimmune response among these subjects[56,57]. Besides, antibiotic therapy reduced stress induced visceral hypersensitivity, enhanced bacterial wall adherence and increased luminal s-IgA levels in dysbiotic mice[90]. Considered together, emotional stress, gut microbiota and host immune system interact with each other to respond with altered bowel motor, sensory and secretory functions observed among IBS subjects.

Food

The experience of certain food ingestion and its following abdominal symptoms are common among the population. For example, acute chili ingestion aggravated abdominal pain and burning symptoms of FGID subjects[91]. Regarding the self-reported food elicited bowel symptoms of IBS subjects, most of them believed that certain diets such as beans, apple, flour, and plum could trigger bowel symptoms, particularly those foods rich in carbohydrates, fat, and biogenic amines such as milk, wine and pork, while women reported more intolerable food items than men[92]. On the other hand, an objective study indicated that IBS patients did not consume different food calories and constituents, but they usually tried to avoid diets rich in fermentable oligo-, di-, monosaccharides, and polyols (FODMAP), and their diets often contained low contents of calcium, magnesium, phosphorus, vitamin B2 and vitamin A[93]. Regarding the relationship between ingested food and gut microbiota composition, a recent study observed that IBS subjects consuming a restriction diet with a lower content of fermentable short-chain carbohydrates for 4 wk had adequate relief of bowel symptoms, while the concentration and proportion of luminal bifidobacteria were diminished together[94]. In summary, food owing to its certain components seems to be a factor leading to IBS, but the food intolerance of IBS subjects does not mean food allergy.

Abuse and separation

Childhood abuses including sexual issue are the significant worldwide health burden. For example, abuse has been a main risk factor leading to health problems including shaken baby syndrome and behavioral regression during the developmental period, while its long-term risks consist of mental health disorders, substance use disorders and chronic physical complains in the later adult life[95]. Unfortunately, both physical and sexual abuses are common and underestimated among IBS patients[96]. In addition, these victims often manifest severe pain perception, psychological distress, and poorer health outcome[97]. Their perceptive pattern was already centrally confirmed *via* advanced neuroimage to show an enhanced nociception[98].

Early life trauma is able to increase future visceral pain perception. Accordingly, maternally separated neonatal rodents are used to create a model to study the

Table 1 Potential drugs and measures to treat irritable bowel syndrome

Category	Functions	Examples
Antispasmodics	Antagonists of muscarinic receptors and calcium channels of smooth muscle	Cimetropium bromide, dicyclomine, hyoscine butylbromide, mebeverine, otilonium bromide, peppermint oil, pinaverium bromide, trimebutine maleate
Antidiarrheals	Agonists of μ-opioid receptors	Loperamide
Laxatives	Osmotic, stimulant	Bisacodyl, lactulose, magnesium citrate, magnesium sulfate, polyethylene glycol
Bulking agents	Water binding to increase stool bulk	Methylcellulose, psyllium, wheat bran
Receptor targeted new drugs	Agonists and antagonists of 5-HT	Alosetron, cilansetron, naronapride, prucalopride, ramosetron, tegaserod
	Chloride channel activators	Lubiprostone
	Agonists of GC-C	Linaclotide
	Antagonists of NK_1 receptors	Ezlopitant, TAK 637
	Agonists of κ-opioid receptors	Asimadoline
	Agonists of α2 adrenergic receptors	AGN-203818, clonidine, solabegron
	Antagonists of CCK_1 receptors	Loxiglumide
	Agonists of somatostatin receptors	Octreotide
Psychiatrics	Tricyclic antidepressants	Amitriptyline, desipramine, doxepin, imipramine, trimipramine
	SSRIs	Citalopram, fluoxetine, paroxetine, venlafaxine
	Psychotherapy	Biofeedback, cognitive behavioral therapy, dynamic psychotherapy, hypnotherapy, relaxation training
Probiotics	To balance gut microbiota	VSL-3, lactobacilli, bifidobacteriae
Fecal transplantation	Living microbiota supplement	Through nasogastric tube, enema or colonoscopy
Anti-inflammation	Mast cell stabilizers, PAR-2 blockers TRPV receptor type 1 and 4 blockers	Capsazepine, GB88, ketotifen, RN1734
Antibiotics	To inhibit gut microorganisms	Neomycin, rifaximin
Miscellaneous	Antinociceptive substance	Melatonin
	Bile acid sequestrant	Cholestyramine
	To diminish inflammation? To absorb bacteria and enterotoxins?	Diosmectite
Food	To enhance immunity?	Kiwifruit
Complementary and alternative medicine	Mysterious	Acupuncture, aromatic therapy, ginger, herb drugs, holistic medicine, homeopathy, massage, reflexology

5-HT: 5-hydroxytryptamine; CCK: Cholecystokinin; GC-C: Guanylate cyclase C; NK: Neurokinin; PAR: Protease-activated receptor; SSRIs: Selective 5-hydroxytryptamine re-uptake inhibitors; TRPV: Transient receptor potential vanilloid.

relationship between early life stress, visceral sensation and depression related disorders including IBS. It was indicated that water avoidance stress increased pain perception and activated somatosensory cortex, periaquaductal gray and hippocampus in the maternally separated rats[99]. In addition, maternally separated rats had significantly increased 5-HT content after colorectal distension[100]. This model also pointed out that the colon of maternally separated rats had elevated circulating levels of interleukin-6 in addition to gut dysfunction[101]. Considered together, neonatal maternal separation appears a stress in rats with exacerbated neurochemical, inflammatory responses, and visceral hyperalgesia in the colon and CNS comparable to IBS subjects. It is of interest whether the neonatal separation story does truly happen in the society leading to IBS. A study to explore the childhood events among IBS adults confirmed that loss and separation during childhood, in the current family and conflicted or dependent maternal relationships were common among some IBS patients[102]. In summary, avoidance of any kind of childhood abuses is necessary to demolish future adult onset of IBS, FGIDs and psychiatric events.

TREATMENT OF IBS

With regard to IBS treatment, patient-centered approach with a strong and effective communication between patients and clinicians has been emphasized to increase the treatment satisfaction and diminish utilization of health care sources[23,103]. In fact, the development of active drugs to exhibit an efficacy greater than placebo in treating heterogeneous IBS is not easily to achieve, because IBS subjects often experience an excellent efficiency up to 40%-50% to placebo treatment[23,104]. Psychologically, placebo effect is believed the total response of treating expectancy, repetitive administration named conditioning and a non-specific psychological effect supported from givers. Now the placebo effect could be well confirmed in the brain *via* functional neuroimage[54]. Table 1 summarizes the multidisciplinary approaches that are optional to treat IBS.

Antispasmodics

Antispasmodics that can block muscarinic receptors and calcium channels of gut smooth muscle cells have been the oldest drugs to treat IBS for decades because of disturbed bowel motility and its effect on abdominal pain are commonly observed among these patients[1,23,34,105,106]. Unfortunately, their effectiveness and recommended evidence are not fair owing to the trial drawbacks including different IBS definitions, limited case number, inappropriate end-points, evaluation methods, dosing, duration, side effect recording, *etc.*[3,23,106]. Apart from hyoscine butylbromide, the only available antispasmodic in United

States, other marketed antispasmodics include dicyclomine, mebeverine, pinaverium, otilonium bromide, peppermint oil, trimebutine maleate, *etc*.[1,2,23,39,106-108]. Overall, a meta-analysis indicated that antispasmodics are beneficial for IBS patients when abdominal pain is the predominant symptom of subjects attempted to treat[109]. Based on their long-term marketing, antispasmodics remain the first-line drugs to treat IBS but their probable anticholinergic side effects are best to warn before the prescription.

Antidiarrheals, laxatives and bulking agents

Disordered defecation has been another concern of IBS subjects and normalization of defecation *via* various approaches such as antidiarrheals for IBS-D and laxatives or bulking agents for IBS-C is recommended[1,23,106]. Regarding the IBS-D treatment using loperamide, it is a synthetic opiate derivative with an agonistic effect on μ-opioid receptors but scant opioid CNS effects. Its antidiarrheal effect comes from directly simulating gut water absorption and is further augmented by an antisecretory activity mediated by calmodulin antagonism, a property not shared by other opioids[110]. Loperamide appears the only antidiarrheal recommended to treat IBS-D during the acute or chronic diarrhea[1,7,106,110,111]. Earlier trials already supported its efficacy over placebo in treating stool consistency, urgency, borborygmi and abdominal pain[112,113]. However, a meta-analysis pointed out that it seems to reduce diarrhea but does not relieve abdominal pain among IBS subjects[109].

Laxatives have long been recommended to treat the constipation concern of IBS-C subjects[1,23,106]. Surprisingly, laxatives are not well evaluated whether they do have effectiveness in treating IBS-C, because most clinical experiences are adopted from those of functional constipation treatments. Only a small-scaled study pointed out that polyethylene glycol *vs* placebo improved stool frequency but not ameliorated abdominal pain among IBS-C subjects[114]. Until now, the evidence to recommend laxatives in treating IBS-C remains controversial[23,106].

Bulking agents including natural and artificial fibers are also recommended to treat constipated subjects including IBS patients. Basically, unabsorbed soluble agents such as psyllium and polycarbophil are dissolved and fermented in colon water to form a gel in turn to shorten colon transit time and to stimulate defecation, whereas insoluble agents such as corn fiber and wheat bran have limited change in gut, but they increase fecal mass to help defecation[115]. Reported trials indicated a limited benefit for constipation and no effect to attenuate other IBS symptoms[116]. Furthermore, a meta-analysis did not support its efficacy in treating IBS symptoms including stool frequency, abdominal pain and bloating[109]. According to the types of bulking agents, another meta-analysis pointed out that soluble fibers improve global symptoms, whereas insoluble fibers even exacerbate the clinical outcome[23,115]. As fermentable substances, the commonly reported side effects of bulking agents such as bloating, abdominal distension and flatulence are best to inform before the prescription[23].

Receptor targeted drugs

Since the end of last century, new drugs targeting receptors known to have pharmacological effects on IBS have been emerging. Of them, 5-HT related drugs including agonists and antagonists are most promising because their efficacies over placebo were critically evaluated based on the high quality controlled trials and finally approved by the authorities[23,106]. For example, IBS-D can be treated using alosetron and cilansetron which have antagonistic activity on 5-HT$_3$ receptors to delay bowel transit, reduce colonic tone and HAPC, blunt gastrocolic reflex and decrease visceral sensation, particularly with obvious therapeutic effect among female patients[7,23,117,118]. Nevertheless, this group should be used with very caution because of the possibility of serious side effects including severe constipation and ischemic colitis. Now they are only restricted to female IBS-D patients when conventional therapies have failed[7,23,106]. Ramosetron is another potent and selective 5-HT$_3$ receptor antagonist that can attenuate abnormal colonic function and abdominal pain in experimental animals. Clinical studies conducted in East Asia confirmed its benefits on abdominal pain/discomfort and bowel habits in both male and female IBS-D patients, but it also had side effect of hard stool. Until now, no ischemic colitis was reported based on a small number of cases exposed to it[23,119].

Regarding IBS-C treatment, tegarserod and prucalopride showed an agonistic activity on 5-HT$_4$ receptor-mediated release of 5-HT from mucosal enterochromaffin cells, which promotes ascending excitatory contraction and descending inhibitory relaxation to enhance bowel motility through a series of chain reactions. Apart from attenuating visceral hypersensitivity, these agonists owing to different affinities with 5-HT$_4$ receptors may account for variable prokinetic potentials and side effects[120-123]. Clinically, 5-HT$_4$ agonists diminish bloating and abdominal pain/discomfort with the improved satisfaction to defecation concerns such as stool consistency and straining[23,106,124]. Unfortunately, tegaserod was withdrawn due to serious cardiovascular adverse events. It is indicated that nonselective 5-HT$_4$ agonists such as cisapride and tegaserod may interact with human ether-à-go-go related cardiac potassium channels to have the chance of causing heart arrhythmia, whereas selective 5-HT$_4$ agonists such as prucalopride and naronapride are believed to have cardiovascular safety[123]. Tegaserod was reintroduced in United States in 2007 under a limited and restricted using for women younger than 55 years and not at risk for cardiovascular events[23,123]. It remains uncertain whether prucalopride can effectively treat IBS-C as tegaserod, although its efficacy was confirmed among functional constipation subjects[106]. Renzapride is a substance to own both activities of 5-HT$_4$ agonist and 5-HT$_3$ antagonist, and its development for IBS-C patients was halted because of the disappointing limited effects in a phase Ⅲ trial[23].

Lubiprostone is a newly approved drug available in United States, United Kingdom and Japan to treat constipated subjects including IBS patients. It is a synthetic bicyclic fatty acid derivative of prostaglandin E1 with the ability to stimulate cystic fibrosis transmembrane conductance regulator (CFTR) dependent chloride channels of enterocytes to increase small intestinal secretion of fluid, mucin and electrolytes and finally to improve bowel functions including defecation[23,46,125]. Lubiprostone is safe and effective to treat constipated subjects, but it has some side effects, with nausea being the most common, followed by diarrhea, abdominal pain, bloating, and even the very rare events of dyspnea and ischemic colitis[23,126,127].

Similarly, linaclotide was marketed in United States and Europe to treat severe constipated patients including IBS patients in 2012[128]. It is a synthetic 14-amino-acid peptide of guanylate cyclase C (GC-C) agonist mainly to increase intestinal fluid secretion and gut transit. Unlike lubiprostone, linaclotide first activates GC-C receptors on the luminal surface of enterocytes to enhance intra- and extracellular levels of cyclic guanosine monophosphate and in turn promote CFTR to secrete chloride and bicarbonate into gut lumen to improve defecation. Interestingly, the activation of GC-C receptors also diminishes visceral pain[23,128,129]. Clinically, linaclotide improves abdominal pain/discomfort, bloating and the defecated symptoms of straining, incomplete defecation and stool consistency of IBS-C patients. Meta-analyses confirmed its superior efficacy over placebo to treat IBS-C and functional constipation[128,130,131]. The most common side effect of linaclotide has been severe diarrhea (20%), thus subjects with a tendency to water and electrolyte imbalance are not indicated. Until now, its long-term safety has not been established yet[128,129,131].

Currently, many new drugs targeting the specific receptors responsible for motility, visceral sensation, gut secretion, neuroimmune and brain-gut axis are being developed to treat IBS. Basically, the key factors in terms of clear mechanisms involving whole pathophysiology, good oral bioavailablity, no CYP dependent metabolism, best once daily, least interaction with food and other drugs, no unwanted metabolites, long-term maintenance ability, good safety records and so forth may determine whether these new drugs can be accepted to treat IBS[132]. Because too many new drugs are under development, only a few examples are briefly introduced here. First, TAK 637 is a selective antagonist of smooth muscle neurokinin 1 receptors that activate intestinal muscle contraction. This agent reduced rabbit abdominal contractions induced by colorectal distension *via* inhibition of neurokinin 1 receptors, mainly located in the spinal cord, and it also reduced colonic transit and defecation in a Mongolian gerbil IBS model. Unfortunately, its development was halted because of serious side events that occurred in two animal species[133]. Second, opioid kappa receptors are located on the cholinergic terminals of ENS with the ability to inhibit cholinergic transmission and gut motility. Asimadoline, an agonist of these receptors, reduces gut wall neurotransmitter releasing to exhibit both analgesic and anti-diarrheal effects[7,132,134]. A recent phase Ⅲ trial on IBS-D patients observed excellent results to treat pain and defecation related concerns such as frequency, urgency and bloating[134]. Third, clonidine initially used to treat hypertension with the commonly reported constipation side effect is a α2 adrenergic receptor agonist. It increased colonic and rectal compliance, and reduced tone, pain, gas sensation and rectal urgency of healthy subjects[135]. A trial also indicated its effect on IBS-D patients with reduced abdominal pain, satisfactory relief of global IBS symptoms and improved disturbed defecation in spite of side effects of drowsiness, dizziness and dry mouth[132,136]. Owing to the obvious CNS effects, clonidine is apparently unable to treat IBS. Other adrenergic agonists such as AGN-203818 and solabegron with the purpose to treat IBS are undergoing evaluation[23].

Psychiatric approaches

Severe and intractable IBS patients who fail conventional therapy may consider the psychiatric approaches such as anxiolytic agents, antidepressants, cognitive behavioral therapy, dynamic psychotherapy and even hypnotherapy[1,23,106,137]. According to the recommendations, antidepressants are only indicated when abdominal pain is the main concern. Its benefits are likely the central antinociceptive effect plus bowel effect[23,106]. When treating IBS patients using either tricyclic antidepressants (TCAs) or selective 5-hydroxytryptamine re-uptake inhibitors (SSRIs), their symptomatic subtypes should be considered. For example, SSRIs such as paroxetine decrease orocecal and whole gut transit times in IBS-C patients. In contrast, TCAs such as imipramine prolong orocecal and whole gut transit times in IBS-D patients[138]. Meta-analyses indicated that IBS global symptoms are improved using both TCAs and SSRIs no matter its subtypes while SSRIs are more tolerable than TCAs owing to their obvious prokinetic effect, but their long-term safety remains unknown[23,106]. Other psychiatric measures are also recommended to treat intractable IBS. Overall, the drawbacks of these non-drug approaches include expert dependence, being unable to have blinding studies, methodological deviation and scant clinical experiences among most gastroenterologists. Nevertheless, experts recommended its good global symptom improvement and less adverse events[1,23]. It may be employed to severe and intractable subjects when all available and conventional treatments have failed.

Probiotics and antibiotics

Since an abnormal composition of gut microbiota exists among IBS patients, modification of gut microbiota components through exogenous supplement or inhibition of them using antibiotics appears promising to treat IBS patients[81,139]. Probiotics prepared as empiric base of "immune-boosting and health-enhancing" for century are live microbial supplements in attempt to improve gut microbial balance[81,140]. Pharmacologically, the benefits

of probiotics consisting of anti-pathogenic ability *via* secretion of bacteriocins, acidification of the colon by fermentation, anti-inflammation to protect gut mucosa, alteration of mucosal response to stress, barrier enhancement, immune-modulating effects, and inhibition of visceral hypersensitivity justify their use to treat IBS[141,142]. Unfortunately, the worldwide probiotic preparations are not standardized. The most commonly used strains and species include *Streptococcus thermophilus, Lactobacillus rhamnosus Lc705, Bifidobacteria, Lactobacillus rhamnosus GG, L., Bifidobacterium animalis ssp., Lactis Bb12,* and non-pathogenic yeasts such as *Saccharomyces boulardii*. However, no two preparations are the same and the extrapolation of therapeutic responses from one to another may be problematic[23,142,143]. It was indicated that probiotic cocktail had potent anti-inflammatory properties of suppressing mucosal inflammation and restoring cytokine balance[143]. Overall, probiotics are safe without serious side effects but the benefit magnitude and the most effective species or strains are undetermined. Multi-species preparations are probable the best to treat IBS[23,84,143-145].

Live fecal microbiota transplantation is an incredible approach to treat various bowel diseases including inflammatory bowel disease, *Clostridium difficile* infection and even IBS. The fecal content can be administered *via* nasogastric tube, enema and colonoscopy[146]. Limited data indicated that constipated patients treated with colonoscopically delivered fecal microbiota had immediately improved defecation, bloating and abdominal pain[147]. It is unknown whether it is applicable to IBS-C subjects. Apart from microorganism supplement, new drugs targeting colonic low-grade inflammation are being developed, *e.g.,* mast cell stabilizer, transient receptor potential vanilloid receptor type 1 and 4 blockers, protease-activated receptor 2 blockers, *etc.* It appears too early to predict their chance of success[7,132].

Antibiotics provide another route to treat imbalanced gut microbiota. For example, rifaximin has been proved in several non-diarrhea IBS controlled trials to improve global symptoms, abdominal pain, dysfunctional defecation and bloating[23,148,149]. Regarding IBS-C patients, neomycin treatment improved global symptoms and constipation. The success of this treatment depended upon the presence and post-treatment elimination of methane[150]. Owing to the chronic and recurrent nature of IBS, the effectiveness and safety of long-term or repeated use of antibiotics to treat IBS remain controversial.

Food therapy

Food restricted approaches such as avoidance of FODMAP items and individual evaluation of the effects of protein-, fat- and carbohydrate-rich/poor diets are recommended to reduce some IBS symptoms[84,93,94]. Likewise, a fermentable short-chain carbohydrates restricted diet significantly improved IBS symptoms of United Kingdom patients[94]. In contrast, another study indicated that dietary manipulation of poorly absorbed short-chain carbohydrates increased total amount of gut gas including hydrogen production to exaggerate the bowel symptoms of Australia IBS patients, thus avoidance of this food constituent is recommended[151]. Food elimination towards IgG antibodies in certain IBS patients effectively reduced bowel symptoms[152,153]. Interestingly, kiwifruit is a natural remedy to own laxative ability, particularly among the elderly population[154]. A study found that 4-wk kiwifruit consumption diminished colon transit time, increased defecation frequency, and finally improved the bowel function of IBS-C subjects[155]. Since kiwifruit may support the immune function to reduce the occurrence and severity of flu-like illness, it is unknown whether its efficacy to treat IBS is relevant to enhanced gut immunity[156]. Overall, the restriction of certain diets may be recommended to all IBS patients, but the routine use of food restriction or supplement without an appropriate drug therapy may not be perfect.

Miscellaneous agents

The intensity of pain perception is usually lower during the night dark hours when blood melatonin level is higher. Consequently, melatonin is considered an antinociceptive substance with the mechanisms broadly involving opioid, benzodiazepine, α(1)- and α(2)-adrenergic, serotonergic, cholinergic and melatonergic (1) and (2) receptors[157]. A short-term oral melatonin treatment improved abdominal pain, distension and abnormal defecation sensation in female IBS patients, whereas the defecation frequency and stool consistency were not affected[158]. Bile acid malabsorption is common among chronic diarrhea subjects and even IBS-D patients. A meta-analysis indicated that this event might be underestimated since about a third of IBS-D patients had moderate to severe bile acid malabsorption[159]. This may explain why cholestyramine is recommended to treat IBS-D patients[1,160]. Mesalazine was observed to reduce the number of mast cells and the subsequent release of mediators and diminish gut permeability and sensitivity in IBS patients, thus a large-scale mesalazine trial is undergoing in an attempt to know whether it can treat IBS-D patients. The final results are expected toward the end of 2013[161]. Diosmectite is inorganic aluminomagnesium silicate clay with a strong adsorbent ability. It is used to treat acute watery diarrhea based on the suggested mechanisms to diminish inflammation and mucolysis, to modify mucus rheologic and to adsorb bacteria, enterotoxins, viruses and other potentially diarrheogenic substances[162]. With regard to IBS treatment, diosmectite diminished abdominal pain and bloating intensity in IBS-D patients, but its effect on the disturbed defecation was not observed[163].

Complementary and alternative medicines

Traditionally, complementary and alternative medicines (CAM) is a medical practice not belonging to the current conventional medicine with therapeutic effects determined by the cultural, ethnic, social, religion, education and economic backgrounds. The CAM theories are markedly deviated from the conventional medicine in terms of

heterogeneity, disease mechanisms, diagnostic approaches, therapeutic measures, judging efficacies, *etc.*[164,165]. Now herb drugs based on Chinese, Indian, Ayurvedic, and Tibetan preparations, acupuncture, aloes, aromatic therapy, ginger, homeopathy, probiotics, peppermint oil, reflexology, massage, colon irrigation, holistic medicine, aromatherapy, Qi gong, bioelectromagnetic field therapy, *etc.* are categorized as CAM[166]. Interestingly, certain CAM members have been acknowledged by the conventional medicine to treat IBS, *e.g.*, probiotics and peppermint oil.

Clinically, many IBS patients do seek CAM before they encounter clinicians[167]. Herb drugs are the most often used but their effects are conflicting. In fact, the therapeutic effects of herb drugs are very hard to evaluate and compare each other since they are criticized with the drawbacks of mixture of variable botanical components, neither purified nor quality control, lack of preclinical animal study, unique preparation as family secret, publication bias, no reported adverse events, absent negative reports, *etc.*[23,168]. For instance, a trial conducted on Australian Caucasians with IBS indicated the very promising effect over placebo in relieving bowel symptoms even after discontinuation[168]. In contrast, herb mixture to treat Chinese IBS patients residing in Hong Kong did not reveal any benefits judged by global symptom and individual bowel symptoms[169]. It is unknown whether certain herb drugs claimed effective to treat IBS have the true pharmacological effect or just enhanced placebo response.

Acupuncture is a well-known old Chinese traditional medicine. Basically, it exhibits the physiological impacts on neural, humoral, opioid and serotonegic pathways with the effects of normalized motility, inhibited acid output, antinociceptive effect, reduced rectal hypersensitivity and altered 5-HT functions[170-172]. Acupuncture looks promising to treat FGIDs including IBS. Apart from Chinese studies, the effects of acupuncture to treat IBS among Western people are conflicting. For example, 10 weekly acupuncture sessions compared to placebo procedure for United Kingdom IBS patients reduced their symptomatic severity and its efficacy even persisted over a 1-year period[173]. Another study using 3-wk true acupuncture and cross-over with another 3-wk sham procedure conducted on United States IBS patients did not support its superiority over sham procedure to treat the global symptom and symptomatic intensities[174]. Overall, meta-analyses repeatedly indicated that acupuncture has no effect to the general wellbeing, individual bowel symptoms and QoL of IBS patients[175-177]. Finally, NICE guidance also does not recommend using acupuncture to treat IBS[178].

Homeopathy is popular among the CAM. Unlike conventional medicine, it means that "a substance is capable of inducing a series of symptoms in a healthy living system, and low doses of the same substance can cure these symptoms under certain circumstances"[179]. Homeopathy is claimed effectively to treat IBS. Now a three-arm trial based on 5 sessions of true homeopathic treatment plus usual care *vs* placebo-homeopathy plus usual care *vs* usual care alone is undergoing in United Kingdom and the final result is expected to resolve whether homeopathy is truly effective to treat IBS[180]. Regarding IBS patients who failed all conventional treatments, CAM may be considered as a supplement or alternative with expected efficacy equal to enhanced placebo effect if they do not have any intolerable or serious side effects.

CONCLUSION

Current Rome Ⅲ criteria-based diagnosis of IBS remains to have limitations, particularly the differentiation from constipation. It probably needs the resolution of coming new criteria. Since IBS is heterogeneous based on the multidimensional pathogeneses, using biopsychosocial dysfunction is effectively to integrate all old and emerging IBS pathogeneses in terms of gut dysmotility, abnormal gut water secretion and gas accumulation, visceral hypersensitivity, impaired mucosal permeability, dysfunctional brain-gut axis, genetic abnormalities, disturbed gut microbiota and immune system, psychological disturbances, impacts from food and various abuses, *etc.* Now multidisciplinary approaches using drugs with different mechanisms of action, imposing psychiatric measures, giving probiotics and antibiotics, possessing diet therapy, and CAM treatment, can be considered individually to treat the major clinical symptoms and other associated concerns.

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Post-infectious irritable bowel syndrome: Mechanistic insights into chronic disturbances following enteric infection

Jennifer K Beatty, Amol Bhargava, Andre G Buret

Jennifer K Beatty, Amol Bhargava, Andre G Buret, Department of Biological Sciences, University of Calgary, Calgary, AB T2N 1N4, Canada
Author contributions: Beatty JK, Bhargava A and Buret AG wrote this paper.
Supported by Natural Sciences and Engineering Research Council of Canada (individual operating and CREATE)
Correspondence to: Andre G Buret, PhD, Professor, Department of Biological Sciences, University of Calgary, 2500 University Drive, NW, Calgary, AB T2N 1N4, Canada. aburet@ucalgary.ca
Telephone: +1-403-2202817 Fax: +1-403-2899311
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Abstract

Irritable bowel syndrome (IBS) is a commonly encountered chronic functional gastrointestinal (GI) disorder. Approximately 10% of IBS patients can trace the onset of their symptoms to a previous a bout of infectious dysentery. The appearance of new IBS symptoms following an infectious event is defined as post-infectious-IBS. Indeed, with the World Health Organization estimating between 2 and 4 billion cases annually, infectious diarrheal disease represents an incredible international healthcare burden. Additionally, compounding evidence suggests many commonly encountered enteropathogens as unique triggers behind IBS symptom generation and underlying pathophysiological features. A growing body of work provides evidence supporting a role for pathogen-mediated modifications in the resident intestinal microbiota, epithelial barrier integrity, effector cell functions, and innate and adaptive immune features, all proposed physiological manifestations that can underlie GI abnormalities in IBS. Enteric pathogens must employ a vast array of machinery to evade host protective immune mechanisms, and illicit successful infections. Consequently, the impact of infectious events on host physiology can be multidimensional in terms of anatomical location, functional scope, and duration. This review offers a unique discussion of the mechanisms employed by many commonly encountered enteric pathogens that cause acute disease, but may also lead to the establishment of chronic GI dysfunction compatible with IBS.

Key words: Post-infectious irritable bowel syndrome; Infectious diarrhea; Enteric pathogen; Inflammatory disorders; Immune alterations

Core tip: This review discusses the long-term consequences of acute enteric infections that may serve to trigger post-infectious irritable bowel syndrome, a routinely diagnosed disorder. This unique discussion elucidates novel initiation mechanisms, underlying pathophysiological features of post-infectious irritable bowel syndrome, employed by commonly encountered enteric pathogens.

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INTRODUCTION

Irritable bowel syndrome (IBS) is among the most commonly encountered chronic functional gastrointestinal (GI) disorders afflicting individuals in westernized nations. Based on the Rome Ⅲ criteria abdominal pain accompanied by sustained changes in bowel habit constitute IBS, whose diagnosis is achieved in the absence of

biochemical markers of disease[1]. Clinical presentation of constipation, diarrhea, or a combination, constitutes the different subtypes of IBS: IBS with constipation (IBS-C), diarrheal IBS subtype (IBS-D), mixed IBS (IBS-M), respectively[2]. Often perceived as a female-dominant disorder, IBS is thought to afflict between 5%-10% of the population[3], especially in westernized nations. Elucidating the mechanisms underlying the typical multifaceted clinical presentation of IBS is a topic of considerable research efforts in the medical community[4]. A growing body of evidence implicates numerous triggering events in contributing to IBS pathophysiology, including an initiating bout of infectious enteritis, low grade inflammation, altered functionalities in GI cell types, increases in epithelial permeability, and alterations in the GI microbiota, although the precise mechanisms of underlying each remain obscure[2,5-8]. Approximately 10% of IBS patients believe that their symptoms began following a bout of infectious dysentery[6], leading to the coinage of the term; Post infectious (Pi)-IBS. While many enteric pathogens cause self-limiting, acute diarrheal disease, subsequent chronic physiological consequences may persist in some individuals[9]. Many commonly encountered enteric pathogens can produce physiological changes that may provide important initiation mechanisms underlying chronic GI conditions, such as Pi-IBS. This article critically reviews the evidence supporting a role for key physiological changes initiated during enteric infection, that may in turn be responsible for IBS symptom.

Pi-IBS

Based on the Rome criteria for diagnosis, any onset of new IBS symptoms subsequently following an infectious event is defined as Pi-IBS[6]. Pi-IBS cases often exhibit characteristics of the IBS-D, and can occur in 4%-31% of patients following acute gastroenteritis[6,10-12]. A large body of work provides evidence supporting a role for pathogen-mediated modifications in the resident intestinal microbiota, epithelial barrier integrity, enterochromaffin cell function, and innate immune features[5,13,14] in Pi-IBS manifestation. Any number of these pathogenic consequences have been reported following enteric infection incited by an array of pathogens such as *Shigella* spp., pathogenic *Escherichia coli*, *Salmonella*, *Campylobacter jejuni*, and *Giardia duodenalis*[14-18]. Enteric pathogens must employ a vast array of machinery to evade the host protective immune mechanisms, and illicit successful infections. Recent work identifying genetic mutations, namely in genes responsible for epithelial and innate immune functionalities, in patients experiencing both the post-infectious, and traditional forms of IBS, point to defects in innate immunity and epithelial homeostasis as an important risk factor for IBS susceptibility[19,20]. The impact of infectious events on host physiology can be multidimensional in terms of anatomical location, functional scope, and duration. Indeed, anatomical, immunological, and neurological dysfunctions, or combinations of such, have all been shown as risk factors determining Pi-IBS manifestation. This review will provide an in-depth discussion surrounding the potential roles in which a variety of commonly encountered enteric pathogens may play in initiating important pathophysiological features of Pi-IBS.

CLINICAL PRESENTATIONS OF IBS FOLLOWING ENTERIC INFECTION: ALTERED INTESTINAL MOTILITY AND HYPERSENSITIVITY

Abnormal bowel habits and abdominal hypersensitivity, or reduced threshold of pain, are the hallmark clinical signs of IBS. The classification of IBS as a functional disorder stems from a lack of determinant histopathological, or structural biomarkers in afflicted patients. The Rome criteria requires the incidence of abdominal pain, accompanied by alterations in bowel habit for complete IBS diagnosis[21].

Altered intestinal motility

Abnormal GI motility is commonly associated with altered bowel habits producing diarrheal, constipation, and mixed IBS subtypes[22]. The potential for dysfunctional intestinal motility in contributing to altered bowel habits in IBS is supported by studies looking at intestinal transit rates between healthy and IBS individuals, with IBS-D subtypes exhibiting enhanced rates of SI transit, and the opposite trend observed for IBS-C patients[22,23]. Moreover, a recent report demonstrated that the normal colorectal reflex (normal increase in rectal tone in response to phasic colonic distention) was largely abolished in IBS patients, regardless of bowel habit, providing some evidence for altered colonic motility in these individuals[24]. Interestingly, muscle hypercontractility and abnormal motility patterns are observed subsequent to *Trichinella spiralis* infection in a commonly used murine model of PI-IBS[13,25-27], suggesting that persistent dysfunctional intestinal motility can be incited following an acute infection.

Abdominal hypersensitivity

Lower thresholds for pain tolerance in IBS patients have been documented along the entire length of the GI tract[22], an effect that is thought to occur in upwards of 60% of afflicted individuals[7]. Hypersensitivity often occurs locally in response to colonic distention[7]. Furthermore, overall visceral hypersensitivity, even upon brief stimuli such as the ingestion of food, is well documented in IBS patients, and may contribute to additional bloating, nausea, and urgency symptoms[8,28].

Stressful events can drastically affect the processing of visceral stimuli[29,30] and result in dysfunctional central neural processes culminating into heightened pain perception. Injury to visceral afferents, for example, is a common cause underlying visceral hypersensitivity[7]. Studies using a rat model of TNBS-induced transient colonic inflammation have highlighted that persistent tis-

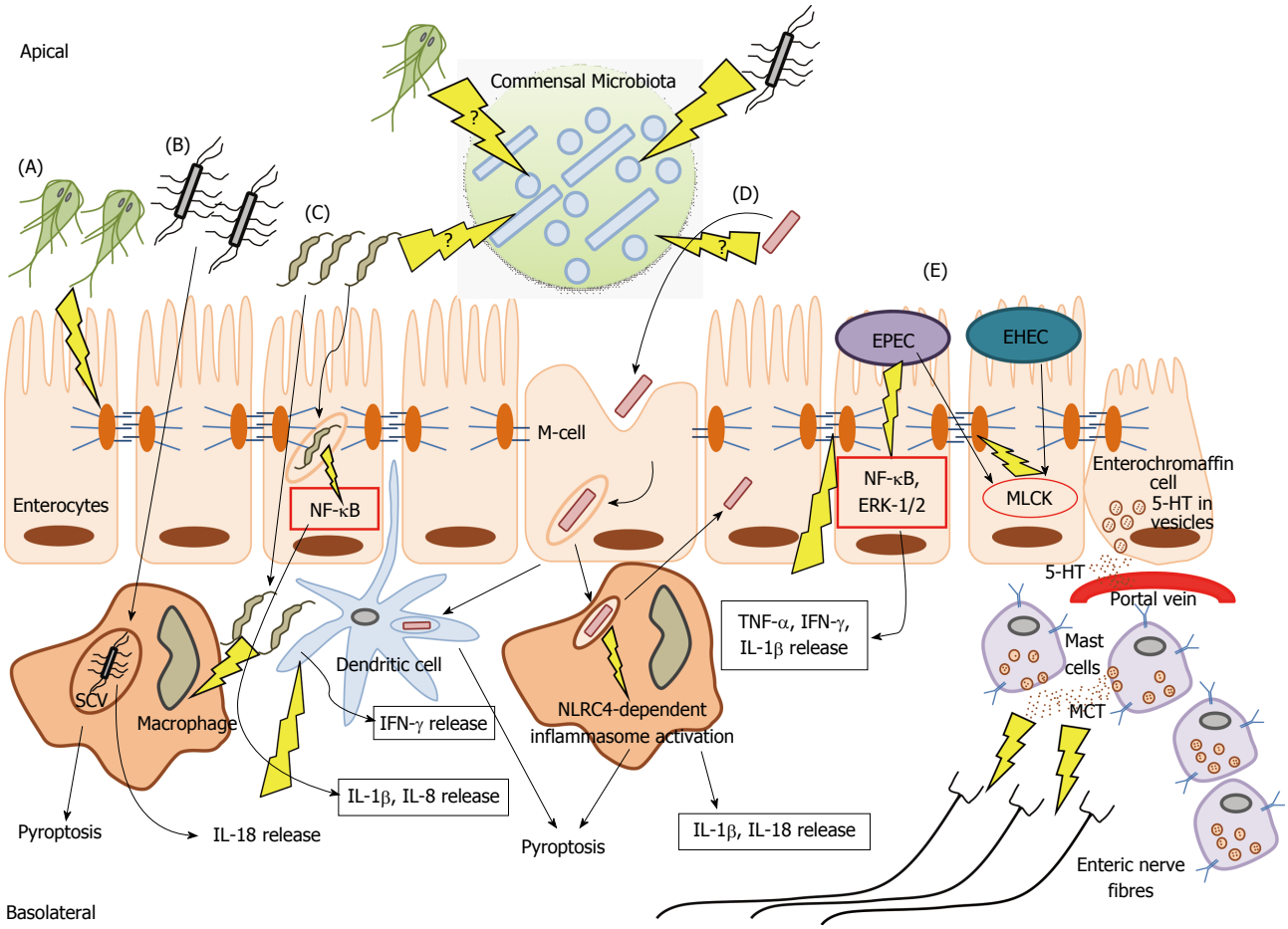

Figure 1 Illustration representing the interaction of several pathogens with the intestinal epithelium and resident immune cells, and their contribution to the development of post-infectious-irritable bowel syndrome. A: *Giardia duodenalis* disrupts tight junctional proteins in the epithelium, in addition to resulting in a decrease in 5-HT-producing enterochromaffin cells; B: *Salmonella* enterica serovar *Typhimurium* invades enterocytes and makes its way to resident macrophages, where upon being phagocytosed it causes interleukin (IL)-18 release, which further stimulates interferon (IFN)-γ release from nearby immune cells, *i.e.*, lamina propria dendritic cells, and macrophage pyroptosis. This pathogen is also able to disrupt the resident microbiota; C: *Campylobacter* jejuni causes disruptions in TLR9 signaling to make epithelial cells more susceptible (would sensitive apply here instead of susceptible?) even to mild pro-inflammatory cytokines. It also activates the NF-κB pathway to result in an IL-1β and IL-8 release. *C. jejuni* has also been shown, particularly in cases of post infectious (Pi)-IBS, to cause a reduction of resident $CD68^+$ macrophages; D: *Shigella flexneri* crosses the epithelium through the M cell and is taken up resident macrophages, where it causes IL-1β and IL-18 release, and pyroptosis in these macrophages. *S. flexneri* has also been associated with increased number of mast cells, secretions of which MCT can activate the enteric nervous system; E: EPEC results in TNF-α, IFN-γ, and IL-1β release *via* NF-κB and ERK-1/2 activation. Both EPEC and EHEC result in MLCK-dependent tight junctional disruption. Intriguingly, *G. duodenalis* (A) and *C. jejuni* (C) have been implicated in the modification of the intestinal microbiota; however, the effects of this modification remain unclear[90]. A variety of combinations of these factors may contribute to the pathogenesis of PI-IBS. MCT: Mass cell tryptase.

sue injury may directly produce heightened visceral pain perception[31]. Importantly, chemically induced colonic inflammation models have stark parallels to many of the physiological events accompanying enteric infections. Initial processes of inflammation, for instance, may act to first sensitize effector, neuronal, and immune cells within the GI tract.

Interestingly, many of the physiological consequences that can result from infectious events within the GI tract have also been proposed as determinants capable of contributing to abnormal motility and hypersensitivity symptoms seen in IBS patients. The major mechanisms currently thought to underlie IBS pathogenesis, and the evidence surrounding possible contributions made to each by distinct enteric pathogens, will be discussed in the following sections (Figure 1).

PATHOPHYSIOLOGICAL FEATURES OF IBS FOLLOWING ENTERIC INFECTION

Immune system alterations

Accumulating evidence suggests subtle alterations in the immune system in both the gut, and peripheral circulation of PI-IBS patients[32]. Pathogen-mediated disruptions of the mucosal barrier have the ability to allow for persistent immune activation within the intestine, largely due to increased exposure to luminal antigens. Likewise, the host inflammatory response towards perceived pathogens, while meant to be protective, may result in detrimental, perpetuated activation of effector cells and inflammatory mediators. The incidence of PI-IBS symptoms in many patients following enteric infection has fuelled interest in looking at persistent immune infiltrate, and/or altered

immune functionalities, as plausible driving forces in the generation of IBS symptoms[33].

Mast cells/macrophages/dendritic cells: Certain enteric pathogens have been shown to promote mast cell accumulation. A recent study found that a large proportion of patients experiencing Shigellosis, caused by invasive *Shigella* spp., go on to develop PI-IBS, and that this effect is accompanied by augmented mast cell numbers[34]. Under normal conditions, mucosal mast cells are highly involved in wound-healing, and defense against pathogens[5]. However, multiple reports document heightened numbers of mast cells within the small[35,36], and large intestines[37-39] of IBS patients. One study, which observed increased mast cells specifically within the duodenum of IBS patients suggested that infiltration of these cells may provide some explanation behind the observation that symptoms differ depending upon the affected site along the GI tract[36]. Also, mast cells can secrete serotonin, therefore increased populations of these cells may provide a link between cellular infiltrate and altered serotonin signaling leading to changes along the brain-gut axis, and dysmotility, characteristic of either IBS-D or IBS-C[36]. Furthermore, augmented numbers of mast cells, and particularly those closely associated with nerve fibers, have been reported in both IBS and Pi-IBS[38] (Figure 1), an effect which may be correlated with enhanced bloating and pain perception symptoms[2,40-42].

The *T. spiralis* mouse model of Pi-IBS has provided important insight into many pathophysiological changes following acute enteric infection. A recent study, for instance, documented numerical and phenotypic alterations in lamina propria dendritic cells (LPDC), following acute *T. spiralis* infection[43]. In what the authors defined as the "Pi-IBS stage" of infection, *i.e.*, no recovery of nematode in the stool, LPDCs exhibited enhanced expression of co-stimulatory molecules, and greater ability to migrate to and drive $CD4^+$ T cell proliferation[43]. Furthermore, the altered LPDC phenotype was proposed to underlie enhanced levels of pro-inflammatory interferon (IFN)-γ, IL-23 and tumor necrosis factor (TNF)-α production in the Pi-IBS stage[43]. The important role that these cells play in directing T-cell responses may have implications in promoting a low-grade inflammatory milieu, and requires further investigation in relation to IBS pathogenesis.

Monocytes and macrophages are at the forefront of initiating an inflammatory response to pathogens, in addition to providing essential directives to the adaptive immune system[5]. In Pi-IBS cases confirmed following *C. jejuni* infection the numbers of resident $CD68^+$ macrophages are diminished, perhaps owing to the cytotoxic nature of the pathogen inside host cells[9]. Likewise, *Shigella spp.*[15,16] and *Salmonella* infections have been implicated in causing Pi-IBS, and both are obligate intracellular pathogens, which preferentially exploit phagocytic machinery of the macrophage. Specifically, *Shigella* is transported into the lamina propria through M cells in the epithelium, and presented to resident macrophages and dendritic cells (DCs) for phagocytosis upon which activation of the nucleotide-binding oligomerization domain (NOD)-like receptor protein (NLRC4) inflammasome occurs[44,45] (Figure 1). Consequently, the resulting activation of pro-inflammatory cytokines, interleukin (IL)-18 and IL-1β, are thought to be major determinants of the high inflammatory conditions characteristic of early *Shigella* infection[45]. Inflammasome activation can also produce heightened rates of macrophage cell death *via* pyroptosis, which acts as an "inflammatory" form of programmed cell death (Figure 1). Thus, *Shigella* infection promotes a high status of inflammation, while simultaneously resulting in the detrimental loss of lamina propria (LP) macrophages. LP macrophages have an important regulatory, and anti-inflammatory role in maintaining intestinal homeostasis[45]. Furthermore, as a consequence of resident LP macrophage depletion, additional circulating monocytes may be recruited to the site of infection, and often differentiate into macrophages possessing a more pro-inflammatory capacity[45]. Considering ample reports documenting low-grade inflammation IBS patients[46,47], pathogen-mediated inflammatory conditions, in addition to the promotion of pro-inflammatory cell phenotypes, may be especially relevant triggers underlying Pi-IBS development.

In contrast to *Shigella*, *Salmonella* is seemingly less cytotoxic to macrophages[48], yet Pi-IBS symptoms have been reported following anywhere between 6%-32% of confirmed infections[2,19]. Following phagocytosis, *Salmonella* forms the characteristic Salmonella Containing Vacuole (SCV) in macrophages, in which it replicates while effectively evading host immune machinery, and pyroptosis[48] (Figure 1). While capable of avoiding certain immune parameters, *Salmonella* still evokes a strong IL-18 response[48] which has important implications in exerting paracrine effects on surrounding immune cells to induce IFN-γ expression, and also result in increased levels of activated T cells in the infected intestine, accumulation of which has been documented in many examinations of IBS[9,32,33,42,49].

Cytokine profiles: Substantial regulation exists within the GI tract in order to maintain a functional balance between pro- and anti-inflammatory mediators under homeostatic conditions. Engagement of the Toll-like receptors (TLRs), NOD-like receptors (NLRs), and other host pathogen-recognition-receptors (PRRs) occurs through ligation by various pathogen-associated-molecular-patterns (PAMPs). *Shigella*, for instance, is known to stimulate excess production of IL-1β from immune cells during infection *via* the NLRC4 inflammasome[44,45] (Figure 1). Also, excessive IL-8 secretion is a hallmark of *Campylobacter* pathogenesis[50], and is initiated upon host recognition of the pathogen-associated lipooligosaccharide[51]. Interestingly, a recent report demonstrated a disruption in TLR9 expression on epithelial cells to be implicated in the enhanced susceptibility to mild pro-inflammatory stimuli post-campylobacteriosis in mice[52]. *C. jejuni* is also know to promote the translocation of non-invasive commensal

bacteria *via* paracellular and transcellular pathways[53,54]. *Campylobacter* has also been shown to activate copious amounts of nuclear factor (NF)-κB and IL-1β from immune cells, *in vitro*[51]. Likewise, recognition of EPEC flagellin and endotoxin results in NF-κB and extracellular signal regulated kinase (ERK)-1/2 –driven IL-8 release, and enhanced TNF-α, IFN-γ and IL-1β in the infected mucosa[55,56] (Figure 1). Interestingly, at least some of the pro-inflammatory cytokines, including TNF-α, IL-1β, and IFN-γ may themselves disrupt the epithelial barrier through alterations of the tight junctions (TJs), and promote increased permeability[57-59]. Thus, residual pro-inflammatory infiltrate following enteric infection combined with the sub-epithelial penetration of commensal bacteria, can create extensive damage to surrounding intestinal tissues, and likely promote chronic pathophysiological consequences. Consequently, many reports have drawn links between altered cytokine profiles and IBS generation[60], and findings include increased levels of pro-inflammatory IL-6, IL-8, and TNF-α in plasma and circulating blood mononuclear secretions from IBS patients[47,61]. Lower detection of typical anti-inflammatory cytokines, IL-10 and transforming growth factor (TGF)-β, at the level of mRNA has also been reported[62]. Also, evidence from the *T. spiralis* Pi-IBS murine infection model has shown greater levels of IFN-γ, IL-23 and TNF-α produced by DCs in the Pi-IBS stage[43]. Additionally, sustained levels of pro-inflammatory mediators have been documented in a 21-d *Citrobacter rodentium* model of murine *E. coli* pathogenesis[63]. Regardless of these promising observations, the implications of pathogen-mediated alterations in normal cytokine profiles in providing sufficient trigger for IBS symptom establishment requires further investigation.

Mucosal barrier alterations

The intestinal epithelium provides an interface between the luminal space and the dynamic environment of the underlying subepithelial compartment. This physical barrier is intricately involved in regulating the controlled passage of vital nutrients, molecules, and water, *via* a semipermeable function maintained by TJs. TJs actively maintain the polarized characteristic of the epithelial barrier, and are composed of over 40 proteins consisting of occludin, junctional adhesion molecule (JAM), and claudins[64]. Patients with a history of infectious events experiencing Pi-IBS show drastic increases in permeability[65,66]. A prospective study, however, following a large waterborne outbreak of bacterial gastroenteritis, incited by mixed infection of EHEC O157:H7 and *C. jejuni*, documented increased permeability to be associated with IBS, regardless of whether symptoms were post-infectiously initiated[65]. Enterohemorrhagic *E. coli* (EHEC) is known to have deleterious impacts on the epithelial barrier through number of mechanisms, including TJ disruptions, and abnormal rates of intestinal epithelial cell (IEC) apoptosis[67,68]. These effects can be mediated directly *via* physical interaction through EHEC formation of characteristic attaching and effacing lesions (A/E lesions), and/or diffusely through toxin release[64,69]. EHEC, and its close relative: Enteropathogenic *E. coli* (EPEC), are known to hijack various pathways regulating the semi-permeable profile of TJs, and both have been shown to activate myosin light chain kinase (MLCK) to produce abnormally leaky barrier functionalities[70-72] (Figure 1). Additionally, *Giardia duodenalis*, a protozoan pathogen recently implicated in promoting Pi-IBS development[18,73], is well-known to disturb homeostatic barrier function through alterations in key TJ elements[74]. Specifically, *Giardia* has been shown to disrupt zonula occludins protein (ZO)-1, numerous transmembrane claudin proteins, and alter F-actin and α-actinin in order to disrupt paracellular flow[75,76] (Figure 1), which may have important implications in providing a mechanistic link between initial giardiasis, and subsequent development of IBS symptoms. Indeed, recent analysis of colonic biopsies from IBS patients indicated decreased expression of ZO-1, which was associated with increased permeability[77]. Moreover, an earlier report examining fecal extracts indicated higher levels of serine proteases in samples from IBS-D patients. When these extracts were applied to healthy colonic mucosa, they could elicit a proteinase activated receptor (PAR)-2 dependent increase in paracellular permeability in mice *via* increased myosin light chain (MLC) phosphorylation and delayed redistribution of ZO-1[78]. Numerous pathogens, including both EPEC and EHEC, produce potentially cytotoxic serine proteases[79], suggesting another possible link between enteric infection and IBS pathogenesis. Proteases are known to be involved in the infectious processes of pathogens such as EHEC and EPEC where they can prove detrimental to the epithelial barrier *via* modifications of the extracellular matrix[80], and or by activating protease-activated receptors, which have been shown to stimulate sensory neurons to produce hypersensitivity reactions[81]. Consequently, the possibility of residual pathogen mediators, such as inherent proteases, contributing to persistent changes in GI function requires further examination.

Enterochromaffin cells: Enterochromaffin cells (ECs) lining the GI mucosa are primary sources of Serotonin (5-HT) within the body. Alterations in the biosynthesis of 5-HT, in its release from ECs and degradation, and/or in its re-uptake, may have severe ramifications and perturb normal GI function[82]. Multiple studies have shown significantly higher 5-HT levels in the plasma of Pi-IBS patients compared with that of healthy controls, even in comparison to patients of the sporadic IBS-C subtype[83]. Recent studies have observed such significant alterations in EC counts and 5-HT levels, that the authors declared Pi-IBS as a distinct IBS subtype[10,11]. Augmented numbers of 5-HT-contatining ECs have been observed in colonic biopsies from patients following *C. jejuni* infection[9]. Up to 25% of *C. jejuni* infections are known to result in IBS[9], and the resulting implications on EC hyperplasia and excessive 5-HT bioavailability suggest a possible

mechanism whereby enteric infection may provide sufficient trigger for IBS symptom generation. Additionally, numerous reports have suggested a defect in the serotonin reuptake transporter (SERT) expression, and function in IBS patients[82,84,85], which may dictate inadequacies in homeostatic serotonin turnover. Interestingly, in the *T. spiralis* model of Pi-IBS, mice develop chronic abnormal motility patterns subsequent to infection, an effect that is accompanied by EC hyperplasia and 5-HT release[6,13], and blocked upon administration of a 5-HT antagonist[86]. In contrast, patients with persisting abdominal symptoms after acute *Giardia* infection have lower duodenal 5-HT-containing ECs, and lower plasma 5-HT postprandially, compared to controls[87], further underscoring the complexity of IBS pathophysiology.

Intestinal microbiota disruptions: The intestinal microbiota have extensive protective capacities[88] that are maintained by a diverse species profile. The characteristic high fat, high protein diets employed by the majority of people living in westernized countries facilitates the establishment of distinct microbiota species profile, as compared to that of those living in rural areas of developing countries, with a polysaccharide-rich diet[89]. Particular bacterial groups, mainly *Bacteroidetes* are known to harbor significant genetic capabilities to hydrolyse xyloses, making it an important constituent of the microbiota of people subsisting on carbohydrate-dominant food sources. The relative sensitivity of these distinct microbiota to enteropathogens, and how in turn disruptions in their respective flora may differentially regulate post-infectious disorders, is unknown.

Interestingly, changes in the relative Firmicutes to Bacteroidetes ratio[90,91], loss of Bifidobacteria spp. and Faecalibacterium[91], and overall diminished diversity[92], are all apparent in the microbiota profile of IBS patients. Additionally, numerous studies have demonstrated small intestinal bacterial overgrowth in IBS patients, where excessive colonization of the small intestine occurs with colonic flora[33,93]. There is the possibility that enteropathogens may disrupt the indigenous microbiota, either directly through pathogen-microbiota interactions, indirectly *via* the host mucosal immune response to the pathogen, or by a combination of the two[94]. For example, *S. enterica* serovar Typhimurium induced the loss of 95% of total bacterial numbers throughout the murine intestinal tract, 7 d following infection[94]. Findings from ongoing research also indicate that G. duodenalis and C. jejuni are able to directly alter species distribution of human commensal microbiota[95]. Pathogenic effects, however, may only provide a suitable trigger, and ultimately require the accompaniment of a host inflammatory response in order to markedly alter the microbiota ecosystem. The necessity of these compounding factors is exemplified in contrasting *C. rodentium* and *C. jejuni* murine infection models, where the former induces overt host inflammation, while the later can successfully colonize without producing inflammatory reactions[96]. It appears then, that both enteropathogen assault, combined with pathogen-mediated intestinal inflammation, can elicit dramatic changes in the total abundance of the intestinal microbiota, and shift in anaerobic:aerobic species[96].

CONSIDERATION

Many studies that classify patients as experiencing Pi-IBS do so based upon questionnaires, highlighting the fact that they rely exclusively on a patient's recall of past medical events, including infections and/or prescription drug use. Some antibiotics, for example, have established causality in disturbing the overall fecal microbial composition through drastic reduction of *Firmicutes* and *Bacteroidetes*, and a corresponding promotion of *Proteobacteria* spp.[97].

Also, the classification of IBS as biopsychosocial disorder challenges the mantra of body and mind being distinct entities, and suggests an equal consideration of both when examining disease manifestation. The risk of developing IBS symptoms following enteric infection may also differ in individuals depending on psychological parameters such as stress level, emotional status, and upbringing. High stress and anxiety levels, for instance, are associated with IBS development following *Campylobacter* infection[98]. Anxiety, as well as depression, is also correlated with altered pain perception in IBS patients[30]. Additionally, anxiety and depressive states in IBS patients were recently shown to lead to changes in serum levels of gastrointestinal hormones. Indeed, the authors suggest increased secretion of somatostatin and vasoactive intestinal peptide seen in IBS patients exhibiting anxiety-depression emotional state ratings, may contribute to altered gastrointestinal motility and function[99]. An important mediator in the endocrine arm of the stress response, corticotropin-releasing factor, may also contribute to Pi-IBS development through direct local action on specific cellular targets, namely mast cells, and consequently lead to the modification the intestinal inflammatory process[100].

Additionally, as Pi-IBS is defined based upon the development of exclusively new IBS symptom presentation, researchers must be certain that no preceding presentation of IBS occurred. Indeed, clear cause-to effect relationship studies need to establish mechanistic causalities in Pi-IBS.

CONCLUSION

Unfortunately, the link between physiological consequences of enteric infection and altered gut function (sensitivity and motility) seen in IBS remains largely circumstantial. As many as 30%-40% of patients experiencing enteritis can go on to develop chronic GI abnormalities compatible with IBS; however, this means that a greater percentage of patients make a full recovery. Susceptibility, in turn, to developing IBS is determined by a number of factors, with enteric pathogens constituting only one possible route of initiation. Regardless of the

heterogeneous initiation mechanisms culminating into disease, the pathophysiological implications of enteric infection provide important clues towards elucidating the mechanics underlying IBS manifestation. Animal models are becoming increasingly appreciated as divergent means in which IBS triggering mechanisms may be elucidated. Indeed, the maternal separation stress model in rodents is well documented in mimicking early life stress that can result in lifelong dysfunctions in the brain-gut axis, and is implicated in predisposing to IBS development[101]. Furthermore, animal models of post-infectious, or post-inflammatory conditions, such as those using *T. spiralis* or TNBS, are proving useful in examining the mechanisms underlying motility and pain perception changes subsequent to diverse stimuli, without the challenges associated with patient recall, or the need for complex psychological status analyses.

This is especially relevant in terms of developing treatment technologies to combat IBS, most of which currently target overt symptomology. Many of the physiological consequences of GI infections represent parallels with fundamental triggering mechanisms currently though to contribute to IBS. Understanding the similarities between remnants of enteric infections, and the detrimental outcomes, can lead to the development of prevention strategies and therapeutic techniques to target IBS generation; before it can even start.

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Irritable bowel syndrome in children: Pathogenesis, diagnosis and evidence-based treatment

Bhupinder Kaur Sandhu, Siba Prosad Paul

Bhupinder Kaur Sandhu, Siba Prosad Paul, Department of Pediatric Gastroenterology, Bristol Royal Hospital for Children, Bristol BS2 8BJ, United Kingdom
Author contributions: Sandhu BK and Paul SP had contributed equally to the article in its design and to its intellectual content.
Correspondence to: Bhupinder Kaur Sandhu, Professor, Department of Pediatric Gastroenterology, Bristol Royal Hospital for Children, Upper Maudlin Street, Bristol BS2 8BJ, United Kingdom. bhupinder.sandhu@uhbristol.nhs.uk
Telephone: +44-117-3428828 Fax: +44-117-3428831
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Abstract

Irritable bowel syndrome (IBS) is the commonest cause of recurrent abdominal pain (RAP) in children in both more developed and developing parts of the world. It is defined by the Rome Ⅲ criteria for functional gastrointestinal disorders. It is characterized by abdominal pain that is improved by defecation and whose onset is associated with a change in stool form and or frequency and is not explained by structural or biochemical abnormalities. It is estimated that 10%-15% of older children and adolescents suffer from IBS. IBS can be considered to be a brain-gut disorder possibly due to complex interaction between environmental and hereditary factors. The diagnosis of IBS is made based on the Rome Ⅲ criteria together with ruling out organic causes of RAP in children such as inflammatory bowel disease and celiac disease. Once the diagnosis of IBS is made, it is important to explain to the parents (and children) that there is no serious underlying disease. This reassurance may be effective treatment in a large number of cases. Lifestyle modifications, stress management, dietary interventions and probiotics may be beneficial in some cases. Although there is limited evidence for efficacy of pharmacological therapies such as antispasmodics and antidiarrheals; these have a role in severe cases. Biopsychosocial therapies have shown encouraging results in initial trials but are beset by limited availability. Further research is necessary to understand the pathophysiology and provide specific focused therapies.

Key words: Recurrent abdominal pain; Irritable bowel syndrome; Brain-gut disorder; Lifestyle modifications; Biopsychosocial therapies; Children; Rome Ⅲ criteria

Core tip: Irritable bowel syndrome (IBS) is the commonest functional gastrointestinal disorder regarding which there is often limited knowledge amongst clinicians. This paper aims to address the clinical challenges that a clinician may face in managing children with IBS. Importance of the application of the Rome Ⅲ criteria and a focused history is necessary to manage IBS. An evidence-based approach for managing children with IBS is highlighted in this article followed by a section on current best practice-authors' personal view. We hope the readers will find this article useful in their clinical practice.

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HISTORICAL PERSPECTIVES AND DEFINITION

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder (FGID) involving bowel function.

Table 1 Rome III diagnostic criteria for childhood irritable bowel syndrome

Abdominal discomfort or pain associated with 2 or more of the following (present at least 25% of the time):
Improved after defecation
Onset of symptoms associated with a change in stool frequency
Onset associated with a change in stool form alternating between diarrhea and constipation
No evidence of an inflammatory, anatomic, metabolic, or neoplastic process that explains the child's symptoms

The above criteria should be fulfilled at least once per week for at least 2 mo before a diagnosis of irritable bowel syndrome is made.

FGIDs are defined as a variable combination of chronic or recurrent gastrointestinal symptoms not explained by any structural or biochemical abnormalities[1,2].

One of the first references to the concept of an "irritable bowel" causing symptoms of diarrhea, abdominal pain, constipation, without any well-recognized infective cause appeared in the *Rocky Mountain Medical Journal* in 1950[3]. This article suggested that IBS is caused by a psychosomatic or mental disorder[3].

Recurrent abdominal pain (RAP) of childhood is an important feature of IBS. RAP was first described by Apley and Naish following their pioneering study of 1000 children in Bristol, United Kingdom[4]. Apley defined RAP as three or more episodes of abdominal pain occurring over a period of at least 3 mo, with pain sufficient to cause some impairment of function[4]. This definition of RAP still stands and is in current use internationally.

Prior to 1995, IBS was not recognized as a concept amongst children and these children were likely to be diagnosed as having RAP. An international group of pediatric gastroenterologists gathered together in Rome in 1995 to define the diagnostic criteria for FGIDs in childhood (including IBS) and this was published in 1999 as part of the larger Rome Ⅱ criteria. The Rome Ⅱ criteria were subsequently modified[1] and the current internationally agreed diagnostic criteria for childhood IBS is known as the Rome Ⅲ criteria and is shown in Table 1.

The difference between Rome Ⅱ and Rome Ⅲ criteria for diagnosing IBS in children is the reduction of the required duration of symptoms from 3 to 2 mo. The consensus of the committee members was that in children 2 mo better reflects clinical experience. This allows primary care physicians to diagnose IBS earlier than 3 mo and also allows 4 wk for an acute infective process and a further 4 wk to develop chronicity of symptoms[1].

Children with IBS may experience a sense of incomplete evacuation after defecation and sit on the toilet for a long time. This symptom may not always be present in children and is therefore not part of the Rome Ⅲ diagnostic criteria.

EPIDEMIOLOGY

IBS is the commonest cause of functional RAP in children in the Western world, accounting for more than 50% of cases of RAP[5,6]. Hyams *et al*[5] first applied the Rome I (adult) criteria for IBS to 171 children previously diagnosed with RAP in a hospital based setting and found that 68% fulfilled the adult criteria for IBS. The same group then carried out a community-based study amongst 507 middle and high school children and identified IBS in 10% children (14% amongst high school and 6% among middle school children)[7].

In a study based in a specialist gastroenterology unit in Bristol, UK (serving the same population as Apley 50 years earlier), IBS was identified as the commonest cause of RAP[6]. Out of 103 children fulfilling the diagnostic criteria for RAP and entering the study, after extensive investigations 72 were found to have no organic pathology. Thirty-seven of these 72 children (51%) fulfilled the diagnostic criteria for IBS; making IBS the commonest cause of RAP even in a specialist hospital setting[6]. This recent study compared to Apley had the advantage of improved screening tests (celiac serology, *Helicobacter pylori* antibody titer, inflammatory markers, serum amylase, and abdominal ultrasonography) as well as previously existing tests such as liver function tests, full blood count; urine and stool analyses. In addition to screening tests, endoscopy and oesophageal pH monitoring were performed where there was a clinical indication[6].

In a Sri Lankan study of 1717 school children aged 10-16 years randomly selected from 4 provinces; 107 children were diagnosed with IBS symptoms as per Rome III criteria. The overall prevalence of IBS was found to be 6.23% with a higher prevalence amongst girls[8].

In a randomized study by clustering samples in China, which involved 5403 children and adolescents aged between 6 to 18 years from 9 schools, the prevalence of IBS using the Rome Ⅱ criteria was found to be 13.25% and it was higher amongst girls (male:female ratio was 1:1.8)[9]. These studies suggest that the prevalence of IBS in children from different geographical settings is similar.

ETIOLOGY OF IBS

The exact etiology of IBS remains to be determined. The debate remains whether it is caused by hereditary or environmental factors. It is possibly due to complex interaction between both. Infection, inflammation, visceral hypersensitivity, allergy and disordered gut motility may all play a part.

In a questionnaire study with 10986 respondents (response completed by patient and their family regarding health problems) and representing 6060 twin pairs; concordance for IBS was significantly higher amongst monozygotic (17.2%) than dizygotic (8.4%) twin pairs[10]. The same study also highlighted that having a mother or father with IBS is a stronger predictor (15.2%) than having a twin. The authors concluded that heredity contributes to IBS, but social learning has an equal or greater influence[10]. Another study of 319 young adults with history of childhood functional abdominal pain (FAP) reported parental history of chronic pain in their childhood. It

also reported an increase in health service utilization for FAP[11].

As there is a strong familial trend noted in IBS, there has been an ongoing interest in finding a genetic link in IBS. So far, a positive association between IBS and interleukin-10 (IL-10) polymorphism has been reported. A study in Taiwan with 94 children with IBS and 102 healthy controls, significantly lower Escherichia coli lipopolysaccharide-induced IL-10 production by peripheral blood mononuclear cells was noted in children with IBS although the study group concluded that this reduction in IL-10 production may not have been fully determined genetically[12]. Patients with a mutation in a sodium channel gene (SCN5A) were found to report gastrointestinal symptoms especially abdominal pain more often, and this mutation may be a contributory factor in IBS[13,14].

An infectious trigger for IBS may also play a role. In a prospective study of 102 children in Russia with *Giardia lamblia* detected in stool by ELISA, the prevalence of post-infectious IBS was found to be 28% in girls and 17% in boys[15]. In a postal questionnaire survey of 576 individuals with a *Salmonella* or *Campylobacter* infection (between 2000-2009), nearly 10% of 189 individuals who responded to the questionnaire reported post-infectious IBS symptoms up to 10 years later[16]. Similar findings were reported in another study after an outbreak of bacterial gastroenteritis (with *Escherichia coli* 0157:H7 and *Camplobacter* species) with 8 year follow-up (2002-2008) which reported increased incidence of IBS after the episode in children and adults[17].

A prospective study recruited subjects following a large outbreak of acute gastroenteritis attributed to foodborne norovirus at the annual meeting of the Canadian Society of Gastroenterology Nurses and Associates (CSGNA)[18]. It then documented development of post-infectious IBS symptoms. The study data showed that although after 3 mo norovirus infection produced new IBS symptoms in about 25% of case; by 6 mo the incidence was no different in infected individuals compared to healthy controls and concluded that IBS following viral gastroenteritis is a transient entity[18].

PATHOPHYSIOLOGY OF IBS

No single clear pathophysiology has been demonstrated to date for IBS in children. Studies both in children and adults have suggested different mechanisms which may contribute to the development of IBS[19]. IBS can be considered to be a brain-gut disorder. It is postulated that a state of dysregulation exists/occurs within the enteric and the central nervous systems in patients with IBS and this results in alteration in sensation, motility, and possibly, immune system dysfunction[19]. It is important to consider the bidirectional brain-gut interactions, the "brain-gut axis" when considering any pharmacological interventions in IBS[20,21].

In a prospective study of 98 children who underwent upper or lower GI endoscopy, serotonin (5-HT) signaling was noted to be altered in IBS with diarrhea but not in functional dyspepsia[22]. In another study of 93 children (aged 7-10 years) with symptoms of FAP or IBS, evidence of increased GI permeability and low-grade GI inflammation were detected; the latter related to the degree to which pain interfered with activities when compared to healthy control (n = 52)[23].

A study of 35 children (aged 10-17.6 years) who fulfilled criteria for IBS demonstrated that abdominal pain is associated with visceral hypersensitivity and abnormal perception of visceral sensations[24]. Studies suggest that patients with IBS describe gut stimuli as being unpleasant or painful at lower intensity levels when compared with non-IBS individuals and this is likely to be neurological in origin[19,25].

In another study of 10 children with 10 age-matched controls, fecal short-chain fatty acid (SCFA) profile of patients with diarrhea predominant IBS (IBSD) was found to have lower concentrations of total SCFA, acetate, and propionate and a higher concentration and percentage of n-butyrate. Fecal flora from these patients produced less SCFA in an *in vitro* fermentation system in response to incubations with various carbohydrates and fibers. Differences in SCFA production by colonic bacterial flora in patients with IBSD may be related to the development of GI symptoms[26].

A recent study in the United States looked at the intestinal microbiomes in stool samples (n = 71) obtained from 22 children with IBS (diagnosed by Rome Ⅲ criteria) and 22 healthy controls[27]. Children who received antibiotics, probiotics, or steroids (oral or inhaled) within 6 mo of sampling were excluded. Stool samples were analyzed using 16S metagenomics by PhyloChip DNA hybridization and deep 454 pyrosequencing. Specific microbiome signatures were associated with pediatric IBS suggesting important association between GI microbes and IBS in children. Children with IBS were characterized by a significantly greater percentage of the class Gammaproteobacteria *Haemophilus parainfluenzae* being a prominent component of this group. It is postulated that the microbiome signature approach may prove to have a diagnostic role in the future[19,27-29].

HISTORY TAKING AND CLINICAL PRESENTATION

The most important step in making a diagnosis of IBS is to elicit a detailed history and compare symptom concordance with the Rome Ⅲ criteria (Table 1). Children and adolescents generally present with RAP along with change in bowel frequency and/or consistency associated with abdominal pain. The pain is classically relieved following defecation. Children with IBS may report a sense of incomplete evacuation (often sitting on the toilet for a long time); however this is not by itself diagnostic of IBS. It is important to enquire about symptoms of bloating and an urgency to go to the toilet as well as the location of pain which is usually central peri-umbilical but may

Table 2 Red flag symptoms

Night time pain or diarrhea
Recurrent unexplained fever
Recurrent or worsening rectal bleeding
Joint pains
History of weight loss and poor growth
Family history of inflammatory bowel disease
Persistence of severe vomiting or diarrhea
Unexplained pallor
Stools that may be difficult to flush away
Delay in onset or progression of puberty

involve the lower abdomen.

Parents sometimes describe their child as a "little worrier"[30]. Older children and adolescents sometimes report that symptoms get worse during periods of emotional stress so it is important to explore psychological issues at school such as bullying, oncoming exams or at home such as financial difficulties, recent parental separation or divorce or ill-health. It is important to ask if there is history of recent gastrointestinal infection as this may be associated with onset of IBS symptoms. This may be particularly relevant in children with diarrhea predominant IBS[31].

It is important to enquire if there is a family history of IBS amongst parents or siblings. An anxiety state may be present in children and also in parents of children presenting with features suggestive of IBS[31-34]. Studies have reported that IBS patients in comparison to healthy controls have higher scores for anxiety, hostile feelings, sadness, depression, interpersonal sensitivity and sleep disturbance[25] and these issues should be explored while eliciting the history.

It is essential to specifically enquire about "red flag" symptoms as these may indicate serious underlying organic pathology[21]. The "red flag" symptoms are listed in Table 2. Very occasionally an organic pathology may coexist with IBS as the latter is relatively common.

If any or combination of the above symptoms are elicited from the history, appropriate investigations are necessary to exclude underlying organic pathology.

CLINICAL SUBTYPES AND DIFFERENTIAL DIAGNOSIS OF IBS

Three clinical subtypes of IBS are encountered in clinical practice: diarrhea predominant IBS is termed as IBSD, constipation predominant IBS is termed as IBSC and IBS with alternating diarrhea and constipation is termed as IBSA. The clinical subtypes are not rigid classification and cross-over from one type to another may be seen during the course of treatment. It is useful, depending on the presenting stool pattern, to assign the children to a clinical subtype as the management in part will depend on the subtypes of IBS.

EXAMINATION OF CHILDREN WITH IBS

The history taking should be followed by a thorough physical examination including plotting the height and weight on an age and sex appropriate growth chart. A confirmed documented significant weight loss should be considered as a red flag sign and is unlikely to be due to IBS[21]. Signs of anemia, jaundice, mouth ulcers, skin rash or arthritis should also be specifically looked for and suggest organic pathology. It is useful to ask the child to point with one finger where the pain is worse and is most frequently felt. In IBS this is often centered around the umbilical region. Inspection of the abdominal wall for scars, distension or masses is necessary. Prominent juicy perianal skin tags or fistulae are indicative of Crohn's disease.

Great care should be taken to rule out any organomegaly, tenderness and/or abdominal mass in the right iliac fossa. If the child is too tense, distraction may be needed in such cases and discussing about other aspects of their life such as school, friends or even a planned holiday may be helpful.

DIAGNOSING CHILDREN WITH IBS

In the absence of a definitive laboratory or radiological diagnostic test, IBS remains a clinical diagnosis. We suggest following investigations to rule out other serious gastrointestinal disorders: serological screening for celiac disease, inflammatory markers (ESR, C-reactive protein, plasma viscosity or orosomucoid) likely to be raised in inflammatory bowel disease (IBD), liver function tests (low serum albumin in IBD) and full blood count (unexplained anemia, blood loss in IBD). In developing countries it is especially important to send a stool sample for microscopy and culture with specific request to look for ova, cyst and parasites (including *Giardia*).

The diagnosis of IBS is made after exclusion of organic causes of abdominal pain and bowel changes based on history and examination particularly ensuring that no red flag symptoms are present (Table 2). These organic causes include lactose intolerance, celiac disease and IBD. Symptoms concordant with the Rome Ⅲ criteria should help clinicians to make a positive diagnosis of IBS and avoid unnecessary investigations.

Specialist investigations such as gastrointestinal endoscopy or radiological evaluation should be reserved for difficult cases where the diagnosis may not be clear from the history, and/or physical examination suggest pathology. These investigations if indicated should be best carried out by pediatric gastroenterologists.

DIFFERENTIAL DIAGNOSES

It is important to differentiate IBS from other causes of RAP by matching symptom concordance with the Rome III criteria for childhood FGIDs[1]. Those with epigastric pain or discomfort not relieved by defecation are classified as functional dyspepsia. Abdominal migraine causes self-limiting episodes of severe abdominal pain interspersed with pain free periods[1]. The remaining group of children whose pattern of abdominal pain does not fit into the above groups is classified as FAP. If other symp-

toms such as headache and limb pain are reported this is labeled as FAP syndrome. Amongst the latter group there are some children with abdominal pain but no other GI symptoms whose pain is made worse by exercise. This pain can be musculoskeletal in origin and may represent the pediatric equivalent of adult abdominal wall pain. Constipation also needs to be considered as it may be associated with overflow spurious diarrhea which may mimic an alternating constipation and diarrhea pattern seen in IBSA.

EVIDENCE BASED TREATMENT FOR CHILDREN WITH IBS

The aim for any therapeutic intervention in IBS should be to improve the quality of life. This includes ensuring the child's pain is minimized and stool consistency and frequency are normalized. The most important step in managing children with IBS is to explain the diagnosis to parents (and the child if age appropriate), explain strategies to cope with stress and provide reassurance that there is no serious underlying disease.

Wherever available and feasible, multidisciplinary team approach should be used to deal with the complex interplay of biopsychosocial factors considered to be involved in the development of IBS in children. It is important to explain the expected benefits of any therapy and give a realistic overview about its expected outcome to parents (and children) before commencing the intervention. Drugs may be needed to treat symptoms including modulating abnormalities in sensorimotor function of enteric nervous system. Following therapeutic interventions have been used in children with IBS, the evidence base for their use is discussed in the next section: (1) dietary interventions; (2) probiotics; (3) drug therapy → peppermint oil, tegaserod, antispasmodics, anti-diarrheal agents, antibiotics; and (4) biopsychosocial therapy → hypnotherapy, cognitive behavior therapy, yoga, acupuncture.

Dietary interventions

Dietary interventions form an important strategy in managing children with IBS. It is important to note that parents generally accept dietary treatment more willingly than drugs.

A recent Cochrane review[35] included seven studies comparing dietary interventions with placebo. Two studies which compared fiber supplements with placebo and had 83 participants, found that the pooled odds ratio for improvement in the frequency of abdominal pain was 1.26 (0.25-6.29). Two studies compared lactose-containing diet with lactose-free diet in 90 participants, but no definite conclusion could be drawn from the way the data was presented. A comparison between Lactobacillus GG and placebo was made in 3 trials, and these gave a pooled odds ratio for improvement of symptoms as 1.17 (95%CI: 0.62-2.21). The Cochrane review conclusion was that there is a lack of high quality evidence on the effectiveness of dietary interventions[35].

Another meta-analysis included 3 randomized controlled trials (RCTs) selected by searching MEDLINE, EMBASE and the Cochrane Library and included 167 children aged 5-17 years[36-39]. This compared use of dietary fiber supplements with placebo for abdominal pain-related FGIDs in children[36]. The reviewers concluded that there is lack of evidence to support the supplementation with fiber as a dietary manipulation for treating children with FGIDs[36].

Fermentable oligo-, di-, mono-saccharides and polyols (FODMAPs) may play a role in triggering gastrointestinal symptoms in IBS patients[40]. The effect of low FODMAP diet was prospectively evaluated using a symptom questionnaire amongst 90 children with IBS with a mean follow up of 15.7 mo. Abdominal pain, bloating, flatulence and diarrhea were significantly improved amongst participants while on low FODMAP diet ($P < 0.001$ for all)[41].

A recent randomized controlled trial in Italy[2] involved treating 60 children (aged 8-16 years) with IBS and RAP with either partially hydrolyzed guar gum (PHGG) or fruit juice. Improvement was seen in 43% children ($n = 30$) given PHGG compared with 5% in control group ($n = 30$) given fruit juice, with normalization of bowel movements in IBS subgroups which was statistically significant. Improvement in abdominal pain was noted but was not statistically significant. Benefit from PHGG was largely seen in IBSA group; similar findings were also reported in an earlier observational study[2,42].

Probiotics

Probiotics have shown some promising results in adult studies with validated efficacy with no reported adverse effects[43,44]. A study of VSL#3 (a probiotic containing 8 beneficial species of bacteria) demonstrated beneficial effect in reducing flatulence scores and retarded colonic transit in patients with IBS and bloating[45].

In an observational study in Germany with 203 children (66 boys, 137 girls) aged 4 to 18 years (mean 10.5 ± 4.5 years) treated with Symbioflor 2 (contains the natural intestinal bacterium E. coli) for an average of 43 d, the treatment was well tolerated and no adverse events were reported[46]. The key IBS symptoms (abdominal pain, stool frequency) as well as the other symptoms (bloating, mucous and blood in stool, need for straining at stools, urge to defecate) improved significantly during treatment and the global assessment of therapy was found to be altogether positive as reported by parents and doctors[46].

In a double-blind, placebo controlled, crossover trial conducted in 5 pediatric tertiary care centers (4 in Italy and 1 in India); 59 children (aged 4-18 years) were randomized to receive either VSL#3 or a placebo for 6 wk. VSL#3 was superior to placebo both in primary (subjective assessment of relief of symptoms) and secondary endpoints (abdominal pain/discomfort, abdominal bloating/gassiness and family assessment of life disruption)[47].

Another randomized double-blind, placebo controlled trial involved 141 children (aged 5-14 years) treated with *Lactobacillus rhamnosus* GG (LGG) or placebo for 8 wk

and then further followed up for 8 wk[48]. At entry and at the end of the trial, children underwent a double-sugar intestinal permeability test. Children treated with LCG showed reduction in abnormal permeability results post treatment. When compared with baseline, children who received LGG reported a significant reduction in both frequency and severity of abdominal pain. At 12 wk, success was achieved in 48 children in LGG group as compared to 37 in placebo group ($P < 0.03$)[48].

A systematic review of RCTs by Spiller on the effectiveness of probiotics postulated that their beneficial effects are due to enhancement of gut barrier function, inhibition of pathogen binding and modulating gut inflammatory response. They may also reduce visceral hypersensitivity associated with both inflammation and psychological stress. Probiotics can also alter colonic fermentation and stabilize colonic microbiota[49].

Another recent systematic review and meta-analysis[45,47,48,50-53] performed to investigate the quantity and quality of the current evidence regarding the effect of different probiotics strains in the treatment of FGIDs in children and adolescents found probiotics to be more effective than placebo in the treatment of patients with abdominal pain-related FGID, especially with respect to patients with IBS. A meta-analysis of 6 RCTs selected by searching MEDLINE, EMBASE, CINAHL, the Cochrane Library, trial registries and proceedings of major meetings[47,48,51,53-55] compared use of LGG with placebo for abdominal pain-related FGIDs (n = 457) in children including 3 RCTs involving IBS specifically (n = 167)[54]. It concluded that children treated with LGG had moderate increase in treatment success with abdominal pain-related FGIDs and this was particularly marked in children with IBS[54].

Drug treatment

A Cochrane review concluded that only weak evidence exists regarding beneficial effects of pharmacological agents in providing relief from symptoms in FAP in children[20]. Evidence relating to some of the pharmacological agents that have been used is discussed here:

Peppermint oil: exerts an antispasmodic action via menthol [main component of peppermint oil (PO)] and acts as a calcium antagonist and results in anti-flatulent action, the exact mechanism of which currently remains unexplained. A review has been carried out of 16 clinical trials investigating the use of 180-200 mg enteric-coated PO in irritable IBS or RAP including 1 study in children[56]. Nine out of 16 studies were randomized double blind cross over trials, five had a randomized double blind parallel group design and two were open labeled studies. Placebo was used in 12 studies and anticholinergics were used as comparator in three studies. Adverse events reported with PO were very specific, but generally mild and transient and included typical GI effects like heartburn and anal/perianal burning or discomfort. The review concluded that 1-2 enteric coated capsules (180-200 mg) daily over 2-4 wk may be the first drug of choice for patients with IBS and constipation and diarrhea[56].

Tegaserod: is a selective 5-HT4 (serotonin) receptor agonist which has shown improvement in children (and adults) with IBSC and chronic idiopathic constipation. A Cochrane review, which included randomized or quasi-randomized controlled trials comparing tegaserod with placebo, no treatment or any other intervention, showed some improvement in overall symptomatology and frequency of bowel movements in those with IBSC or chronic constipation[57]. However, due to its significantly increased risk of cardiovascular ischemic events, tegaserod is not licensed in many countries[58] and is not recommended.

Antispasmodics agents: Have been shown to have a role in IBSD and attenuate heightened baseline and postprandial contractility. Mebeverine is licensed in the United Kingdom and is generally well tolerated; and can be used on an as required basis before meals. A systematic review[59] which searched medical databases and all relevant literature from 1965 to June 2009 for any placebo-controlled clinical trials of mebeverine, identified 14 relevant papers (8 were randomized trials with 555 patients) and concluded that mebeverine is mostly well tolerated with no significant adverse effects; however, its efficacy in global improvement of IBS was not found to be statistically significant[59].

In a recent randomized study from Turkey involving 78 children (selected out of a total of 345 children aged 4-18 years who were diagnosed with IBS on basis of Rome Ⅲ criteria), clinical recovery was seen in 94.9% of 39 children treated with trimebutine maleate at the end of 3 wk when compared to the non-medicated group where spontaneous recovery was seen in only 20.5% children[60]. Children in this study predominantly had IBSC and the authors concluded that trimebutine maleate is an effective agent for treating childhood IBS.

Anti-diarrheal agents: Have a limited role in managing children with IBS but may be tried in children with IBSD where diarrhea and increased bowel frequency interferes with activities of life. Loperamide, an opiate analogue, is most commonly used and acts by stimulating inhibitory presynaptic receptors in the enteric nervous system resulting in inhibition of peristalsis and intestinal secretion. Adult studies have found loperamide to be effective in reducing diarrhea in IBS patients but did not alleviate symptoms of abdominal pain.

Antibiotics: Role of antibiotics in treatment of children with IBS remains controversial. The only rationale behind antibiotic therapy is to eradicate small intestinal bacterial overgrowth. The big question remains as to what antibiotic to use as haphazard prescription of antibiotic therapy may not be effective and will lead to antibiotic resistance. In an adult (aged ⩾ 18 years) study improvement in resolution of symptoms (bloating, abdominal

pain, and loose or watery stools.) were noted in IBS patients treated with Rifaximin for at least 2 wk[61]. Similar beneficial results were later replicated in a study of 50 children with IBS symptoms whose visual analogue scale (VAS) score to evaluate symptoms (abdominal pain, constipation, diarrhea, bloating, flatulence) showed improvement and normalization of lactulose hydrogen/methane breath test (66% cases) after 1 month treatment with 600 mg of Rifaximin[62].

Amitriptyline: Amitriptyline (AMI), a tricyclic antidepressant (TCA), has been found to be effective in adults with IBS in producing global improvement, increasing feelings of well-being, reducing abdominal pain, and increasing satisfaction with bowel movements. The beneficial effects of antidepressants can be explained by partial increments in the central pain threshold. Other mechanisms by which antidepressants might exert their effects include anticholinergic effects (may result in improvement of diarrhea), regulation of GI transit and peripheral anti-neuropathic effects. In a randomized double-blind placebo controlled trial of 33 participants (24 females) aged 12 to 18 years, it was found that patients who received amitriptyline were more likely to experience improvement from baseline in overall quality of life at 6, 10, and 13 wk (P = 0.019, 0.004, and 0.013)[63]. They also reported reduction in IBS-associated diarrhea at 6 and 10 wk, a reduction in periumbilical pain at 10 wk, and a reduction in right lower quadrant pain at 6, 10, and 13 wk[63].

Biopsychosocial modifying therapies

Hypnotherapy: Studies have shown that hypnotherapy may produce a beneficial effect in children with IBS which persists for at least five years after cessation of therapy[64-68]. A randomized controlled study which compared hypnotherapy (n = 27) with standard medical treatment (n = 23) and followed up for a mean duration of 4.8 years showed that 68% of children in the hypnotherapy group remained in remission as compared to only 20% in the control group[64]. It is postulated that hypnotherapy normalizes altered visceral sensation, reduces colonic phasic contractions and reverses the patients' negative thoughts about their condition. Another prospective randomized controlled trial in Germany[69] with 38 children aged 6 to 12 years evaluated a brief hypnotherapeutic-behavioral intervention program in 20 children (recruited for therapy) and compared their response to a waiting list condition (n = 18, served as control). Children in the treatment group reported a significantly greater reduction of pain scores and pain-related disability (55%) than children of the waiting list condition (5.6%)[69].

A recent systemic review which included three RCTs comparing hypnotherapy to control treatment with sample sizes between 22 to 52 children found that all trials demonstrated statistically significant improvement in abdominal pain scores in hypnotherapy group[70]. While one trial reported statistically significant improvement in the quality of life, two trials reported improvement in school attendance and the benefit was persistent even after 1 year of completion of therapy. The authors go on to recommend hypnotherapy as the first line in the management of children with IBS[70].

Cognitive behavioral therapy: Many children with IBS receive psychological interventions[70]. A Cochrane review which included six trials conducted in children aged between 5 to 18 years with RAP comparing Cognitive behavioral therapy (CBT) with standard therapies such as dietary interventions, pharmacological interventions, *etc.* concluded that CBT may be a useful intervention for children with RAP and IBS[71]. However, the evidence remains weak and behavioral therapies are beset by unavailability of therapists and the need for multiple numbers of sessions.

Yoga: Yoga can be considered as a form of behavioral therapy and consists of general relaxation exercises, breathing exercises, focused training for abdominal relaxation and positive reinforcement by focusing thoughts on a single topic and good experiences. In a pilot study[72], 20 children aged between 8-18 years were trained Hatha yoga by a children's yoga teacher and received 10 yoga sessions and also practiced at home. Yoga exercises were found to be effective in children with RAP and IBS resulting in significant reduction of pain intensity and frequency[72].

Acupuncture: This is considered to relieve pain by release of endogenous opiates and triggering of serotoninergic inhibitory pathways. A study compared differences in the therapeutic effect of Tianshu acupuncture (ST 25) (n = 20) and Dachangshu acupuncture (BL 25) and western medication with Trimebutine maleate (n = 20). Acupuncture was found to relieve symptoms of IBS and was reported to be superior to medication[73].

A recent Cochrane review which included 17 randomized controlled studies (including the one above) with 1806 adult participants, greater benefits were reported by participants treated with acupuncture as compared to the two antispasmodic drugs (pinaverium bromide and trimebutine maleate). However, five sham-controlled RCTs comparing acupuncture with sham acupuncture showed no significant difference[74].

Current best practice-authors' personal view

There is no universally proven therapy that will work in all children with IBS. We start with a detailed focused history of the symptoms including family, social and educational history of the child and make enquiries regarding other members in the family who may be suffering from IBS or other FGIDs. Use of Rome Ⅲ criteria and targeted enquiries regarding the possible presence of red flag signs are also made.

This is followed by a thorough physical examination and review of growth and development including pubertal assessment where appropriate and an assessment of

child's mental status.

Basic investigations are carried out to rule out organic causes. Our practice is to do a full blood count, liver and renal function test, inflammatory markers, amylase, celiac screen and in cases of diarrhea a stool culture and stool reducing substances. Specialist investigations such as ultra sound scan of abdomen, MRI scan, gastrointestinal endoscopy, colonic transit test, *etc*., are only carried out if organic pathology is suspected and the test is appropriate.

Majority of children will improve with a positive diagnosis of IBS (based on Rome Ⅲ criteria) with counseling, education about IBS and personalized pain, stress and other management advice and need no other treatment. It is important to spend time with the patient and their family in explaining the diagnosis of IBS, categorically mention that all the investigations done so far have been negative and that there is nothing seriously wrong with their tummy and it will improve.

For a small subset of patients with severe disabling symptoms finding an effective treatment will remain a challenge and few strategies may need to be tried before symptom control is achieved. Lack of a single proven intervention for all cases highlights the complexity of psycho-pathophysiology of IBS.

We favor an integrated bio-psycho-social approach. It is important to educate the family about IBS and address emotional or environmental issues that may be triggering symptoms of IBS and/or making them worse. The need to achieve may be leading to stress and counseling may be necessary. Clinicians need to invest time early in the diagnosis in exploring and addressing other issues such as bullying at school, difficulties in relationship with parents or peers, unrealistic academic expectations, *etc*.

A dietary history including type and amount of food and drinks taken should be recorded and appropriate changes to the diet suggested involving the dietician if needed. High fiber diet may have a beneficial role in IBSC while diet low in fiber may be beneficial in patient with IBSD. It is important to explain that a high fiber diet is often associated with intestinal gas production, increased cramps and flatulence and may not be tolerated by some patients. If there is a suspicion of dairy intolerance, lactose free diet may be useful; a trial of 2-4 wk should be enough to get a response. A trial of PHGG may be beneficial in patients with IBSA in regulating stool type and frequency.

Probiotics such as VSL#3 or LGG are safe to use and are worth considering especially when IBS symptoms have been triggered off by an episode of gastroenteritis.

Social individualized support for child and family may be necessary in difficult cases. A multi-disciplinary team comprising of pediatric gastroenterologist, dietician, social care, education, psychologist, will be necessary in such cases and the chances of achieving success are better. Financial difficulties that a family may be facing are also worth exploring and addressing. It is also important to involve parents in supporting their children with IBS for positive reinforcement and distraction. The positive effect of distraction was evident from a randomized controlled study where symptom complaints of pain by children (aged 8-16 years) with FAP (n = 104) and well children (n = 119) nearly doubled in the group where parents were trained to give attention and were reduced by half in the distraction group[75].

Pharmacotherapy: There is only limited evidence regarding effectiveness of pharmacological treatments. Smooth muscle relaxants such as peppermint oil and trimebutine may be helpful in children where abdominal pain or spasms are a major problem. In difficult cases with low mood or severe symptoms, membrane stabilizer such as low dose amitriptyline may be necessary. Loperamide on a required basis is useful in children with IBSD. Antibiotics should be reserved for cases where there is strong suspicion of small intestinal bacterial overgrowth or giardiasis.

Biopsychological therapy: Hypnotherapy and CBT have shown promising results in selective cases[76]. Yoga or acupuncture may also be beneficial. However all these need specialist trained pediatric therapists who may not be easily available in most centers. The lack of trained therapists may be solved by such therapies being delivered by pre-recorded therapies in DVDs to be used at home. This suggestion is supported by a study of 34 children aged 6-15 years with FAP[77] who were randomly assigned to receive standard medical care with or without self directed home-based audio-recorded guided imagery hypnotherapy treatment. Guided imagery treatment plus medical care was reported to be superior (63.1%) as compared to standard medical care only (26.7% successful) for the treatment of abdominal pain in FGIDs, and treatment effects were sustained over a long period (6 mo after completion of therapy)[77].

Future direction: IBS no longer remains a condition thought to be affecting adults and adolescents only and is being increasingly recognized as a common condition in young children in developing and more developed countries. There is a need for research to fully understand the pathophysiology of IBS in children. There is a need to understand subsets of IBS so as to deliver specifically targeted effective treatments. There is also a need for well planned randomized placebo controlled evaluations of pharmacological, psychological and other biopsychosocial therapies in children with IBS taking into account subsets of RAP and IBS.

CONCLUSION

IBS remains a clinical diagnosis of exclusion and can sometimes present a challenge because of the nature and range of associated symptoms and their interpretation amongst parents and pediatricians. A detailed focused history and use of Rome Ⅲ criteria helps to clarify uncer-

tainties about the diagnosis. Investigations should be kept to the minimum and used for selected cases to exclude other serious pathologies that may present with similar features. Successful management of IBS in children involves the biopsychosocial approach with enough time initially spent at explaining and reassuring the child and the parents. Therapy needs to be individualized to patient needs and it is important that the expected benefits and possible side-effects are explained to the family before initiating therapy. In difficult cases a multi-disciplinary team approach is needed.

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Irritable bowel syndrome: Relations with functional, mental, and somatoform disorders

Constanze Hausteiner-Wiehle, Peter Henningsen

Constanze Hausteiner-Wiehle, Psychosomatic Consultation Service, Murnau Trauma Center, 82418 Murnau, Germany
Constanze Hausteiner-Wiehle, Peter Henningsen, Department of Psychosomatic Medicine and Psychotherapy, Klinikum rechts der Isar, Technische Universität München, 81675 Munich, Germany
Author contributions: Hausteiner-Wiehle C and Henningsen P contributed equally to this work.
Correspondence to: Constanze Hausteiner-Wiehle, PhD, Psychosomatic Consultation Service, Neurocenter, Murnau Trauma Center, Prof.-Küntscher-Str. 8, 82418 Murnau, Germany. c.hausteiner-wiehle@tum.de
Telephone: +49-8841-480 Fax: +49-8841-482116
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Abstract

This review describes the conceptual and clinical relations between irritable bowel syndrome (IBS), other functional, somatoform, and mental disorders, and points to appropriate future conceptualizations. IBS is considered to be a functional somatic syndrome (FSS) with a considerable symptom overlap with other FSSs like chronic fatigue syndrome or fibromyalgia syndrome. IBS patients show an increased prevalence of psychiatric symptoms and disorders, especially depression and anxiety. IBS is largely congruent with the concepts of somatoform and somatic symptom disorders. Roughly 50% of IBS patients complain of gastrointestinal symptoms only and have no psychiatric comorbidity. IBS concepts, treatment approaches, as well as health care structures should acknowledge its variability and multidimensionality by: (1) awareness of additional extraintestinal and psychobehavioral symptoms in patients with IBS; (2) general and collaborative care rather than specialist and separated care; and (3) implementation of "interface disorders" to abandon the dualistic classification of purely organic or purely mental disorders.

Key words: Functional somatic syndrome; Somatoform disorder; Somatic symptom disorder; Bodily distress syndrome; Interface; Irritable bowel syndrome

Core tip: Irritable bowel syndrome should be seen as a potentially multidimensional condition, even if cases with an uncomplicated, solely gastrointestinal course occur. Often, patients' general mental and physical functioning, participation, as well as quality of life are also affected.

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INTRODUCTION

About 50% of patients with irritable bowel syndrome (IBS) report additional somatic and mental symptoms once they are asked for[1,2]. Often, the additional symptoms call for a supplementary diagnosis of a somatoform disorder, anxiety or depressive disorder, or another functional somatic syndrome. Several reviews show the types and frequencies of IBS comorbidities[3-10]; possible pathophysiological and psychophysiological relations such as enhanced pain perception, altered regional brain activation, infectious etiologies, dysregulations in immune and neuroendocrine function, and genetic susceptibility are discussed[3-10]. However, a clear concept that binds togeth-

er the various manifestations has not yet been proposed.

IBS AND OTHER FUNCTIONAL SOMATIC SYNDROMES

In clinical as much as in nonclinical populations, IBS shows a high symptom overlap with other functional somatic syndromes (FSSs). For example: (1) 16% of 270 IBS patients fulfil the criteria for temporomandibular disorder (TMD), and 64% of 25 TMD patients also have IBS[11,12]; (2) the frequency of fibromyalgia syndrome (FMS) in IBS patients is reported to be 20%-65%[4-21], and among FMS patients, 25%-81% have an additional IBS[4,16,21-23]. The co-occurrence of both syndromes appears to be more common in women than in men[24]; and (3) many patients with IBS also suffer from chronic fatigue. According to the literature, 36%-63% of IBS patients have chronic fatigue, 14% have chronic fatigue syndrome (CFS)[11,25], and 35%-92% of CFS patients also have IBS[3,4,21,26-30].

The symptom overlap is never exact; on average, the symptoms of IBS and other FSSs show an overlap of < 50%. A cross-sectional study among almost 4000 twins in the United States showed that patients with IBS have less comorbidity with other FSSs than patients with CFS, low back pain, chronic tension headache, FMS, and TMD[31].

Therefore, neither the so-called "lumpers" nor the "splitters" can so far offer a convincing concept of the relation between IBS and other FSSs[32]. Lumpers follow the "single-syndrome hypothesis" that the different FSSs are manifestations of one overarching disease; most likely a somatoform disorder. Splitters, on the other side, prefer the view of FSSs as distinct physical diseases.

IBS, AND MENTAL SYMPTOMS AND DISORDERS

IBS patients report not only extraintestinal somatic, but also mental symptoms. The latter include predominantly depressive symptoms (including exhaustion, sleeping problems, and loss of appetite) and anxiety (including nervousness, worrying, rumination, and panic attacks). Research on the comorbidity of IBS and mental disorders has been ongoing for approximately 40 years[33,34]. Many IBS patients meet the full criteria for the respective mental disorders or suffer from subsyndromal, but nevertheless clinically relevant forms[35].

The total lifetime prevalence for at least one mental disorder in IBS patients is reported to range from 38% to 100%[36]. The majority of studies, which do not exclude mental disorders at the outset, report rates of > 90%[33,34,36-42]. In particular, the results vary depending on the level of healthcare from 6% to 70% for depressive disorders and from 5% to 50% for anxiety disorders. The prevalence of a panic disorder (with its characteristic episodic vegetative symptoms) among IBS patients is reported to range between 0% and 41%[36,37,39,40,43,44]. Most studies report an increased prevalence of trauma disorders (such as post-traumatic stress disorder) between 8% and 36% among IBS patients[41,45-47]. Conversely, patients with panic attacks have an increased risk to suffer from IBS with a prevalence of 17%-47%; patients with depressive disorders suffer from IBS in 17%-59% of cases; and patients with generalized anxiety disorder also fulfil the criteria for IBS in 17%-37% of cases[48-53].

A high comorbidity with depressive, anxiety, and trauma disorders has been shown for all FSSs, but IBS especially appears to be associated with eating disorders: between one-half and two-thirds of patients with a current or former eating disorder also meet the criteria for IBS[54,55].

Summing up, approximately 50% of IBS patients also show clinically relevant symptoms of mental distress.

SOMATOFORM DISORDERS

According to the current issue of the International Classification of Disease (ICD-10), an somatoform disorder (SFD) can be diagnosed in a patient who has unexplained symptoms (which are persistent and disabling), together with persistent requests for medical investigations (in spite of repeated negative findings and reassurances by doctors that the symptoms have no physical basis). The category of SFDs is not much older than Rome I; it was introduced as a mental disorder in 1980. Like the Rome process, it is also a symptom-based classification that explicitly tries to avoid etiological assumptions[56].

The trait characteristic "somatization" is defined as "a tendency to experience and communicate psychological distress in the form of somatic symptoms and to seek medical help for them"[57]. IBS patients score higher on somatization than healthy controls, but lower than patients with somatoform disorders; somatization it is a significant psychological factor directly associated with IBS severity[58,59]. With "somatoform autonomous dysfunction of the gastrointestinal system" (F45.32), the ICD-10 classification of somatoform disorders provides a distinct category for patients who have "symptoms as if they were due to a physical disorder of the gastrointestinal system or organ, based upon objective signs of autonomic arousal, and nonspecific or changing in nature" - a definition that is almost automatically met by IBS patients[60]. Fifteen to 48% of IBS patients fulfill the criteria for somatization disorder, which is the most severe form of SFDs[33,34,36,39,40,61,62].

In summary, it is again a high percentage of IBS patients who meet the criteria for an SFD. However, a large group of patients does not fit this diagnosis. It is more difficult to give precise numbers because the case definitions of SFDs are vague.

In the new edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) from May 2013, the former category of "somatoform disorders" was renamed and largely revised[63]: A "somatic symptom disorder (SSD)" is defined by: (1) one or more somatic

symptoms that are distressing and/or result in significant disruption of daily life; (2) excessive thoughts, feelings, or behaviors related to the somatic symptoms or associated health concerns; and (3) disproportionate and persistent thoughts about the seriousness of one's symptoms, persistently high level of anxiety about health or symptoms, or excessive time and energy devoted to these symptoms or health concerns.

Notably, now there is no demand for a lack of "medical" explanation of symptoms anymore. This means that the concept of a "somatic symptom disorder" describes the common phenomenon that somatic symptoms are combined with psychobehavioral characteristics. The diagnosis can be the primary diagnosis in patients or it can be the secondary diagnosis in patients who have a defined organic illness.

The frequency with which IBS patients fulfill the criteria for SSD has not yet been investigated.

FUNCTIONAL IMPAIRMENT, DISTRESS, AND PROGNOSIS

Awareness of the psychological dimension of IBS appears to have high clinical relevance. The presence of other bodily symptoms beyond the IBS core symptomatology is associated with higher functional impairment, more psychological distress, and lower quality of life[1,64]. Psychiatric comorbidity is associated with more severe gastrointestinal symptoms and contributes to poorer therapeutic outcomes[44,65,66]. There is, in fact, evidence that it is rather psychological factors, such as somatization, trait anxiety, maladaptive coping and catastrophizing, than somatic factors that correlate with severity of IBS and poorer outcomes[44,59,67-69].

The severity of IBS core symptoms is not necessarily related to the extent of overlapping bodily symptoms. There are cases of severe IBS with further functional bodily symptoms as well as without those symptoms[56,67].

TAXONOMIC STRUCTURE OF IBS

The term IBS is considered to be relatively noncontroversial in comparison with many other terminological discussions regarding FSSs. It summarizes several conceptually important aspects: (1) the target organ, where the core symptomatology is centered, is clearly specified as the bowel; (2) the term irritable implies a pathophysiological mechanism, that is irritability, or (hyper-)sensitivity; and (3) the term syndrome describes an association of several clinically recognizable features. These typically occur together, so that the presence of one or more features implies the presence of the other features. The term IBS acknowledges the presence of a general principle (irritability), a specific location (bowel) and variability of the clinical picture.

Studies looking into the taxonomic structure of IBS and other FSSs have found that: (1) several latent variables fitted observations best (including a separate latent variable for irritable-bowel-like syndromes); and (2) there is one common, higher-order, general factor explaining large parts of the syndrome's variance[70-73]. Witthöft *et al*[73] promoted a bifactor model of different FSSs. This model consists of a general factor and symptom specific factors. The general factor most likely has a cognitive, affective, or neurobiological component of symptom perception; the symptom-specific factors might reflect physiological factors such as infections, prior organic diseases, or other environmental factors[73]. IBS appears to be associated particularly with the factor gastrointestinal symptom and with the factor general symptom distress, but not with other symptom factors[73]. Witthöft *et al*[73] noted that the absence of a significant association between the other specific symptom factors and IBS did not mean that these symptoms were of no importance. They rather suggested that many symptoms (in this case, those asked for in the PHQ-15) were associated with IBS (*e.g.*, symptoms of pain and fatigue)[73]. However, when symptom-specific (*i.e.*, incremental) components of variance were considered (as implied by the bifactor model), only gastrointestinal symptoms predicted IBS over and above the factor general symptom distress[73].

But how can the relation of peripheral and central mechanisms, of sensation and processing, and the influence of affects and cognition be conceptualized?

Rapps *et al*[74] suggested that central nervous processes could modulate signals from the periphery. This central modulation of peripheral input could underlie the conscious experience of symptoms[74]. However, visceral (and other) pain should not only be seen as pure sensation. Rather, it should be seen as a homoeostatic emotion that indicates disturbances in the internal milieu of the body in its interaction with the environment; just like the present level of arousal and anxiety[75]. Thus, IBS, other FSSs, and somatoform disorders could be conceptualized as disorders of interoception, that is, disorders of the sense of the physiological condition of the body[56].

CURRENT AND FUTURE IBS CONCEPTS

The classificatory approach to IBS has evolved over the past 17 years and the Rome process has become a multifaceted enterprise. The Rome process collects high-level scientific evidence on etiology, diagnosis, and treatment[76]. This process has recognized the importance of symptoms as a basis for classification, which is independent of assumed etiology and somatization. Reporting multiple extraintestinal bodily symptoms plays an important role in defining the severity of IBS[77]. Albeit, the Rome Ⅲ classification currently requires gastrointestinal symptoms only.

Future conceptualizations have the chance to cover the various manifestations of IBS. For example, the new German IBS guidelines recently introduced an IBS definition that additionally requires psychobehavioral and functional criteria, for example, help-seeking behavior and/or worry, and a significant impairment of quality of

life[78]. With this conceptualization, IBS is moved closer to SFDs or SSDs.

It has been suggested that the Rome classification of IBS could define the uncomplicated prototype of complaints (centered around bowel function and abdominal pain), whereas the new DSM-5 diagnosis of SSD could define the complicated prototype (with multiple bodily complaints and certain affective, cognitive and behavioral characteristics)[56]. However, this approach tends to ignore the many shades of patients in between, that is, those who have mainly gastrointestinal problems with a few concerns and slightly impaired quality of life; those with several gastrointestinal and one or two extraintestinal complaints; or those with two functional syndromes and mild depression. An overarching category of general (medical-psychiatry) interface disorders could be a helpful conceptualization for the many phenomena that are neither only somatic nor only mental[32,56,79]. The ICD-11, awaited in 2015, offers a new chance to do that.

The concept of a bodily distress syndrome (BDS) offers another scientifically coherent common basis for the classification of different dimensional graduations of IBS[80]. BDS is divided into a single-organ type and a multi-organ type, depending on the number and location of symptoms. A BDS diagnosis is provided when the symptoms impair the patient's level of functioning and participation (and thus, appropriate need of action is defined). Thereby, the concept differentiates between mere indisposition, or mild, transient symptoms, or clinically relevant illness. In a stratified sample of 978 consecutive patients from neurological and medical departments and from primary care, this concept captured 98% of the IBS cases as BDS, gastrointestinal type[80].

CLINICAL IMPLICATIONS

Such multidimensional conceptualizations of IBS could clear the way for a stepped, collaborative care for IBS patients, along with several implications for their clinical management[32,81]: (1) All mental and bodily symptoms, including those beyond the IBS core symptoms, as well as psychosocial strain, the level of functioning/participation, and quality of life should be asked for early in the course of the illness and regardless of the examiner's subspecialty; (2) The whole range of symptoms and problems should be considered when making a diagnosis. Comorbidity should be documented; (3) The whole range of symptoms and problems should be borne in mind when a treatment plan is established; and (4) When indicated, other specialists should be brought in for both diagnostics and treatment, for example, in the form of rheumatology, infection, or psychosomatic medicine consultations.

CONCLUSION

There is little doubt that both the following groups exist: (1) patients with IBS who have mild to severe gastrointestinal symptoms without or with only few other bodily complaints or psychobehavioral features; and (2) patients in whom IBS-like symptoms are part of a broader picture of multiple, changing bodily symptoms accompanied by anxiety, depression, and dysfunctional illness-related affects, cognition, and behavior. There also exists a tendency for those who are involved in the care of these patients to overstretch their preferred approach, that is, to use only the concept of IBS for patients with multiple symptoms, or to use the concept of SFDs or somatization for patients with punched-out functional gastrointestinal symptoms. That is to say that the classification of IBS (and of FSSs in general) is not only a medical, but also a political issue, because a case definition implicates the "right" specialist that is supposed to care for (and to get reimbursed for) the patient - a general practitioner, a gastroenterologist, a psychotherapist, or a psychiatrist, respectively[56].

Overall, now there is less separation between the perspectives of IBS, FSS, and SFDs than there used to be, and we should take advantage of this development. What we need is a far-sighted, balanced, truly psychosomatic approach. We need a high awareness for gastroenterological, but also extraintestinal and psychobehavioral symptoms in patients with IBS. We need gastroenterologists to know about the concepts of SFD/SSD, psychiatrists to know about the symptomatic characteristics of IBS patients, and both of them talking to each other and knowing their limitations. We need more generalist and collaborative care to overcome pure specialist care. We need to abandon our dualistic classification of either organic or mental disorders. A simple definition of patient groups with uncomplicated or complicated IBS depending on bodily and psychological comorbidity, cognition, behavior, and degree of impairment might be a first step[32].

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Role of antispasmodics in the treatment of irritable bowel syndrome

Anita Annaházi, Richárd Róka, András Rosztóczy, Tibor Wittmann

Anita Annaházi, Richárd Róka, András Rosztóczy, Tibor Wittmann, First Department of Medicine, University of Szeged, 6720 Szeged, Hungary
Author contributions: All of the authors drafted and revised the article and revised and approved the final version.
Correspondence to: Tibor Wittmann, MD, PhD, Professor, First Department of Medicine, University of Szeged, Korányi fasor 8-10, 6720 Szeged, Hungary. wittmann.tibor@med.u-szeged.hu
Telephone: +36-62-545189 Fax: +36-62-545185
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Abstract

Irritable bowel syndrome (IBS) is a long-lasting, relapsing disorder characterized by abdominal pain/discomfort and altered bowel habits. Intestinal motility impairment and visceral hypersensitivity are the key factors among its multifactorial pathogenesis, both of which require effective treatment. Voltage-gated calcium channels mediate smooth muscle contraction and endocrine secretion and play important roles in neuronal transmission. Antispasmodics are a group of drugs that have been used in the treatment of IBS for decades. Alverine citrate, a spasmolytic, decreases the sensitivity of smooth muscle contractile proteins to calcium, and it is a selective 5-HT$_{1A}$ receptor antagonist. Alverine, in combination with simethicone, has been demonstrated to effectively reduce abdominal pain and discomfort in a large placebo-controlled trial. Mebeverine is a musculotropic agent that potently blocks intestinal peristalsis. Non-placebo-controlled trials have shown positive effects of mebeverine in IBS regarding symptom control; nevertheless, in recent placebo-controlled studies, mebeverine did not exhibit superiority over placebo. Otilonium bromide is poorly absorbed from the GI tract, where it acts locally as an L-type calcium channel blocker, an antimuscarinic and a tachykinin NK2 receptor antagonist. Otilonium has effectively reduced pain and improved defecation alterations in placebo-controlled trials in IBS patients. Pinaverium bromide is also an L-type calcium channel blocker that acts locally in the GI tract. Pinaverium improves motility disorders and consequently reduces stool problems in IBS patients. Phloroglucinol and trimethylphloroglucinol are non-specific antispasmodics that reduced pain in IBS patients in a placebo-controlled trial. Antispasmodics have excellent safety profiles. T-type calcium channel blockers can abolish visceral hypersensitivity in animal models, which makes them potential candidates for the development of novel therapeutic agents in the treatment of IBS.

Key words: Irritable bowel syndrome; Motility; Treatment; Calcium channel blockers; Spasmolytics

Core tip: Treatment of irritable bowel syndrome (IBS) must target intestinal motility alterations and visceral hypersensitivity. Antispasmodics have been used in the treatment of IBS for decades, and large placebo-controlled trials have recently been conducted on their efficacy. Alverine citrate, in combination with simethicone, effectively reduced abdominal pain and discomfort; while otilonium bromide also improved defecation problems. Pinaverium bromide regulated impaired motility and reduced stool complaints. Phloroglucinol and trimethylphloroglucinol reduced pain in IBS patients. Mebeverine was recently found to be effective only in non-placebo-controlled trials. Antispasmodics are considered safe. T-type calcium channel blockers could represent a future therapeutic option in IBS treatment.

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INTRODUCTION

Irritable bowel syndrome (IBS) is a chronic gastrointestinal (GI) disorder affecting a significant proportion of the global population, with a calculated prevalence of approximately 11.2%[1]. IBS has a large impact on health-related quality of life, resulting in reduced work productivity, elevated absenteeism and increased health care use and costs[2,3]. IBS can also seriously impair the patient-doctor relationship[4], because ineffective symptom control can diminish clinicians' credibility and prompt the patient to seek further opinions[5]. IBS has a long course and high relapse rates, with more than half of IBS patients reporting the same symptom profile after 1 and 7 years and a further 25% having persistent minor IBS symptoms[6]. Regarding the long term persistence of IBS, effective long-term therapies are of great economic importance in both Eastern and Western countries; however, drug trials have revealed an extremely high relapse rate in this disease[7]. Despite intensive research aiming to find new therapeutic pathways, the present possibilities have mostly focused on symptom suppression, and only a few drugs have been found to be more effective than placebo over the long term.

A heterogeneous group of drugs called "antispasmodics" or "spasmolytics" such as direct smooth muscle relaxants (*e.g.*, papaverine, mebeverine, peppermint oil), anticholinergic agents (*e.g.*, butylscopolamine, hyoscine, cimetropium bromide, pirenzepine) and calcium channel blockers (*e.g.*, alverine citrate, otilonium bromide, pinaverium bromide), have been used in therapy for IBS for decades. The aim of these drugs is to reduce defecation symptoms by increasing colonic transit time, improving stool consistency and reducing stool frequency. The pharmacological action of these agents is not always clear, and the mechanisms are often mixed. Nevertheless, meta-analyses performed on studies comparing antispasmodics to placebo or other treatments have uniformly confirmed the positive effects of these drugs, and their side effect profiles have been excellent[8]. Recent adverse reactions, such as severe constipation, ischemic colitis and possible deaths[9], have led to the transient withdrawal of alosetron, a 5-HT_3 receptor antagonist, and fatal ventricular arrhythmias caused the retraction of the mixed 5-HT_3 antagonist/5-HT_4 agonist cisapride in many countries, thus highlighting antispasmodics as attractive and reliable therapeutic options.

The accurate diagnosis of IBS has been a major pitfall in designing clinical trials in the past because a precise definition has been lacking. After the proposal of previous diagnostic criteria by Manning *et al*[10] and later by Drossmann *et al*[11], the Rome criteria were developed, emphasizing the importance of a positive diagnosis based on symptoms. Currently, the diagnostic criteria for IBS based on the Rome Ⅲ system are recurrent abdominal pain or discomfort for at least 3 d per month over the previous 3 mo, associated with two or more of the following: improvement with defecation; and onset associated with a change in frequency of stool; and onset associated with a change in form (appearance) of stool[12]. These criteria must be fulfilled for the previous 3 mo, with symptom onset at least 6 mo prior to diagnosis. Depending on the predominant bowel symptom, IBS can be classified as IBS with constipation (IBS-C, 20%-30% of patients), IBS with diarrhea (IBS-D, 20%-30% of patients) or IBS with "mixed" constipation and diarrhea (IBS-M, up to 45% of patients)[13].

In this review, we aimed to collect and summarize the available data on the efficacy and safety of modern antispasmodics in the treatment of IBS, focusing on placebo-controlled clinical trials using valid patient selection criteria.

PATHOPHYSIOLOGY OF IBS

Profound research over the last few decades has revealed a multifactorial pathogenesis. Preceding enteric infections, altered colonic or small intestinal bacterial flora, increased gut permeability and immune activation may play a role in the development of the disease[14-16]. Signals from the GI tract are processed in the brain, which in turn can influence GI motility, secretion and immune function[17]. This brain-gut axis is essential for the healthy regulation of the GI system, and its structural or functional alteration can lead to the development of disorders such as IBS[18]. Therefore, psychological factors and chronic stress can also be involved in triggering symptoms[19], in association with alterations in the activity of specific brain regions[20,21]. Nevertheless, abnormal intestinal motility and visceral hypersensitivity remain key factors in the pathogenesis of the disease[22]. The origin of visceral hypersensitivity seems to be complex. Intraluminal factors, such as serine-proteases, can increase colonic permeability in IBS-D patients by activating protease-activated receptor-2, resulting in visceral hypersensitivity[23]. Increased colonic permeability in IBS-D patients has been correlated with stool frequency, which also suggests a role in symptom generation[24]. Luminal cysteine-proteases have been shown to increase colonic permeability through the degradation of tight junction proteins, resulting in visceral hypersensitivity in IBS-C patients, possibly through local microinflammation[25]. Colonic mucosal immune activation, which is characterized by mast cell, intraepithelial lymphocyte and lamina propria lymphocyte counts, was found to be significantly higher in IBS-D than in healthy controls[26]. This immune activation was similar to inactive inflammatory bowel disease. Mast cells have been implicated in the development of IBS: the number of degranulating mast cells in colonic mucosa and their spontaneous release of trypsin and histamine were markedly increased in IBS patients compared with controls[27]. Furthermore, mast cells in close proximity to nerve end-

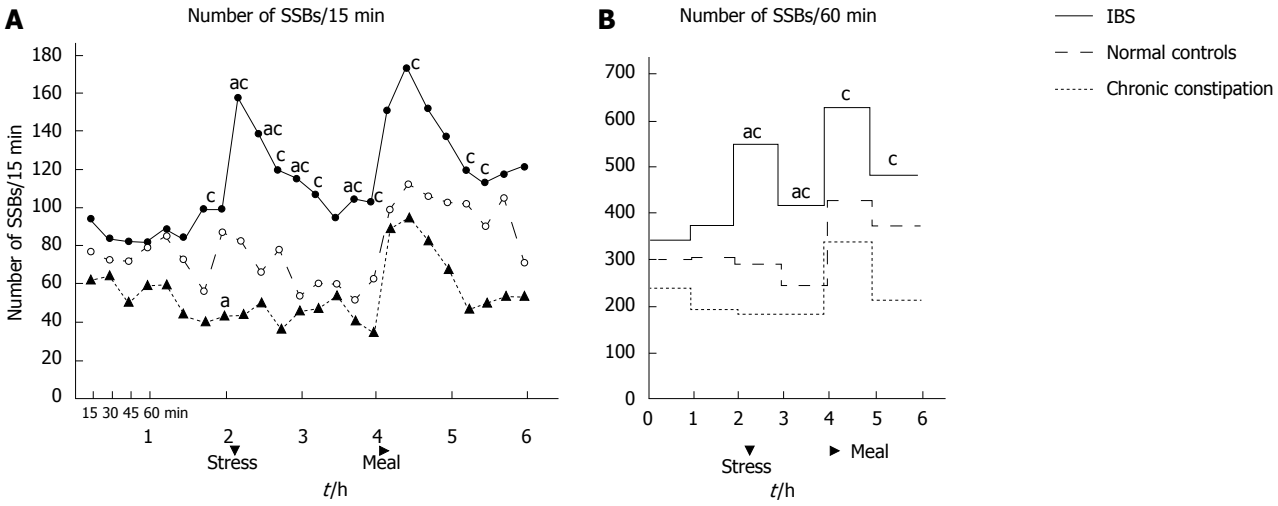

Figure 1 The number of short spike bursts measured by colonic intraluminal electromyography[34]. The values were calculated over the period of 15 (A) and 60 min (B). Each group included 8 patients. The 6-h screening session consisted of three 2-h periods: a control period; a period of stress (during which a cold pressor test was performed for 15 min); and a post-prandial period (patients ingested a 800 kcal meal). SSBs: Short spike bursts. $^{a}P < 0.05$ *vs* normal controls; $^{c}P < 0.05$ *vs* chronic constipation patients.

ings have been significantly correlated with the severity and frequency of abdominal pain/discomfort in IBS patients. Enterochromaffin cells can also play important roles in the development of visceral hypersensitivity by producing and releasing serotonin, which activates 5-HT_3 receptors located on afferent sensory neurons[28]. Furthermore, the activation of 5-HT_4 receptors on sensory afferent neurons triggers the peristaltic reflex, whereas 5-HT_4 receptors on colonic smooth muscle mediate relaxation[29,30]. Motility disturbances in IBS patients have been well known for decades. Baseline muscular tone was found to be higher in IBS-D and IBS-M but not in IBS-C compared with healthy individuals[31]. Further abnormalities in colonic motility patterns are characterized by hyperreactivity - namely, a prolonged increase in colonic motor activity after meals, an exaggerated increase in motor activity in response to stressors or cholecystokinin (CCK) and increased motor activity in response to balloon distention[32]. Stress alone can be an important factor in the pathogenesis of motility disturbances, as suggested by long-duration restraint in rats having been shown to induce rapid, dramatic changes in small bowel motility, with gradually increasing differences in colonic motility as measured by electromyography[33]. Colonic motility changes were still present 60 h after such restraint stress, suggesting that these persistent alterations could form the basis of the development of functional disorders. In a study measuring electromyographic activity in the left colon with an intraluminal probe, a large increase in short-spike bursts was induced by stress (*via* the cold pressor test) in IBS-C patients but not in controls or in chronically constipated patients, and this difference remained significant for 2 h after the stress episode (Figure 1)[34]. Ingestion of a standard meal has provoked the increase of aboral migrating long spike bursts in control subjects, but this propulsive motor effect was largely depressed in IBS-C patients[35]. In a subsequent study, repetitive distention of the distal sigmoid colon below the sensory threshold in IBS patients induced exaggerated colonic motility[36]. Small bowel motility was also impaired, as the repetitive distention inhibited motility of the small intestine in healthy subjects, whereas no such effect was observed in IBS patients[36]. Specific patterns of small bowel motor activity have also been described in IBS patients, such as ileal propulsive waves and clusters of jejunal pressure activity, which have usually been associated with abdominal cramping and pain[37]. Visceral hypersensitivity by itself is not painful, but it can lead to abdominal pain in IBS patients by the effect of an intense stimulus, such as an exaggerated colonic contraction[38]. However, a clear connection between visceral hypersensitivity and motility disturbances could not been established, and these two factors have usually been considered independent, both requiring effective treatment[31].

VOLTAGE-GATED CALCIUM CHANNELS

Voltage-gated calcium channels are ion channels mediating calcium influx in response to membrane depolarization, and they regulate intracellular processes, such as contraction, secretion, neurotransmission and gene expression, in a variety of cells[39]. Calcium-channels are traditionally classified by their current properties and pharmacology[40]. The L (long-lasting)-type calcium channel is a large-conductance channel that produces long-lasting current at strong depolarizations, and it is generally inhibited by dihydropyridine (DHP) derivatives[41]. L-type currents are most important for muscle and endocrine cells, in which they mediate contraction and secretion[39]. In neurons and cardiac pacemaker cells, L-type currents can also be found to activate at lower voltages. N (neuronal)-type currents are also long-lasting, but they require strongly negative potentials for the complete removal of inactivation and strong depolarizations for activation, and they are not blocked by DHP[41]. In Purkinje cells, three further channels have been identi-

fied. P-type currents are blocked by low concentrations of ω-agatoxin, whereas the Q-type is only responsive to high concentrations. Residual currents, which were resistant to all known calcium-blockers at the time of their discovery, were called R (resistant)-type. The last group of voltage-gated calcium channels, the T (transient)-type, is characterized by a small and transient conductance activated upon weak depolarizations[41]. These currents are responsible for modulation of the action potential and for the performance of pacemaker activities.

The medical use of calcium antagonists started in the 1980s with DHP-type antagonists, which block L-type channels, in the treatment of hypertension by exploiting their properties as vasodilators[40]. Because a Ca^{2+} increase in smooth muscle is required for contraction, calcium antagonists induce relaxation of blood vessels, followed by a consequent reduction in blood pressure. Further, relaxation of the coronary arteries increases coronary flow, acting against angina pectoris. Calcium antagonists have no effect on skeletal muscles; however, they slightly influence cardiac muscle by decreasing pacemaker activity and conduction. Based on the well-known gastrointestinal motility impairments in IBS, calcium antagonists used for cardiovascular conditions appeared to be potential options for relieving symptoms by relaxing the colonic smooth muscles. Therefore, in the late 1980s, nicardipine was proposed for the treatment of irritable bowel syndrome, based on its spasmolytic properties[42]. Nonetheless, cardiovascular side effects have seriously limited the application of such calcium antagonists, thereby inspiring researchers to identify substances that act selectively on the gastrointestinal tract.

MODERN ANTISPASMODICS WITHOUT CARDIOVASCULAR SIDE EFFECTS

Alverine citrate

Experimental studies: Alverine citrate is an antispasmodic drug that inhibits calcium uptake and modulates smooth muscle activity. An experimental study on anesthetized cats showed that alverine acts on vagal sensory endings of the GI tract, where it decreases the responses of mechanoreceptors to mechanical and chemical stimuli[43]. Because chemically induced responses and smooth muscle contraction are both calcium dependent, decreased chemical sensitivity and smooth muscle relaxation can be explained by reduced calcium influx. In addition, a calcium-independent mechanism of action, such as selective 5-HT_{1A} receptor antagonism, might also be present, which has been demonstrated in rats using a 5-HT agonist-induced hypersensitivity model[44]. However, the picture is more complex than first thought - in guinea pig urinary bladder preparations, the frequency of spontaneous contractions in endogenously active smooth muscle was surprisingly increased by low doses of alverine citrate, whereas contraction amplitude was decreased. Higher doses of the drug could suppress both the frequency and amplitude of contractions; nevertheless, the authors speculated that the doses used in clinical practice would not reach this concentration in humans. They concluded that alverine citrate should be considered a true spasmolytic, because it suppresses the duration of spontaneous contractions of the gut, preventing local ischemia and reflectoric pain in the colonic wall evoked by "spasms". In addition, it has also been noted that alverine can increase calcium influx during action potentials by inhibiting the inactivation of calcium channels, but it reduces the sensitivity of contractile proteins to calcium, consequently suppressing the evoked muscular activity. Stress-induced colonic motility changes are an important factor in the pathogenesis of IBS; therefore, the clinical effects of alverine and the antifoaming agent, simethicone were tested in a rat model of colonic hypersensitivity induced by acute restraint stress[45]. Treatment with simethicone (200 mg/kg *po*) or alverine citrate (10 mg/kg *po*) reduced stress-induced increases in colonic permeability and hypersensitivity to distension, but lower doses were ineffective[45]. However, the combination of inactive doses of simethicone (100 mg/kg) with low doses of alverine (7 mg/kg) completely abolished the effects of stress, suggesting a synergistic action.

Clinical trials: Clinical studies on alverine citrate in IBS have been scarce (Table 1). In a randomized, placebo-controlled, double-blind clinical trial conducted in three British centers, after a 2-wk screening period, IBS patients selected according to the modified Rome criteria received 12 wk of treatment with 120 mg alverine citrate three times daily[46]. The patients completed diary cards about abdominal pain or discomfort, bloating, bowel movements, stool consistency, and general well-being; furthermore, the severity and frequency of abdominal pain, bloating, nausea and early satiety were assessed at study visits four times during treatment. Although abdominal pain, bloating and general well-being were all slightly more improved in the alverine-treated group than in the placebo group, when comparing the first diary card to the third, the difference was not statistically significant. This finding might be explained by the placebo effect being exceptionally high, sometimes reaching almost 70%, which would require a study with a much great number of participants to detect a possible positive effect of alverine. Regarding safety, no serious adverse events were reported in the study, and more patients experienced adverse events in the placebo group (48.1%) than in the alverine-treated group (39.6%). Using the well-known spasmolytic properties of alverine, a randomized, placebo-controlled trial showed that pretreatment of patients over 5 d with 60 mg alverine citrate plus 300 mg simethicone three times daily reduced intubation time during colonoscopy by 19%; nonetheless, it did not affect scores for pain, spasm, difficulty or cleanliness[47]. The combination of alverine with simethicone was also tested in a double-blind, placebo-controlled, randomized trial conducted in 17 sites in Hungary and Poland[48]. A total of 412 IBS patients meeting the Rome Ⅲ criteria received a combi-

Table 1 Characteristics and primary outcomes of randomized, double-blind, placebo controlled clinical trials in irritable bowel syndrome patients

Ref.	IBS population	Selection criteria	Treatment	Dose	Duration	Outcome
Mitchell *et al*[46]	All subtypes	Modified Rome	Alverine citrate *vs* placebo	120 mg *tid*	12 wk	No significant difference compared to placebo
Wittmann *et al*[48]	All subtypes	Rome Ⅲ	Alverine citrate + simethicone *vs* placebo	60 mg *tid* + 300 mg *tid*	4 wk	Significantly reduced abdominal pain and discomfort compared to placebo More therapy responders, regardless of stool pattern, compared to placebo
Connel *et al*[52]	All subtypes		Mebeverine *vs* placebo	100 mg *qid*	12 wk	Superior in controlling IBS symptoms compared to placebo
Kruis *et al*[58]	All subtypes		Mebeverine *vs* placebo *vs* Wheat bran	400 mg daily	16 wk	No significant difference compared to placebo
Enck *et al*[59]	All subtypes		Mebeverine *vs* placebo *vs* Dietary fiber		16 wk	Therapy response rate lower than placebo
Everitt *et al*[61]	All subtypes	Rome Ⅲ	Mebeverine *vs* methylcellulose *vs* placebo with/without cognitive behavioral therapy web site (assisted or not)	135 mg *tid* 3 tbl. *bid*	6 wk	No significant difference between drugs Significantly increased enablement at 6 and 12 wk in website group compared to no website group, significantly more participants scored their subjective assessment of global relief as improved at 12 wk in website group compared to no website group.
Baldi *et al*[69]	Abdominal pain predominant		Otilonium bromide *vs* placebo	40 mg *tid*		No significant difference in abdominal pain, bloating and general well-being compared to placebo, but significantly reduced sigmoid motility
Battaglia *et al*[70]	All subtypes	Drossman	Otilonium bromide *vs* placebo	40 mg *tid*	15 wk	Significantly better compared to placebo in reduction of abdominal pain frequency, global score improvement of abdominal pain and discomfort, therapy responder rate, reduced tenderness of the sigmoid colon, higher general well-being and global judgement of investigators; superior in improving severity of diarrhea/constipation, number of evacuations and mucus in stool; more effective in treating diarrhea, but not constipation
Clave *et al*[72]	All subtypes	Rome Ⅱ	Otilonium bromide *vs* placebo	40 mg *tid*	15 wk	Reduced abdominal pain frequency and bloating and improved stool frequency and patient global assessment compared to placebo; lower symptom recurrence after treatment
Awad *et al*[85]	All subtypes		Pinaverium bromide *vs* placebo	50 mg *tid*		Significantly reduced post-prandial rectal spike amplitude plus frequency and spontaneous recto-anal inhibitory reflex frequency compared to placebo
Chassany *et al*[98]	All subtypes	Rome Ⅱ	Phloroglucinol + trimethylphloroglucinol *vs* placebo	62.2 mg + 80 mg *tid*	1 wk	Significantly higher relative decrease of pain intensity and responder rate in the phloroglucinol plus trimethylphloroglucinol group compared to placebo; persisting treatment effect in a higher percent of patients treated with phloroglucinol plus trimethylphloroglucinol
Cha *et al*[99]	IBS-D	Rome Ⅲ	Phloroglucinol *vs* placebo	80 mg *tid*	2 wk	Significantly improved subjects' global assessment and decreased stool frequency

Characteristics and primary outcomes of randomized, double-blind, placebo controlled clinical trials in irritable bowel syndrome (IBS) patients with alverine citrate, mebeverine, otilonium bromide, pinaverium bromide and phloroglucinol. IBS-D: IBS with diarrhea.

nation of 60 mg alverine citrate and 300 mg simethicone or placebo three times per day for 4 wk. Combined alverine citrate and simethicone treatment achieved a higher reduction in abdominal pain and discomfort, as measured using visual analog scale (VAS) scores, and significantly more patients responded to therapy than to placebo, regardless of stool pattern. A visible, but not statistically significant, trend was also observed, showing greater improvement in IBS life impact scores with combination therapy than with placebo. No severe drug-related adverse events were noted in the study, and the numbers of adverse events were similar in both groups.

Mebeverine

Experimental studies: Mebeverine is a beta-phenyletylamine derivative of reserpine, which has relatively specific effects on smooth muscle cells without atropine-like side effects in humans[49]. It directly blocks voltage-operated sodium channels and inhibits intracellular calcium accumulation[49,50]. It is three times more potent than papaverine in inhibiting the peristaltic reflex of the guinea-pig ileum[51], but further animal studies on its pharmacological effect have been lacking.

Clinical trials: Mebeverine became treatment of inter-

est for IBS in the 1960s. In an early study by Connell[52], *iv* mebeverine decreased all sigmoid colonic motility, especially in hyperactive subjects, and it had less or no effect in hypoactive subjects. In a subsequent part of the study, mebeverine was superior to placebo at each time point over 12 wk of treatment in IBS patients in terms of symptom improvement and general well-being. Using prolonged ambulant manometry in 12 IBS patients and 6 healthy controls, compared to a placebo period, mebeverine had no significant effects on interdigestive small bowel motor parameters in controls; in contrast, a higher phase 2 motility index was observed in both IBS-D and IBS-C patients, and phase 3 motility was also affected[53]. These alterations in small bowel motile activity by mebeverine suggest possible spasmolytic and prokinetic effects in IBS patients.

Regarding symptom control in IBS, non-placebo-controlled studies have shown positive results. Significant improvement was observed after 6 wk of treatment with both the plain and sustained-release forms of mebeverine, with a minimal number of adverse events[54]. When comparing pinaverium bromide to mebeverine in 91 IBS-D patients, the improvements in global well-being were similar in the two groups, the daily defecation frequencies were markedly decreased, and stool consistencies became well formed in both groups, while no significant side effects were observed[55]. In a clinical trial comparing the effects of ramosteron, a 5-HT_3 receptor antagonist, to those of mebeverine in patients with IBS-D, both treatments were equally effective in reducing abdominal pain/discomfort and urgency and improving the stool form score and stool frequency compared to baselines[56].

However, when the effects of mebeverine have been compared to placebo and not compared to another drug or measured by self-control, the results have been controversial. A recent systematic review, including eight randomized trials, revealed that clinical improvement and relief of abdominal pain by mebeverine treatment were not statistically significant compared to placebo[57-59]. No differences were found in the effectiveness of 200 and 135 mg mebeverine doses. Tolerability was excellent, without significant adverse effects. Similarly, no positive effects of mebeverine over placebo were seen in an exploratory study performed in 135 IBS patients fulfilling the Rome Ⅲ criteria who were recruited from general practice, when mebeverine, methylcellulose and placebo were compared, with or without the combination of a cognitive behavioral therapy-based self-management web site (with or without additional telephone and e-mail support)[60,61]. Disappointingly, the use of the web site also did not improve IBS symptom severity scores or quality of life scores significantly over the "no web site" group; nevertheless, there was a visible trend toward continued improvement in the self-management group (particularly those with telephone support) throughout the study, while the "no web site" group and the medication groups seemed to lose their therapeutic gains from weeks 6 to 12. However, in a study performed in London, personal sessions of cognitive behavioral therapy were beneficial in addition to mebeverine, and the effects persisted for up to six months after therapy, both in terms of symptom relief and improvement in social and work disability[62]. Depression and anxiety predict poor outcomes in mebeverine-treated IBS patients, and in cases of patients with unhelpful coping behaviors (*e.g.*, avoidance), the combination of mebeverine with cognitive-behavioral therapy could be useful[63].

Otilonium bromide

Experimental studies: Otilonium bromide is weakly absorbable from the GI tract due to its quaternal ammonium structure; thus, it is almost completely excreted in the feces[64]. In experimental studies, it accumulated in the walls of the GI tract after oral administration, with minimal systemic absorption[65]. Its effects are rather complex, consisting mainly of L-type calcium-channel blockade, but binding to muscarinic M1, M2, M4 and M5 receptors has also been observed[66]. Antagonism of M3-coupled calcium signals in human colonic crypt cells suggested an anti-secretory action in IBS-D patients[67]. Additionally, by antagonism of tachykinin NK-2 receptors, otilonium not only causes spasmolysis but also reduces peripheral sensory afferent transmission to the central nervous system[64]. These effects suggest that otilonium could be effective in reducing both of the main symptoms of IBS: spasms and abdominal pain.

Clinical trials: In a small study of 15 IBS patients, one week of treatment with otilonium bromide significantly increased the pain threshold of IBS patients to anorectal distension, while thresholds for first sensation and stool remained unchanged[68]. In a multicenter, double-blind, placebo-controlled trial with 72 IBS patients in Italy, treatment with 40 mg otilonium bromide three times daily significantly decreased abdominal pain and bloating, improved well-being and global assessment, while it markedly increased the pain threshold during sigmoid distension. Nevertheless, these results did not differ from those of the placebo group[69]. However, otilonium significantly reduced sigmoid motility during distension, whereas placebo did not, suggesting the need for larger studies, a different setup or more accurate patient selection. Seven years later, the results of a larger trial were published in Italy, including 375 IBS patients selected by the Drossman criteria, when a 2-wk placebo run-in period was included to exclude patients with low compliance or with quickly resolving symptoms[70]. After randomization, the patients received 40 mg otilonium bromide or placebo three times daily for 15 wk, and their symptoms were assessed at weeks 5, 10 and 15. Abdominal pain frequency was reduced in both of the groups, with a statistically significant difference in favor of otilonium after 10 and 15 wk of treatment. The global score improvements in abdominal pain and discomfort were significantly greater in the otilonium group throughout the

whole study. Therapy was successful in significantly more patients treated with otilonium than in those treated with placebo. Defecation disturbances improved similarly in both groups. Tenderness of the sigmoid colon, general well-being and global judgements by the investigators all differentially improved in the otilonium-treated group. Extended analysis of the data from this study with different analysis forms 3 years later revealed that otilonium had therapeutic gains over placebo not only in terms of pain intensity, pain frequency and meteorism but also regarding the severity of diarrhea/constipation, the number of evacuations and the presence of mucus in stool[71]. When sorting patients according to stool habits, otilonium was more effective than placebo in treating diarrhea but was only as effective as placebo in managing constipation.

Otilonium bromide in irritable bowel syndrome (OBIS) was a recent international clinical trial in which patients diagnosed according to the Rome Ⅱ criteria received 40 mg otilonium bromide three times a day or placebo over 15 wk after randomization[72,73]. Otilonium bromide effectively reduced abdominal pain frequency and bloating, while improving stool frequency and patients' global assessments, compared to placebo. The prominent outcome of this study was the efficacy of otilonium in dramatically reducing abdominal pain frequency from more than half of the days to less than one day per week, compared to the persistent 1-3 episodes in the placebo group. Otilonium had no significant effects on pain severity, stool consistency or mucus in the stool. During the 10-wk follow-up period after finishing treatment, the likelihood of symptom recurrence was significantly higher in the placebo group than in the otilonium group. This finding might be explained by the elongated persistence of otilonium in the colonic wall due to its lipophilic properties. No serious adverse events occurred in the study, and only three adverse events, consisting of dry mouth or nausea, were judged by the investigator to be related to otilonium's side effects.

When compared to other spasmolytics in a meta-analysis, otilonium bromide performed outstandingly among 12 different antispasmodics in terms of IBS symptom control[74]. In a double-blind, randomized, active-controlled trial conducted in China of IBS patients selected according to the Rome Ⅱ criteria, the results confirmed the similar, but not superior, efficacy of otilonium to that of mebeverine in the management of the frequency and intensity of abdominal pain, and abdominal bloating, flatulence and satisfactory stool frequency were all improved similarly by both therapies[75]. The most common side effects of dry mouth and nausea/dizziness - recorded in previous studies as well-might have been caused by peripheral and central muscarinic antagonism, respectively, and could be explained by the known ability of otilonium to bind to muscarinic receptors[66].

Pinaverium bromide

Experimental studies: Pinaverium bromide is also a quaternary ammonium derivate that is poorly absorbed, with pronounced pharmacological effects in the gastrointestinal tract instead of the cardiovascular system[76]. It has a low absorption rate from the GI tract, corroborated by hepato-biliary excretion[64]. It has been shown that its effects are very similar to those of the established L-type calcium-channel blockers (nitrendipine, diltiazem, D600); that is, it reduces the plateau phase of slow waves, thereby inhibiting calcium influx and preventing consequent contractions[77]. Pinaverium has been shown to inhibit the contractile response in dog and rat colonic smooth muscle preparations to acetylcholine, the neurotransmitter of cholinergic intrinsic nerves[77,78]. Similarly, in colonic smooth muscle cells isolated from normal or inflamed human colons, pinaverium bromide inhibits contraction induced by different agonists (CCK 8, carbachol or KCl)[79]. In inflamed colonic cells, pinaverium exerts more pronounced inhibition than the non-GI-selective L-type calcium channel blockers nicardipine and diltiazem. This effect of pinaverium on colonic smooth muscle cells is mediated mainly by the inhibition of calcium influx through L-type calcium channels, thereby inhibiting contractions induced by acetylcholine or KCl in rat preparations[80]. Stress plays an important role in the pathogenesis of IBS; therefore, colonic smooth muscle preparations from cold restraint-stressed rats have also been examined, revealing that the hypermotility observed after stress is mostly related to increased calcium influx into the cells[80]. This observation supports the use of pinaverium in IBS, in which pathological colonic hypermotility must be suppressed. Furthermore, in rats chronically fitted with intraparietal electrodes in the proximal colon, pinaverium bromide has been found to have no effect on colonic long spike bursts in the fasting state, but it inhibits increases in colonic spike burst frequency induced by a meal or by CCK-8[81]. However, it loses its effects in capsaicin-pretreated animals, showing the participation of sensory afferent neurons in the mechanism of action, which might also explain the efficacy of pinaverium bromide in treating the two main features of IBS: motility disorders and gut hypersensitivity.

Clinical trials: Pinaverium bromide has been used for managing functional bowel disorders for decades, with double-blind studies performed as early as 1977[82]. Its effects on colonic smooth muscle have been well established by animal experiments; however, intensive research has only started to characterize its mechanism of modifying GI motility in humans. In an early pilot study performed in 12 IBS patients, colonic motility was detected by surface electromyography over a 2-h fasting period and a 2-h postprandial period following a standard meal, before and after 10 d of treatment with 50 mg pinaverium bromide three times daily[83]. The leading symptoms, such as abdominal pain, bloating and altered bowel habits, started to ameliorate on day 4 of treatment. Abnormal colonic motility patterns (viz., increased frequency and amplitude of contraction, arrhythmia in

motoric activity), which were particularly pronounced post-prandially, diminished after 10 d of treatment. In a continuation of this pilot study, the authors studied 22 IBS patients and 7 healthy controls[84]. The healthy controls received no treatment but served as controls for electromyographic measurements. The study protocol was as previously described, except for the length of pinaverium bromide therapy, which was extended to 14 d. The results showed increased fasting and postprandial colonic motility parameters in IBS patients compared to controls, which was effectively reduced by 14 d of pinaverium bromide therapy. Abdominal pain and bloating were also significantly improved by treatment. Stool frequency was normalized by pinaverium bromide therapy in both diarrheic and constipated IBS patients. The effects of pinaverium bromide on intestinal motility were affirmed by a further randomized, double-blind, placebo-controlled trial on IBS patients[85]. Pinaverium bromide was administered in a 50 mg dose (*po*, *tid*), and myoelectrical and mechanical activities of the rectum and the internal anal sphincter were recorded before treatment, in the fasting state and at 2 h post-prandially. Post-prandial rectal spike amplitude and frequency, as well as the frequency of the spontaneous recto-anal inhibitory reflex, were significantly decreased after treatment with pinaverium bromide. Pinaverium bromide was also able to change colonic transit and colonic responses to food in IBS patients, as demonstrated by a technique using orally ingested radiopaque markers visible on plain abdominal X-rays[86]. Beneficial effects of pinaverium bromide treatment were also demonstrated by an open trial, in which 61 treated IBS patients experienced significantly reduced abdominal pain, improved stool consistency, reduced defecation straining and urgency, and decreased mucus in stool, with good drug tolerance and few side effects[87]. The clinical efficacy of pinaverium bromide was also evaluated using a statistical technique new to the field-namely, by employing polar vectors on data from a phase Ⅳ clinical trial with 1677 Rome Ⅲ IBS patients receiving pinaverium bromide combined with simethicone[88]. The results showed amelioration of stool frequency and consistency in IBS-C, IBS-D and IBS-M patients; furthermore, the intensity of abdominal pain and bloating was also significantly reduced.

When comparing pinaverium to otilonium bromide in IBS, both treatments were similarly useful in reducing the intensity of pain and in regulating bowel movements, but otilonium was superior to pinaverium in terms of decreasing pain frequency[89]. The side effects were similar in the two groups. The use of pinaverium has generally been considered safe; however, the drug is not licensed for use in pregnant women. In a letter reporting ten involuntary cases of pregnant women taking pinaverium bromide due to dispensing errors, nine individuals delivered healthy babies, while the tenth experienced a spontaneous abortion 1 week after the ingestion of pinaverium[90]. Several women complained of abdominal pain and constipation in parallel with pinaverium use.

Phloroglucinol

Experimental studies: Phloroglucinol is a phenol derivative with non-specific antispasmodic properties, together with its methylated form trimethylphloroglucinol. The mechanism of action is most likely based on the direct inhibition of the voltage-dependent calcium channels of smooth muscle; however, the modulation of prostaglandin or nitric oxide release has also been suggested[91]. Although it has long been used in clinical practice as an antispasmodic for painful urogenital and gastrointestinal conditions, in an early study on anesthetized rats, phloroglucinol was found to be inactive toward the contraction of the duodenum, ileum and colon[92]. Similarly, in anesthetized dogs, phloroglucinol plus trimethyl-phloroglucinol failed to antagonize acetylcholine-induced contraction of the colon[93].

Clinical trials: In parallel with animal studies, phloroglucinol plus trimethyl-phloroglucinol had no clear effects in humans on ascending and sigmoid colon hypermotility evoked by neostigmine[94]. However, in 20 IBS patients, *iv* phloroglucinol effectively reduced postprandial rectosigmoid motility increases after a test meal, compared to placebo[95]. In another study of IBS patients, phloroglucinol inhibited phasic contractions provoked by intrarectally injected glycerol, but it did not modify colonic tone[96]. In an open-label study of 100 IBS patients selected according to the Rome Ⅱ criteria, *po* 50 mg phloroglucinol was administered three times daily for two months[97]. The 68 patients who completed the study reported significant improvement in abdominal pain, frequency of stools per day, urgency, passage of mucus per the rectum, sense of incomplete defecation and bloating. Nevertheless, straining was unchanged. Further, a multicenter, randomized, double-blind, placebo-controlled trial examined the effects of phloroglucinol/trimethylphloroglucinol (62.2 mg P plus 80 mg TMP three times daily) or placebo for 7 d in 307 IBS patients diagnosed using the Rome Ⅱ criteria[98]. The relative decrease in pain intensity and the responder rate were significantly higher in the P/TMP-treated group, compared to the placebo-treated group. Further, the treatment effect persisted up to the 7th day in a higher percentage of patients treated with P/TMP than in those treated with placebo. The frequency and severity of adverse events did not differ between the two treatment groups, and no adverse events were considered sufficiently serious to stop treatment. Finally, according to a preliminary report, 72 patients with D-IBS, based on the ROME Ⅲ criteria, were involved in a double-blind, placebo-controlled trial and were treated with placebo or phloroglucinol (80 mg) three times daily for 14 d after a 1-wk run-in period[99]. Significantly more patients reported "moderate or more improvement" in Subjects Global Assessment in the phloroglucinol group than in the placebo group over the 2-wk period of treatment and the 1-wk post-treatment period. Stool frequency decreased significantly in the phloroglucinol group, compared to the placebo group. Individual symptom scores and stool

consistency also improved significantly, but they did not differ from those of the placebo group. Regarding its safety, a French epidemiologic study of phloroglucinol in pregnancy did not find evidence of a teratogenic risk in humans[100].

FUTURE PERSPECTIVES: T-TYPE CALCIUM CHANNELS

The low-voltage-activated or T-type Ca^{2+} channels (T-channels) are a subclass of voltage-gated Ca^{2+} channels named after their characteristic of being activated by small depolarizations of the plasma membrane[101,102]. They can also generate neuronal spontaneous firing and pacemaker activities, and they generally control excitability[101,102]. In mammals, T-channels are encoded by three pore-forming calcium-channel $\alpha 1$ subunit genes: CaV3.1, CaV3.2 and CaV3.3. The CaV3.2 subtype is expressed in the cell bodies and nerve endings of somatic afferent fibers, where it plays a role in regulating neuronal excitability and modifying pain perception[103,104]. Knockout of the CaV3.2 gene results in decreased mechanical, thermal and chemical sensitivity in mice, compared to their wild-type littermates[105], whereas systemic injections of mibefradil, a T-channel antagonist, induces mechanical and thermal antinociception in rats without affecting their sensorimotor abilities[106]. Interestingly, ethosuximide, an anti-epileptic and relatively selective T-channel blocker, elicits near-complete reversal of mechanical allodynia/hyperalgesia in a rat model of painful peripheral neuropathy induced by the chemotherapeutic agent paclitaxel, whereas opiates and the NMDA receptor antagonist MK-801 are only slightly or not effective in this model[107]. Despite the importance of T-channels in somatic pain perception, their roles in visceral perception and gastrointestinal pathologies have not been well established. Recently, an interesting study demonstrated the possible role of T-channels in the pathophysiology of IBS[108]. IBS was modeled in rats using intracolonic sodium butyrate injections, a method that induces colonic hypersensitivity by reproducing the elevated colonic butyrate concentrations found in a subset of IBS patients resulting from butyrogenic enteric flora[109]. CaV3.2 knockdown treatment prevented butyrate-induced hypersensitivity without modifying colonic sensitivity in control rats, suggesting that CaV3.2 channels do not significantly participate in colonic sensitivity under healthy conditions[108]. Further, the T-channel blocker mibefradil reversed butyrate-mediated colonic hypersensitivity by both intrathecal and topical routes. Similarly, intraperitoneal administration of other T-channel antagonists, ethosuximide and NP078585, produced robust antihyperalgesic effects[108]. T-channels were up-regulated in the dorsal root ganglions (DRGs) of butyrate-treated animals, and neuronal T-type current density was also increased, emphasizing the participation of T-channels in the mechanism of colonic hypersensitivity[108]. Based on these results, the antinociceptive effects of TTA-A2, a state-dependent CaV3 blocker, were tested recently *in vitro* in cell cultures and in mice DRGs, showing that TTA-A2 potently inhibited recombinant and native T-currents in sensory neurons expressing CaV3.2-like T-type channels, consequently decreasing their excitability[110]. Moreover, in the previously described rat IBS model, systemic administration of TTA-A2 robustly abolished butyrate-induced hypersensitivity and induced a statistically significant dose-dependent antihyperalgesic effect[110]. These results demonstrate that T-channel blockers are promising candidates for further research into novel analgesics that could be potentially useful for treating the characteristic symptoms of IBS, such as visceral pain and discomfort.

CONCLUSION

In conclusion, antispasmodics without cardiovascular actions, such as alverine citrate, mebeverine, otilonium bromide, pinaverium bromide and phloroglucinol, are widely used in therapy for IBS. Their effects are mostly based on their spasmolytic properties *via* the inhibition of calcium influx into smooth muscle cells. Further, otilonium could have direct inhibitory effects on primary sensory afferents, thus reducing hypersensitivity, which is a common feature in IBS. Otilonium and pinaverium are quaternary ammonium derivatives that are poorly absorbed from the GI tract, therefore mainly acting locally. Clinical trials with antispasmodics in IBS have sometimes been controversial, which can be explained by the marked placebo effect in many cases. Nevertheless, the overall results have generally been positive, showing that antispasmodics are able to regulate GI motility disturbances, defecation alterations and abdominal pain/discomfort, with excellent safety profiles. A new generation of calcium-channel blockers acting on T-type calcium channels could represent a novel therapeutic pathway in the future for the management of IBS.

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Acupuncture-moxibustion in treating irritable bowel syndrome: How does it work?

Xiao-Peng Ma, Jue Hong, Cai-Ping An, Dan Zhang, Yan Huang, Huan-Gan Wu, Cui-Hong Zhang, Sian Meeuwsen

Xiao-Peng Ma, Jue Hong, Dan Zhang, Huan-Gan Wu, Cui-Hong Zhang, Key Laboratory of Acupuncture-moxibustion and Immunology, Shanghai Research Institute of Acupuncture and Meridian, Shanghai 200030, China
Cai-Ping An, Department of Acupuncture and Moxibustion, Obstetrics and Gynecology Hospital of Fudan University, Shanghai 200011, China
Yan Huang, Graduate School, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China
Sian Meeuwsen, Research Department, Bodystat Ltd., Douglas IM99 1DQ, United Kingdom
Author contributions: Ma XP, Wu HG and Zhang CH made the concept of the manuscript; Ma XP, Hong J, Zhang D, An CP and Huang Y collected the data; Ma XP, Hong J and An CP collated the data and wrote the manuscript together; Zhang CH and Wu HG approved the final version of the manuscript; Meeuwsen S edited the language.
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Correspondence to: Cui-Hong Zhang, PhD, Key Laboratory of Acupuncture-moxibustion and Immunology, Shanghai Research Institute of Acupuncture and Meridian, South Wanping Road, Shanghai 200030, China. zjtnyx@126.com
Telephone: +86-21-64690257 Fax: +86-21-64382181
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Abstract

Irritable bowel syndrome (IBS) is a functional intestinal disease characterized by abdominal pain or discomfort and altered bowel habits. It has drawn great attention because of its high prevalence, reoccurring symptoms, and severe influence on patients' lives. Many clinical studies have demonstrated the efficacy of acupuncture-moxibustion in treating IBS. Increasing attention has been paid to research regarding the action mechanisms of acupuncture-moxibustion for IBS, and the adoption of modern techniques has achieved some progress. This article reviews the latest advances among action mechanism studies from the perspectives of gastrointestinal motility, visceral hypersensitivity, the brain-gut axis, the neuroendocrine system, and the immune system. It is shown that acupuncture-moxibustion can effectively regulate the above items, and thus, this treatment should have a high efficacy in the treatment of IBS. This article also identifies existing problems in current mechanism research and raises several ideas for future studies. Further revelations regarding these action mechanisms will promote the application of acupuncture-moxibustion in treating IBS.

Key words: Irritable bowel syndrome; Acupuncture-moxibustion; Mechanism study; Gastrointestinal motility; Visceral hypersensitivity; Brain-gut axis

Core tip: This is a review of the latest advances made towards identifying the action mechanisms of acupuncture-moxibustion in treating irritable bowel syndrome (IBS). How does this ancient therapy affect gastrointestinal motility, visceral hypersensitivity, the brain-gut axis, the neuroendocrine system, the immune system, and other factors involved in the pathogenesis of IBS? This paper details answers to these questions.

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INTRODUCTION

Irritable bowel syndrome (IBS) is a common chronic functional gastrointestinal condition that is majorly char-

acterized by abdominal pain, bloating, and disturbed defecation and is coupled with psychological conditions. Falling under the scope of "abdominal pain", "diarrhea", or "constipation" in traditional Chinese medicine (TCM), it exists worldwide with a relatively high prevalence[1-3]. Because its recurrent symptoms severely affect the quality of life (QoL) of patients, driving them to consult extensive medical resources, IBS has drawn great global attention. A great amount of epidemiological research from various countries at different times has revealed that the prevalence and distributing characteristics of IBS vary according to different countries, regions, and populations. According to the Rome Ⅲ diagnostic criteria, the global IBS prevalence is between 5% and 20%[4,5].

The pathogenesis of IBS remains unknown. However, during recent years, pathophysiological research has increasingly indicated that multiple factors, such as genetic factors, psychological factors, diet, infections, immunity, and the brain-gut axis, can combine in a complex manner, leading to the visceral hypersensitivity and gastrointestinal dysmotility that are manifested by corresponding symptoms[6-9].

Acupuncture-moxibustion is a crucial part of TCM, comprising both acupuncture and moxibustion methods. As external treatments of TCM, acupuncture and moxibustion act by stimulating acupoints to unblock the meridians and collaterals, regulating the function of qi and blood, supporting health and expelling pathogens. When acupuncture needles are inserted into acupoints, needling manipulations, such as twirling and lifting-thrusting needles, are usually adopted to treat diseases. After the acupoints are targeted, moxibustion allows for the further treatment of diseases by the thermal stimulation generated by ignited moxa. Thus far, a large number of studies have proven the efficacy of acupuncture-moxibustion in attenuating the symptoms of IBS without producing obvious adverse effects[10]. Meta-analyses have also revealed that the therapeutic efficacy of acupuncture plus moxibustion is better than that of Western medications for IBS[11-14]. One clinical study also showed that acupuncture enhanced and extended the prescribed regimen's efficacy in treating IBS[15]. As its therapeutic efficacy has been confirmed, the action mechanisms of this traditional therapy have become the focus of much current research.

REGULATION OF GASTROINTESTINAL MOTILITY

The gastrointestinal dysmotility in IBS can be further classified into four groups based on their clinical features: spastic colon syndrome, functional diarrhea, diarrhea-predominant spastic colon syndrome, and midgut dysmotility[16]. Stress reactions can either enhance or attenuate dysmotility as well as subsequent symptoms. The pathophysiology of IBS involves dysmotility of both the colon and the small intestine, and the migratory motor complex (MMC) cycles have been reported to be shorter in diarrhea-predominant IBS (D-IBS) patients but longer in constipation-predominant IBS (C-IBS) patients[17].

In a clinical study, 10 patients with D-IBS conforming to the Rome Ⅲ diagnostic criteria received acupuncture and were observed for the changes in their borborygmus frequency and colonic peristalsis after acupuncture intervention. Compared with 10 healthy controls, the borborygmus frequency and colonic peristalsis were significantly higher before acupuncture ($P < 0.01$). However, after the acupuncture treatment, the two metrics of the IBS patients had been downregulated ($P < 0.05$). These results indicate that acupuncture can immediately regulate colonic peristalsis in D-IBS patients[18]. An electrocolonogram (ECOM) revealed that acupuncture at Zusanli (ST 36) (Figure 1A) was able to produce a virtuous bidirectional regulation of the ECOM in IBS cases of different TCM syndromes. Before treatment, IBS patients with splenic deficiency due to dampness had a decreased frequency of peak (Fp) in the sigmoid colon, suggesting that the tension of sigmoid colon should be low. The amplitude of peak (Ap), Fp, and the average zero-crossing frequency (Fz) increased after acupuncture, revealing that acupuncture at ST 36 can enhance colon contraction. In contrast, Ap, Fp, and Fz were abnormally high in IBS patients due to liver-intestine qi stagnation before acupuncture, suggesting that the sigmoid colon was hyperactive and the intestine wall was extremely contracted. After acupuncture, Ap, Fp, Fz dropped significantly, revealing that acupuncture at ST 36 downregulated colonic motility[19].

In animal experiments, IBS rat models were designed to observe the effect of electroacupuncture on intestinal dysmotility. Bilateral ST 36 and Shangjuxu (ST 37) (Figure 2A) were treated with electroacupuncture by selecting sparse-intense waves [100 Hz/2 Hz; 1, 2, 3 mA (increased by every 10 min)]. Each session lasted 30 min, and sham electroacupuncture was adopted in the controls. Compared with normal controls before treatment, the colonic peristalsis was significantly higher in adult IBS rats ($P < 0.05$). After 30 min of electroacupuncture treatment, the colonic peristalsis of the IBS rats had dropped ($P < 0.05$), while the IBS rats in the sham electroacupuncture group showed no obvious changes in colonic peristalsis ($P > 0.05$). The above study showed that IBS rat models had an abnormally increased intestinal motility that was significantly suppressed by electroacupuncture[20]. Another experiment revealed that herb-partitioned moxibustion can enhance gastric emptying and small intestinal propulsion in rats with functional gastrointestinal disorders (FGIDs) due to liver depression and spleen deficiency[21].

The above studies all illustrate that acupuncture-moxibustion has positive regulatory effects on gastrointestinal dysmotility, constituting one of the most crucial mechanisms of acupuncture-moxibustion in treating IBS.

REGULATION OF VISCERAL HYPERSENSITIVITY

Visceral hypersensitivity refers to the decreased pain threshold of inner organs and more intense experience

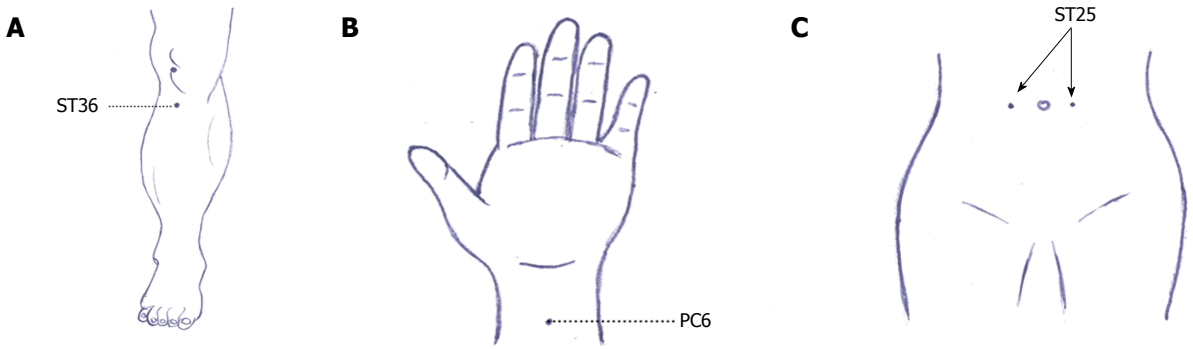

Figure 1 Acupoints of human. A: Acupoint Zusanli (ST 36); B: Acupoint Neiguan (PC 6); C: Acupoint Tianshu (ST 25).

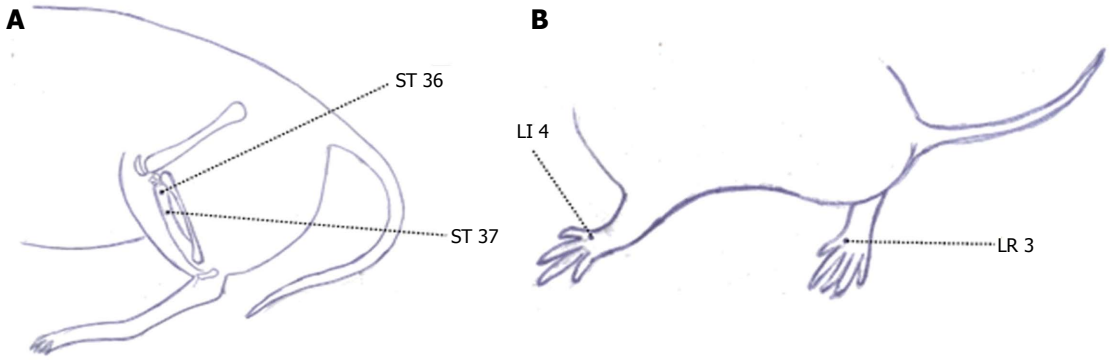

Figure 2 Acupoints of rat. A: Acupoint Zusanli (ST 36) and Shangjuxu (ST 37); B: Acupoint Taichong (LR 3) and Hegu (LI 4).

of stimuli. IBS patients of various subtypes and healthy volunteers underwent rectal noxious stimulation with an air balloon and a water balloon. IBS patients were found to have significantly lower thresholds for abdominal discomfort than healthy volunteers, and the hypersensitivity subgroups had significantly lower thresholds than the normosensitive subgroups[22,23]. Another study discovered that hypersensitive IBS patients had more severe gastric conditions than normosensitive patients[24].

A clinical study on D-IBS found that transcutaneous electrical acustimulation (TEAS) at Neiguan (PC 6) (Figure 1B) and ST 36 significantly increased the threshold for the rectal sensation of gas, the desire to defecate, the sensation of pain, improved rectal perception, and attenuated visceral hypersensitivity[25]. D-IBS patients usually experienced the urge to defecate and a decreased pain threshold. Short-term transcutaneous electrical nerve stimulation (TENS) was able to increase the threshold of rectal perception. After a 2-mo TENS intervention, the threshold of rectal perception had obviously increased in the D-IBS patients, while the defecation frequency and pain intensity had obviously decreased. The psychological scores dropped to a normal level[26]. In animal experiments, the abdominal withdrawal reflex (AWR) and abdominal myoelectric activity (AMA) were adopted to evaluate intestinal sensitivity. Studies on electroacupuncture or moxibustion as IBS interventions all showed that the acupuncture-moxibustion effectively alleviated visceral hypersensitivity in IBS rats[27-30].

REGULATION OF THE BRAIN-GUT AXIS AND THE NEUROENDOCRINE SYSTEM

The role of the brain-gut axis has drawn great attention regarding the pathogenesis of IBS. Because the inducing factors of IBS, such as gastrointestinal dysmotility, visceral hypersensitivity, infection, and mental conditions, are all included in this system, the potential for breakthroughs in studying the pathogenesis of IBS is considerable. The brain-gut axis is a complex, bidirectional signaling system between the central nervous system (CNS) and the gastrointestinal system. The regulation of gastrointestinal function *via* the brain-gut axis is called brain-gut interaction.

Regulation of brain-gut peptides

Brain-gut interaction is realized by multiple neural transmitters that reside in the endocrine cells of the CNS, enteric nervous system (ENS) and the gastrointestinal tract. They work as both neurotransmitters and hormones and are termed brain-gut peptides. Brain-gut peptides work extensively to regulate gastrointestinal activities and are thus closely related to IBS. The major excitatory neurotransmitters include histamine, 5-HT, substance P (SP), calcitonin gene-related peptide (CGRP), and corticotropin-releasing factor-related peptide (CRF). The major inhibitory neurotransmitters include cholecystokinin (CCK), NO, norepinephrine (NE), and vasoactive

peptide (VIP)[31].

Approximately 95% of 5-HT arise from the enterochromaffin cells (EC) of the intestinal mucosa, which are involved in the regulation of intestinal movement and perception. Changes in the 5-HT signal system are among the pathophysiological feature of IBS[32]. A clinical study was conducted to observe the change in colonic mucosal 5-HT among D-IBS patients and to assess the efficacy of herb-partitioned moxibustion. The results showed that IBS patients had a significantly increased expression of 5-HT in the colonic mucosa, while herb-partitioned moxibustion simultaneously improved IBS symptoms and downregulated the level of 5-HT[33]. Meanwhile, herb-partitioned moxibustion has been shown to downregulate the concentration of serum 5-HT in IBS patients[34]. Laboratory studies[35,36] also discovered that electroacupuncture enhanced the pain threshold of rats with chronic visceral hypersensitivity (CVH), downregulated the abnormally increased 5-HT level in colons, and enhanced the expression of 5-HT4 receptor and serotonin transporter (SERT), although the 5-HT3 receptor was insignificantly influenced. Therefore, we can conclude that electroacupuncture improves visceral hypersensitivity and stress-induced colonic dysfunction *via* the 5-HT signal system. Another study also showed that both herb-partitioned moxibustion and suspended moxibustion can increase the pain threshold and relieve hypersensitivity in CVH rats[37].

CRF and its receptor both play important roles in the onset and development of IBS and may work synergistically *via* the CNS and peripheral systems[38]. As a key factor in the pathogenesis of IBS[39], CRF can induce repeated defecation in rats and mice, although this activity can be blocked by its antagonist[40]. CRF also alters the rectal perceptions of human beings[41]. Anxiety, depression, altered colonic movement, and visceral algesthesia are involved in the CRF/CRF1 signal pathway in the brain, whereas the activation of central and peripheral CRF2 receptor can inhibit this pathway[42]. We discovered that electroacupuncture effectively downregulated the hypothalamic CRF concentrations of CVH IBS rats[43]. The TENS-related experiment also confirmed that TENS can downregulate the hyper-expression of CRF in the PVN of rats[44].

Moreover, the concentrations of other brain-gut peptides, *e.g.*, SP, VIP, and neuropeptide Y (NPY), are also changed in IBS patients, and these changes all play crucial roles in the pathogenesis of IBS[45]. It has been reported that electroacupuncture can regulate the secretion of colonic SP, SP receptor, and VIP in CVH IBS models[46]. It has also been found that acupuncture at ST 37 can reduce the concentrations of serum motilin (MTL) and somatostatin (SS) in CVH IBS rats[47]. A D-IBS model was developed by chronic mild restraint stress plus neonatal maternal separation and gastric administration of *Fan Xie Ye* (Folium Sennae) to observe the effect of acupuncture at bilateral ST 36 and Taichong (LR 3) (Figure 2B). The results showed that acupuncture at ST 36 and LR 3 inhibited the production of SS, SP, and VIP while increasing the level of NPY, which may be related to the efficacy of acupuncture in treating IBS[48,49]. Moreover, the effect of acupuncture on brain-gut peptides may be specific. A study observed the effect of electroacupuncture at ST 36 and Hegu (LI 4) (Figure 2B) on colonic NPY and SS of IBS rats prepared by the rectal administration of acetic acid (AA). The results showed that, after electroacupuncture, the hypothalamic NPY increased significantly in both electroacupuncture groups, while the colonic NPY level showed no obvious change. The colonic SS decreased in both electroacupuncture groups after intervention, while the plasma SS level only dropped significantly in the electroacupuncture group of ST 36, not in the LI 4 group[50].

The above results indicate that acupuncture-moxibustion can improve intestinal motility and visceral sensitivity by regulating brain-gut peptide levels in the CNS, intestines, and blood.

Regulation of nervous system

Effect on brain activation: IBS patients all experience visceral hypersensitivity of different levels. Over the recent years, studies on CVH IBS have proven that the abnormal activities of pain processing systems, including the anterior cingulate cortex (ACC), prefrontal cortex, insular cortex, thalamus, dorsal pons, and periaqueductal gray matter, are more or less associated with the IBS pathogenesis[51-53].

Functional magnetic resonance imaging (fMRI) has realized its capacity for non-invasive study on the effect of acupuncture-moxibustion on brain activation in IBS patients. By using PET to observe the functional changes of the visceral sensory center under rectal distension in D-IBS patients and the effect of electroacupuncture at Tianshu (ST 25) (Figure 1C) on the visceral sensory center, the study revealed that IBS patients had an increased glucose metabolism in the bilateral superior temporal gyri, right middle occipital gyrus, right superior frontal gyrus, and bilateral middle frontal gyri, but not in the visceral sensory center. Rectal distension enhanced the glucose metabolism in the prefrontal cortex, left cingulate gyrus, anterior and posterior central gyri, and temporal gyrus and also activated part of the visceral sensory center, including the anterior cingulate gyrus. Electroacupuncture at ST 25 significantly downregulated the glucose metabolism in the left cingulate gyrus, right insula, right parahippocampal gyrus, precuneus, and right caudate nucleus[54]. SPECT was adopted to observe the effects of eye acupuncture on cerebral blood flow in D-IBS patients. It was discovered that the blood flow in bilateral thalamus dropped significantly after eye acupuncture, indicating that a reduction of the blood flow in specific brain regions was involved in the action mechanisms of eye acupuncture in treating D-IBS[55]. Researchers once used fMRI to observe the effect of electroacupuncture and sham electroacupuncture on brain activities in IBS patients in different sessions (pre-intervention, during

intervention, and post-intervention). A blank control group was set up for comparative study. Of the participants who underwent rectal distention, there was no regional difference in brain activation between the electroacupuncture and sham electroacupuncture groups at baseline. In the electroacupuncture group, increased brain activation from the baseline was observed in the perigenual cingulate cortex, the bilateral prefrontal cortex and the temporal lobes, while high activation was noted at the right insula and bilateral somatosensory cortex during intervention. In the sham electroacupuncture group, increased brain activity was observed only in the anterior cingulate cortex, bilateral prefrontal cortex and left somatosensory cortex. The electroacupuncture group had significantly higher brain activation in the right insula and thalamus than the sham electroacupuncture group. After intervention, the brain activation in the electroacupuncture group significantly dropped in all regions; however, in the sham electroacupuncture group, the brain activation decreased in the temporal lobes and anterior cingulate gyrus but increased in the prefrontal cortex. No significant difference in brain activation was detected between the two groups. Of the participants who did not receive a rectal distension, there was no significant difference in the brain activation between the electroacupuncture and sham electroacupuncture groups, regardless of the different sessions[56].

The locus ceruleus is a nucleus located on the brain stem and plays an important role in producing norepinephrine. It is also closely related to stress reactions. When observing the effects of electroacupuncture at ST 37 on the neuronal discharge of the locus ceruleus in rats with acute restraint stress undergoing noxious rectal distension, scholars found that acupuncture at ST 37 inhibited the neuronal discharge that was activated by noxious rectal distension ($P < 0.01$). This result indicates that electroacupuncture may regulate rat colonic functions by downregulating the activation of neurons in the locus ceruleus[57]. A novel study observed the excitability of visceral sensory neurons in the rostral ventromedial medulla (RVM) and the *N*-methyl-*D*-aspartate receptor 1 (NR1) in IBS rats, before and after electroacupuncture at ST 36 and ST 37. It was noted that electroacupuncture significantly inhibited the hyper-excitability of the neurons and the expression of NR1 in RVM. This result suggests that the inhibition of NR1 should be an important factor in reducing the hyper-excitability of the neurons in RVM, as well as one of the action mechanisms of acupuncture in alleviating visceral hyperalgesia[58].

Regulation of neuronal excitability in spinal cord: The dorsal horn of the spinal cord is a crucial contributor to visceral and somatic perception. Thick myelinated primary afferent fibers (Aα, β) and thin myelinated/unmyelinated primary afferent fibers (Aδ and C), projection neurons (T), and inhibitory interneurons in the substantia gelatinosa form a neural web for the segmental regulation of the spinal cord. In IBS, the increased response of dorsal horn cells to the current afferent impulses and old under-threshold afferent impulses leads to an increased response to non-noxious stimulation, enlarged perception region, and decreased activation threshold. An action mechanism study on acupuncture-moxibustion for alleviating visceral hyperalgesia adopted colorectal distension (CRD) as the noxious stimulation. The study found that CRD activated the convergent neurons in the dorsal horn; the mechanical stimulation to contra-lateral body surface and hand acupuncture at ST 36 inhibited this noxious response. Thus, acupuncture and noxious stimulation are believed to meet and interact on the level of the spinal cord, and acupuncture can inhibit the neuronal activation induced by noxious stimulation in an action involving the spinal cord at even higher levels[59]. C-Fos is a proto-oncogene that is the human homolog of the retroviral oncogene v-fos. The expression of the c-fos protein is strengthened during neuronal activation, and its production is considered the biological marker for the activation of nociceptive neurons[60]. Compared to normal rats, CVH IBS rats had significantly more activated c-fos neurons in the superficial laminae (SDH, laminae I and Ⅱ), nucleus proprius (NP, laminae Ⅲ and Ⅳ), and neck of the dorsal horn (NECK, laminae V and Ⅵ) in the spinal segments of L_6-S_2 and in the neck of the dorsal horn of T_{13}-L_2 ($P < 0.05$). Electroacupuncture significantly downregulated the number of activated neurons of c-fos in the SDH, NP, and NECK of L_6-S_2 and in the sub-region of NECK of T_{13}-L_2 ($P < 0.05$). Sham electroacupuncture produced no notable effects on the expression of c-fos protein. Therefore, electroacupuncture can significantly inhibit the hyper-excitability of visceral sensory neurons in the dorsal horns of IBS rats, which qualifies as one of the action mechanisms of acupuncture in attenuating chronic visceral hyperalgesia[61].

In addition, the *N*-methyl-*D*-aspartic acid (NMDA) receptor (NR) also participates in the sustainment of functional chronic visceral hyperalgesia. As an excitatory neurotransmitter receptor in the CNS of mammals, the NMDA receptor mediates the excitability of glutamic acid and other endogenous acidic amino acids. A hetero-oligomer composed of NR1 and NR2, NMDAR is widely distributed in the brain, spinal cord, and peripheral nervous system. NR1 is an essential component, while NR2 modulates the functional feature of the whole receptor[62]. The phosphorylation site of the NMDA receptor is located on serine-896, a sub-unit of NR1. Its phosphorylation is involved in the regulatory effect of orphanin on pain perception in the spinal cord, and the activated receptor plays a specific role in the development of visceral hyperalgesia[63]. Zhou *et al*[64] prepared IBS CVH models using neonatal SD rats at the age of 9 d. The study showed that the rat models had significantly higher expressions of NR1 mRNA in the lumbar enlargement of the spinal cord than normal controls ($P < 0.05$). While pre-treatment with electroacupuncture downregulated these expressions to a normal level, sham-electroacupuncture had no obvious effect. These results

indicate that electroacupuncture could realize its efficacy in treating IBS CVH by regulating the expressions of NR1 mRNA in the spinal cord. Tian *et al*[65] conducted a controlled study by giving electroacupuncture and sham electroacupuncture to rat models of IBS CVH, discovering that electroacupuncture downregulated the phosphorylation of NR1 in rats' $L_{4\text{-}5}$. Hence, the effect of electroacupuncture in alleviating CVH should be related to the downregulation of the phosphorylation of the NMDA receptor in the spinal cord.

Regulation of the ENS: The ENS plays an important role in the pathogenesis of IBS. The ENS is embedded throughout the intestine wall, from the mucosa to the serosa. Its sensory neurons report on the mechanical and chemical conditions, while the motor neurons control peristalsis and secretion[66]. ENS is composed of submucosal and myenteric plexuses. The submucosal plexuses are located in the submucosa, with motor neurons producing acetylcholine (ACh) and VIP; the myenteric plexuses are located between the inner and outer layers of the muscularis externa and contain excitatory neurons (transmitted by ACh, SP, *etc*.) and inhibitory neurons (transmitted by VIP, NO, *etc*.). The ENS is mainly in charge of peristalsis and secretions, as well as the regulation of visceral sensitivity. The reduction of neurons in the submucosal and myenteric plexuses has been discovered to be the plausible common pathogenic factor of D-IBS and C-IBS[67,68]. Electroacupuncture can increase the number of neurons in the myenteric plexuses of C-IBS rats[69], but the effect of acupuncture-moxibustion on the ENS neurons of D-IBS rats requires further study. ENS also regulates certain gastrointestinal activities via neurotransmitters, such as 5-HT, Ach, norepinephrine (NE), ATP, and multiple neuropeptides. Acupuncture-moxibustion produces a positive regulatory effect on the intestinal secretion of 5-HT, SP[36], SPR, VIP[46], SS[48], NPY[49], and AchE[70]; therefore, this procedure is able to correct the gastrointestinal dysmotility and visceral hypersensitivity.

Regulation of intestinal endocrine cells

EC is the predominant endocrine cell in the intestine. With the largest number and broadest distribution, ECs work to synthesize and contain 5-HT and produce peptides such as SP. Many studies[71,72] have shown that IBS patients can significantly increase the number of active ECs. C-IBS rat models were developed by the gastric administration of normal saline at 0 ℃-4 ℃ to observe the effects of electroacupuncture on the activation of colonic EC. The mean optical density (MOD) of the colonic EC has also been found to be significantly increased in the model rats ($P < 0.05$), and electroacupuncture has been shown to downregulate this increased MOD ($P < 0.05$). These results indicate that the correction of the abnormal status of the colonic EC may be one of the action mechanisms of electroacupuncture in treating C-IBS[73]. A study with IBS rat models developed by restraint and stress also showed that IBS rats increased the expression of the colonic EC, while eye acupuncture effectively downregulated these levels[74].

REGULATION OF IMMUNOLOGICAL FUNCTION

The activation of the immune system is deeply involved in IBS[75] and is majorly manifested by the abnormal expression of immune cells and active substances, *e.g*., T-lymphocytes and the subgroups, immunoglobulins, inflammatory cytokines, *etc*. A study determined the concentrations of serum IgM, IgG, IgA, C3 and C4, as well as the subgroups of T-lymphocytes in IBS patients, before and after herb-partitioned moxibustion. The results showed that IBS patients had significantly higher serum IgM concentrations than the normal controls, while the IgG, IgA, C3, and C4 concentrations were statistically equal between the two groups. The IgM levels remarkably decreased after herb-partitioned moxibustion, while the concentrations of the other items remained the same. The lymphocyte transformation of the IBS patients was significantly lower than that of the normal controls, and moxibustion restored the lymphocyte transformation to approach normal levels. Herb-partitioned moxibustion also significantly increased the T_8^+ in peripheral blood ($P < 0.01$), and the abnormal ratio of T_4^+/T_8^+ was effectively corrected. These results indicate that herb-partitioned moxibustion can correct the abnormal immune dysfunction of IBS[76]. IL-18, IL-23, and TNF-α are pro-inflammatory mediators. It has been reported that acupuncture-moxibustion can downregulate the expressions of serum IL-18, IL-23, and TNF-α in elderly IBS patients[77].

As a key cell in inducing intestinal dysfunction and paresthesia, the mast cell (MC) plays a crucial role in the immunopathological changes of IBS. The activated intestinal mucosal MC participates in the formation of visceral hypersensitivity in IBS, which constitutes a key portion of the pathogenesis of IBS[78]. It was found that CVH IBS rats had significantly lower visceral pain thresholds and an increased number of colonic mucosal MC compared to normal controls. Concurrently, electroacupuncture markedly reduced the number of MC, indicating that electroacupuncture can effectively regulate the production of colonic MC[27,46].

INTESTINAL FLORA IMBALANCE

The composition of the intestinal flora varies in individuals. IBS patients have been shown to lack Lactobacilli and Bifidobacterium[79]. Other scholars have found that the over-growth of small intestinal bacteria may also represent an important factor in inducing IBS[80]. Little research has reported on acupuncture-moxibustion interventions for IBS. However, Wang *et al*[81] found that the prevalence of Lactobacilli and Bifidobacterium increased in rats with ulcerative colitis (UC) after herb-partitioned moxibustion, indicating that moxibustion can produce certain regulatory effects on the intestinal flora. However,

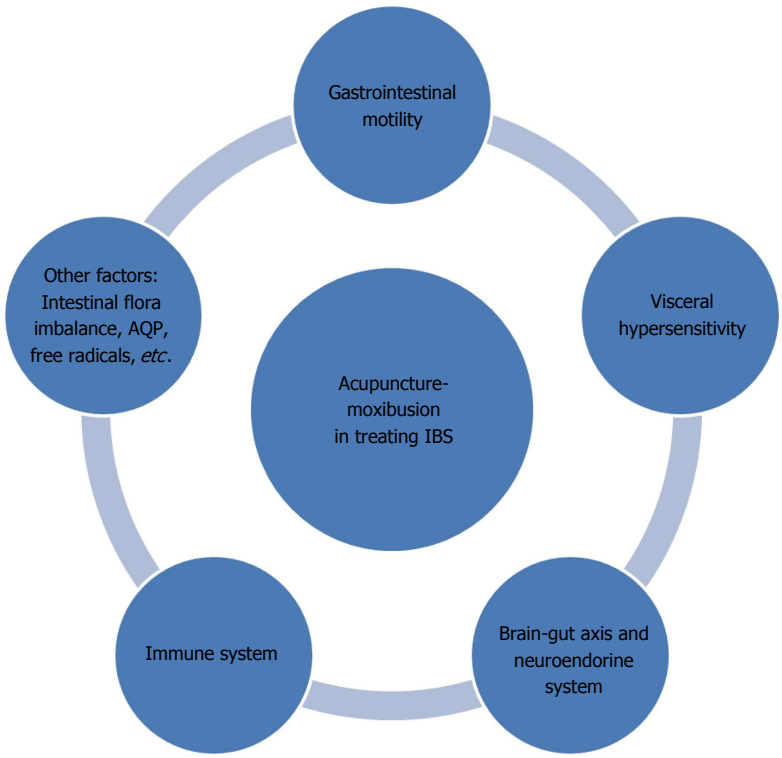

Figure 3 Multiple regulating channels of acupuncture-moxibustion in treating irritable bowel syndrome. IBS: Irritable bowel syndrome; AQP: Aquaporins.

further evidence supporting this indication is required.

OTHER ACTION MECHANISMS

Aquaporins (AQP) are integral membrane proteins from a larger family of major intrinsic proteins (MIP) that form pores in the membranes of biological cells. AQP3, AQP8, and AQP4 are broadly resident in human intestinal tissues, where they work to regulate the absorption and secretion of the intestines. By developing diarrhea-predominant IBS rat models with chronic stress and restraints, scholars found that IBS rats had significantly lower expressions of colonic AQP8 and AQP3 mRNA and protein. However, as eye acupuncture significantly increased the expressions of colonic AQP8 and AQP3 mRNA and protein, we believe that fluid transportation disturbances mediated by AQP8 and AQP3 could possibly be an important factor in developing diarrhea-predominant IBS models. The action mechanisms of eye acupuncture for IBS may involve the regulation of the expressions of colonic AQP8 and AQP3[82,83].

Due to the shortage of free radical scavengers in the blood of IBS patients, gastrointestinal nerves are easily damaged by free radicals. Scholars have investigated the relationship between free radicals and IBS, as well as the effects of acupuncture-moxibustion treatment, by determining the levels of spasm superoxide dismutase (SOD), malonyldialdehyde (MDA), and NO in IBS patients. The results showed that acupuncture-moxibustion is effective in treating IBS. Spasm SOD was significantly increased, while MDA and NO were significantly decreased after acupuncture-moxibustion. These data suggest that acupuncture-moxibustion can enhance the antioxidant capacity of the body, eliminating the accumulated free radicals and maintaining the stability of the intracellular environment and the functional status of the body[84].

CONCLUSION

Despite the high prevalence and vast influence of IBS, its pathophysiological action mechanisms have not been clearly understood. None of the theories put forth in the past, including intestinal dysmotility, visceral hypersensitivity, interaction of brain-gut, and dysimmunity, can fully explain the pathogenesis of IBS, as this entity is likely due to the interactive contributions of multiple factors. This article has reviewed the action mechanisms of acupuncture-moxibustion for IBS regarding the regulation of gastrointestinal motility, visceral hypersensitivity, brain-gut axis, neuroendocrine, and immunity and suggests that the treatment efficacy should be closely related to the regulation of the above aspects (Figure 3). Based on the current research, we can see that the study of the mechanisms used by acupuncture-moxibustion to treat IBS has been thoroughly pursued. The particular accomplishments of this research have provided scientific evidence to elucidate IBS's action mechanisms.

As acupuncture-moxibustion treats the body as a whole system through acupoints, its action mechanisms for the treatment of IBS could involve multiple segments, layers, and targets. Indeed, the current literature supports this perspective. These mechanisms were stud-

ied from various disciplinary perspectives and the variety of acupuncture-moxibustion methods makes it impossible to study systemic and comprehensive issues related to the action mechanisms of this method. Meanwhile, separate from medications, acupuncture-moxibustion is efficacious because it stimulates acupoints on the surface of body. Current studies have mainly focused on the regulation and mechanisms of the target organs after acupuncture-moxibustion has stimulated the appropriate acupoints. However, the precise action mechanisms of acupuncture-moxibustion used to stimulate the acupoints and the pathways through which IBS can be managed remain unknown. These questions still require further research to properly address them. Furthermore, both acupuncture and moxibustion, either in combination or alone, are effective in attenuating the symptoms of IBS. Is there actually any difference between these two treatments in activating acupoints and intervening in IBS? Although acupuncture and moxibustion both act through acupoints, they stimulate the acupoints in different ways, acupuncture through mechanical stimulation and moxibustion through thermal stimulation. Each of these questions should be kept in mind during further investigations. Moreover, we lack a universally approved IBS animal model[85]. The current models cannot fully reflect the clinical manifestations and pathogenic mechanisms of IBS. The use of different animal models and varying acupuncture-moxibustion methods has also caused the current research to be open to doubt and difficult to replicate. The ideal IBS animal model must integrate all of the associated factors of IBS, including its physiology, cognition, emotion, behavior, *etc*.[86,87].

By clarifying of the etiology and pathogenesis of IBS, establishing an ideal IBS animal model, standardizing an acupuncture-moxibustion intervention, and applying modern medical research methods and results, we should be able to better study the action mechanisms of acupuncture-moxibustion in the treatment of IBS and in the activation of acupoints, which will also help promote the application of acupuncture-moxibustion in the treatment of IBS.

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Gender-related differences in irritable bowel syndrome: Potential mechanisms of sex hormones

Mathieu Meleine, Julien Matricon

Mathieu Meleine, Department of Inflammation, Nutrition and Gut-Brain Axis Dysfunction, Inserm Unit 1073, Faculté de Médecine et Pharmacie, Université de Rouen, 76183 Rouen, France
Mathieu Meleine, Julien Matricon, Clermont Université, Université d'Auvergne, 63001 Clermont-Ferrand, France
Julien Matricon, Department of Pharmacology. University of Texas Health Science Center, San Antonio, TX 78229-3900, United States
Author contributions: Meleine M and Matricon J were jointly involved in bibliographic research, data extraction and manuscript writing.
Correspondence to: Mathieu Meleine, PhD, Department of Inflammation, Nutrition and Gut-Brain Axis Dysfunction, Inserm Unit 1073, Faculté de Médecine et Pharmacie, Université de Rouen, 22 Boulevard Gambetta, Cedex 1, 76183 Rouen, France. mathieu.meleine@hotmail.fr
Telephone: +33-2-35148240 Fax: +33-2-35148226
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Abstract

According to epidemiological studies, twice as many women as men are affected by irritable bowel syndrome (IBS) in western countries, suggesting a role for sex hormones in IBS pathophysiology. Despite growing evidence about the implications of sex hormones in IBS symptom modulation, data on mechanisms by which they influence disease development are sparse. This review aims to determine the state of knowledge about the role of sex hormones in sensorimotor dysfunctions and to address the possible interplay of sex hormones with common risk factors associated with IBS. The scientific bibliography was searched using the following keywords: irritable bowel syndrome, sex, gender, ovarian hormone, estradiol, progesterone, testosterone, symptoms, pain, sensitivity, motility, permeability, stress, immune system, brain activity, spinal, supraspinal, imaging. Ovarian hormones variations along the menstrual cycle affect sensorimotor gastrointestinal function in both healthy and IBS populations. They can modulate pain processing by interacting with neuromodulator systems and the emotional system responsible for visceral pain perception. These hormones can also modulate the susceptibility to stress, which is a pivotal factor in IBS occurrence and symptom severity. For instance, estrogen-dependent hyper-responsiveness to stress can promote immune activation or impairments of gut barrier function. In conclusion, whereas it is important to keep in mind that ovarian hormones cannot be considered as a causal factor of IBS, they arguably modulate IBS onset and symptomatology. However, our understanding of the underlying mechanisms remains limited and studies assessing the link between IBS symptoms and ovarian hormone levels are needed to improve our knowledge of the disease evolution with regard to gender. Further studies assessing the role of male hormones are also needed to understand fully the role of sex hormones in IBS. Finally, investigation of brain-gut interactions is critical to decipher how stress, ovarian hormones, and female brain processing of pain can translate into gut dysfunctions.

Key words: Irritable bowel syndrome; Pathophysiology; Gender; Sex hormones; Gut; Sensori-motricity; Neurosensitization; Stress; Immune activation; Permeabilization

Core tip: This review summarizes the current knowledge on the role of ovarian hormones in the pathophysiology of irritable bowel syndrome (IBS). A better understanding of gender differences in IBS may help unveil some key mechanisms contributing to IBS development. We present data on: (1) the modulatory role of ovarian hormones on IBS symptoms; (2) influence of ovarian hormones on risk factors associated with IBS; and (3) potential mechanisms of action, by which ovarian hormones can modulate and/or induce IBS symptoms.

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INTRODUCTION

Irritable bowel syndrome (IBS) is the most common functional gastrointestinal (GI) disorder. IBS is typically characterized by chronic abdominal pain and bowel habit disturbance[1]. The chronicity and the nature of IBS symptoms lead to major impairment in patients' quality of life and to a significant financial burden for the public healthcare system[2]. However, IBS management remains difficult because of the current lack of appropriate treatment[3]. Consequently, understanding the pathophysiological mechanisms underlying IBS is a key issue for drug development[4,5].

Epidemiological data on sex ratio in IBS provides an intriguing and often overlooked potential avenue for deciphering the pathophysiological mechanisms of IBS: the role of sex hormones in IBS etiology. Indeed, IBS is predominantly diagnosed in women, with a female to male sex ratio ranging from 2:1 (questionnaire-based diagnostics) to 4:1 (practice-based diagnostics)[6]. It is also noteworthy that many if not all the comorbid diseases associated with IBS also share this female predominance. To name the most common, fibromyalgia[7], migraine[8], other functional GI disorders such as functional dyspepsia[9], chronic pelvic pain[10], chronic fatigue syndrome[11], and depression[12] all have sex ratio skewed towards female gender[13].

Ovarian hormones can modulate intestinal function and transit speed[14-18]. These properties make ovarian hormones an interesting suspect for explaining gender differences in IBS. There is a strong correlation between IBS and dysmenorrhea[19] and variations in ovarian hormone levels during the menstrual cycle have been shown to modulate IBS symptomatology in women[17,20]. In particular, exacerbation of abdominal pain during perimenses (*i.e.*, the menstruation phase period characterized by low ovarian hormone levels) has been consistently observed when compared to other phases of the menstrual cycle[6,16,21-23]. Interestingly, the same findings have also been observed in inflammatory bowel disease, another chronic intestinal disease[24]. Lower ovarian hormone levels characterize the perimenses period, therefore, these observations suggest that female sex hormones have a protective role against IBS-associated pain[25]. However, studies in this field of research are scarce and the mechanisms of action of ovarian hormones are unclear. For instance, no correlation has been found between plasma ovarian hormone levels and premenstrual symptoms[16,20,26], suggesting more complex interactions between hormones and the gut sensorimotor system.

The goal of this review is to summarize the current knowledge on the influence of ovarian hormones on the pathophysiology of IBS. A better understanding of gender differences in IBS may be a useful approach to unveil some key mechanisms contributing to its development, and eventually, may provide new therapeutic strategies. In this review, we present data on: (1) the modulatory role of ovarian hormones on IBS symptoms; (2) influence of ovarian hormones on risk factors (*e.g.*, stress, permeability and immune system dysfunction) associated with IBS; and (3) potential mechanisms of action, by which ovarian hormones can modulate and/or induce IBS symptoms.

RESEARCH

Search strategy

A multidisciplinary search of the clinical and preclinical bibliography was conducted using Medline/PubMed to identify studies assessing involvement of sex hormones in the pathophysiology of IBS. The search was restricted to studies published in English up to October 2013. The following key words were used to identify original articles with potential relevance to gender differences in IBS, effect of menstrual cycle, menopausal status or hormonal treatments on IBS symptomatology: irritable bowel syndrome, sex, gender, ovarian hormone, estradiol, progesterone, testosterone, symptoms, pain, sensitivity, motility, permeability, stress, immune system, brain activity, spinal, supraspinal, imaging.

Data extraction and exclusion criteria

The bibliography search yielded a total of 473 articles. The articles retrieved by the Medline/PubMed search were evaluated based on their title and abstract. Studies including patients diagnosed using Rome Ⅰ, Ⅱ or Ⅲ criteria were included. Individual case reports, abstracts and studies published in languages other than English were excluded. Additionally, relevant publications cited in reviews, but which were not captured by the search strategy, were also considered. After selection, 175 papers were analyzed and used to write the review. The outcome measure was the description of ovarian-dependent changes in gut immunity, gut sensori-motricity, gut permeability, or pain processing, which were compared between IBS patients and healthy controls.

RESULTS

The first part of this review describes associations between gender and IBS intestinal symptoms, and aims at deciphering the influence of ovarian hormones on sensorimotor dysfunction. Sensorimotor dysfunction is the key feature of IBS pathophysiology and results in altered bowel habits and visceral hypersensitivity, the two major symptoms leading patients to seek medical attention. In the second part, the review details the impact of gender and ovarian hormones on central and peripheral alterations frequently associated with IBS, namely stress, gut barrier permeability and immune system activation.

Role of ovarian hormones in GI motility impairments in IBS

Female gender and influence of ovarian hormones on GI motility: Generally, women display slow GI transit compared to men, with delayed gastric emptying[27] and reduced colonic transit time[28]. This gender difference in GI motility could be, at least partially, due to ovarian hormone variations during the menstrual cycle. Indeed, it has been shown in healthy women that GI transit duration tends to be prolonged during the luteal phase and at onset of menses compared to the follicular phase[29], although this finding was not consistently replicated[30,31]. Further support is given to the idea of a modulatory role of ovarian hormones on GI motility by the frequent association between hormonal changes during pregnancy and the co-occurrence of GI motility disorders[32,33]. GI transit time is significantly prolonged in the third trimester of pregnancy when ovarian hormone levels are increased, compared to the postpartum period[32]. Although mechanical causes inherent to morphological changes during pregnancy can account for bowel habit disturbances[34], it seems that the endocrinological changes are more likely to be the accountable causes of GI motor impairments. Preclinical studies showed an increase in the release of NO in the vascular compartment and from the nonadrenergic, noncholinergic nerves, innervating the proximal colon, during late pregnancy compared with mid-pregnancy[35]. This NO increase is responsible for motility decrease in the GI tract and is thought to be mediated by estradiol[36]. Other animal studies confirmed the correlation between hormonal fluctuations and GI motility. In naive female rats, a biphasic transit pattern was observed during estrus cycling with a slow transit during proestrus and estrus (*i.e.*, the period preceding ovulation and during which estrogen levels rise up) compared to metestrus-diestrus phases (*i.e.*, the period following ovulation, characterized by decreased estrogen levels and increased progesterone levels)[37]. Moreover, pregnant animals have a transit time comparable to those of rats in proestrus-estrus phases, when estrogen levels are increased[37], thus supporting clinical observations.

GI complaints during the menstrual cycle can be related to motility changes and/or altered perception of intestinal motor events. These abnormalities can be, at least partially, mediated by changes in ovarian hormone levels as demonstrated by clinical and animal studies highlighting a differential role of these hormones on GI motility. Indeed, it has been shown that estrogens inhibit colonic smooth muscle contractility *via* a nongenomic mechanism as suggested by the rapid onset and reversible action of the ovarian/sex steroids[38]. Animal studies further showed that estrogens have a peripheral action on smooth muscle contractility. For instance, in ileal tissue isolated from ovariectomized rats, contractions induced by the cholinergic agonist carbachol were impaired in rats treated with estradiol for 3 d. This alteration is due to the inhibition by estradiol of RhoA signaling, a small GTPase protein known to regulate smooth muscle contractility[39]. Inhibitory effects of estrogen on colonic contractile activity have been confirmed in isolated rabbit distal colon in which estradiol potentiates the inhibitory effects of oxytocin on distal colonic contraction[40]. Interestingly, estradiol had no effects on the oxytocin-induced decrease in motility in the proximal colon[41], suggesting a fine regulation of intestinal motility by ovarian hormones depending on GI segments. Oxytocin and estradiol display diverse and sometimes opposite effects depending on species, methods and gut segments considered, adding complexity to our limited understanding of these mechanisms[42,43]. Thus, another report demonstrated that in rats, systemic administration of oxytocin excited colonic motility during proestrus and estrus when estradiol concentration is elevated but not during diestrus when estradiol concentration is low[44].

Estrogen can also affect intestinal motility *via* the involvement of peptidergic hormones. For instance, in ovacter-iomized rats, estradiol benzoate (EB), an estrogen-like hormone, inhibited GI transit and this inhibition was correlated with cholecystokinin (CCK) plasma concentration. CCK(A) receptor antagonists attenuated EB-induced inhibition of GI transit while CCK(B) receptor antagonists had no effects. These results suggest that GI transit estradiol-induced inhibition involves CCK and CCK(A) receptor activation[42].

Similarly to estrogen, data from studies performed in different species suggest an inhibitory effect of progesterone on GI tract motility[45-49]. However, it is noteworthy that depending on the dose, progesterone displays opposite effects. Indeed, while a decrease in GI motility is generally described with high doses, low dose of progesterone seems to induce a motility increase[47]. Several mechanisms have been proposed to explain the differential effects of progesterone. In the guinea pig distal colon, progesterone inhibits transit by altering the normal levels of prostaglandins that induce contraction (PGF2α) and relaxation (PGE2) of intestinal muscles. This is the result of changes in the pattern of G proteins which regulate prostaglandins expression[48]. Similar mechanisms have been observed in intestinal muscle cells from women with chronic constipation, in which G proteins promoting contractions were downregulated and inhibitory G proteins were, by contrast, upregulated. In this study, these abnormalities were reproduced *in vitro* by pretreatment of normal colonic muscle cells with progesterone, suggesting a direct involvement of progesterone and its receptor activation on colonic muscle contractility[48]. Interplay between progesterone and the serotoninergic system could also underlie altered bowel habits in women. Serotonin (5-hydroxytryptamine or 5-HT) is known to play a key role in the motor function of the GI tract by regulating smooth muscle contractility[50]. It has been shown that progesterone administration globally increased 5-HT levels by decreasing the level of serotonin transporter (SERT) which participates in 5-HT reuptake[51], monoamine oxidase mRNA[52,53] expression, and increasing the availability of 5-HT precursor, tryptophan[53]. This effect of proges-

terone on the serotoninergic system is surprising because 5-HT, in many cases, promotes peristalsis. However, recent studies demonstrated that in women with slow transit constipation (STC), progesterone receptors were overexpressed in colonic muscle[54] and epithelial cells[55]. SERT levels were lower and 5-HT concentration was higher than in healthy controls. These results are difficult to explain but the authors hypothesized that increased levels of 5-HT are mostly ineffective in female patients with STC because of the overexpression of progesterone receptors in muscle cells, which impairs the contraction of the circular muscle layer[55]. Nevertheless, the interaction between progesterone, 5-HT and muscle contractions remains to be clarified.

Influence of ovarian hormones on motility in IBS pathophysiology: The aforementioned studies were conducted in healthy volunteers, naive animals or isolated organs. Only few studies assessing the effects of ovarian hormones on intestinal motility are available in IBS patients or in animal models of impaired motility. Female IBS patients are more likely to report constipation-related symptoms including abdominal distension, bloating, infrequent stools and hard stools than men with IBS[56]. In contrast, men report more frequently diarrhea-related symptoms of loose stools and increased stool frequency[57]. Interestingly, at the time of menses, when ovarian hormones are low, diarrhea symptoms become more prevalent in women than constipation does[56]. It is important to notice that menstrual cycle effects on motility symptoms were similar in healthy women and IBS patients but symptom severity was greater in women with IBS[56]. The 5-HT system plays a pivotal role in motility impairment associated with IBS, because it has been shown that postprandial platelet-depleted plasma 5-HT concentration was abnormally elevated in IBS patients with diarrhea (IBS-D)[58-60] but reduced in IBS patients with constipation (IBS-C)[60,61] compared with healthy volunteers. These observations are consistent with animal studies reporting accelerated GI transit induced by increased 5-HT levels[62]. Houghton *et al*[63] highlighted the influence of the menstrual status on plasma 5-HT concentration by showing that IBS-D women who exhibit lower estrogen and progesterone levels at time of menses have an increased 5-HT plasma concentration compared to IBS-D women with high ovarian hormone levels. Although no correlation with motility symptoms were made in this study, it pointed out differential 5-HT metabolism depending on the cycle phases considered in IBS-D patients. Given the involvement of 5-HT system in GI motor function, these data suggest a dynamic role for ovarian hormones in GI transit impairments observed in IBS-D patients.

Key points: Women display slow GI transit compared to men; transit time is prolonged when ovarian hormone levels are high; estrogen and progesterone inhibit smooth muscle contraction**;** progesterone modulates the colonic 5HT system, which is known to regulate peristalsis**;** female IBS patients report constipation-related symptoms more often than men, except at the time of menses, when ovarian hormone levels are low**;** ovarian hormones may contribute to altered motility in IBS-D patients by interacting with the 5HT system.

Role of ovarian hormones in visceral hypersensitivity in IBS

Influence of gender and sex hormones on visceral pain: Over the past 20 years, clinical studies regarding the influence of gender on pain responses have built up a body of evidence demonstrating that women are at greater risk for many pain conditions than men. It is now well established that prevalence of most common forms of pain is higher in women than men, and women display increased sensitivity to several forms of experimentally induced pain including visceral pain[13,64,65]. Accordingly, women are more likely to report abdominal pain, increased pain perception and discomfort to colorectal distension (CRD), and pain-related IBS symptoms than men do[56,66,67]. Similar observations have been made in rodents, in which females have greater visceromotor response to CRD than males[68-72]. The influence of gonadal hormones on nociception could explain these gender-related differences. Accordingly, animal studies showed that hormonal status is able to modulate visceral and somatic sensitivity to different nociceptive stimuli[73-77].

Modulation of peripheral pain processing by ovarian hormones: Altered visceromotor response to CRD in rodents after ovariectomization indicate that ovarian hormones modulate gut sensitivity[78-80] and this is corroborated by changes in visceral sensitivity during the estrus cycle. Indeed, in female rats, visceral sensitivity is increased during proestrus compared to met/diestrus[81,82]. In ovariectomized rats, estradiol administration increases visceromotor response to a visceral painful stimulation consisting of colon or bladder distension[75,76,78,79,83-87], whereas progesterone is thought to counteract the effects of estradiol by an antinociceptive action[83]. However, depending on the dose, ovarian hormones can have opposite effects, and if estrogens are thought to be pronociceptive at physiological concentrations, one study showed that estradiol can be antinociceptive at supraphysiological levels[88]. Furthermore, conflicting results come from studies in different species. For instance, unlike rats, ovariectomized mice displayed long lasting visceral hyperalgesia, which was reversed by estradiol administration[26].

Estrogen receptor (ER)α and ERβ are both expressed at a peripheral level in small-diameter dorsal root ganglion (DRG) neurons conducting nociceptive information[89]. ER activation can modulate nociception by regulating the expression of ion channels and receptors in sensory neurons. Estradiol can inhibit high-voltage activated calcium channel of L and N type expressed on primary afferents[90]. Moreover, the ATP purinoceptor P2X3 and the capsaicin-sensitive transient receptor

potential vanilloid type 1 (TRPV1) receptor, known to participate in visceral nociceptive integration, are both downregulated in ERα and ERβ knock-out (KO) mice[91]. Further studies demonstrated that ATP-induced [Ca^{2+}] in DRG neurons is attenuated following estradiol administration in wild type and ERβ KO but not in ERα KO mice[92,93], suggesting that this attenuation depends on ERα specifically. Thus, an interaction between P2X3 and ERα in primary sensory neurons could underlie gender differences in visceral nociception. Finally, recent research highlighted the potential role of a new estrogen receptor called GPR30 in estrogen-dependent visceral hypersensitivity induced by 5-HT[94].

Modulation of central pain processing by ovarian hormones: Modulation of visceral nociceptive signal by sex hormones at the spinal cord level is also complex. ERα is expressed predominantly in the superficial dorsal horn, which receives information from nociceptive sensory neurons, while ERβ is expressed in deeper layers suggesting a differential function for these receptors in nociceptive processing. Spinal ERs are thought to play an important role in the modulation of visceral sensitivity as suggested by the finding that administration of the ERα agonist 4,4',4"-[4-propyl-(1H)-pyrazole-1,3,5-triyl]trisphenol *via* intrathecal route increases visceromotor response to CRD in ovariectomized rats[87]. In contrast, administration of the ERβ agonist diaryl proprionitrile attenuates the response of viscероceptive dorsal horn neurons and the visceromotor response during CRD, suggesting antinociceptive action of ER[95]. The proposed mechanism of action of ERs on visceral sensitivity at the spinal level is gating of synaptic transmission by modulation of ion channel activity[71,84,96]. Some studies have explored the possible interaction between ERα and the NR1 subunit of the glutamate N-methyl-D-aspartate receptor (NMDA). Both receptors are coexpressed in dorsal horn neurons, and it was shown that estradiol increases spinal processing of visceral nociception by upregulating NR1 expression and activity following PKA-mediated NR1 phosphorylation. In contrast, ovariectomy increases the potency of the NMDA receptor antagonist (2R)-amino-5-phosphonovaleric acid in modulating the visceromotor response to CRD[71,84]. Taken together, these data confirm the pronociceptive effect induced by spinal ERα activation in the rat.

In the brain, a recent study showed in ovariectomized rats receiving implants of estradiol that elevated levels of estradiol in the amygdala correlated with increased visceromotor response to CRD. Estradiol administration to adjacent brain areas had no such effect and did not affect somatic sensitivity[80]. The underlying mechanisms of the central action of estrogens in the amygdala have not been investigated yet but could involve opioid receptors, which are highly expressed in this brain area[97-99] and have been implicated in the estrogen-dependent differential effects of morphine[70,100].

Gender and ovarian hormones influences on hypersensitivity in IBS

Gender differences in peripheral visceral pain processing and IBS pathophysiology: Gender-related differences in somatic and visceral sensitivity in the general population have been widely described, however, the link between gender and pain-related symptoms in IBS is still discussed. The most recent meta-analysis[56] did not reveal any difference in visceral-pain-related symptoms between men and women in the IBS population. This finding contrasts with several studies reporting greater abdominal pain and a reduction in discomfort thresholds during phasic CRD in women compared with men[66,67,101]. Discrepancies between these studies may be attributed to the fact that menstrual cycle phase, menopausal status and hormonal therapy were not taken into account.

Only a limited number of studies have investigated correlations between pain perception and hormonal status. Yet, sex hormones are likely to play an important role in the pathogenesis of visceral hypersensitivity in IBS. This is supported by observations that premenopausal patients present exacerbation of their abdominal pain symptoms at the time of menses[102,103]. Moreover, when considering the different phases of the cycle, worsening abdominal pain and increased rectal perception in response to CRD specifically are observed at the time of menses compared with other phases in IBS patients[25]. Interestingly, it was shown in healthy volunteers that rectal sensitivity was not influenced by the menstrual cycle, suggesting that ovarian hormone fluctuations only affect lower GI sensitivity in pathological conditions[22]. Globally, a negative correlation between female gonadal hormone levels and pain severity is pointed out in several studies supporting a protective role of ovarian hormones. For example, a recent analysis using a population-based questionnaire reported that abdominal pain/discomfort increases after menopause, when ovarian hormones levels fall down, compared to premenopausal women[104]. Nevertheless, some data are conflicting because another study reported that abdominal pain is reduced in women over 50 years old, suggesting a benefit of menstrual cycle cessation[105]. Discrepancies may result from different clinical designs, because the studies used different diagnostic criteria for IBS and different recruitment strategies (*i.e.*, 10-year longitudinal follow-up *vs* single questionnaire). In a recent meta-analysis comparing relative risk of individual IBS symptoms between men and women at different times of the cycle, Adeyemo *et al*[56] concluded that sensitivity was globally increased during menses compared to other phases of the cycle but further analysis is needed to conclude definitively on the involvement of hormonal status in visceral pain. It is noteworthy that these clinical observations are not consistent with preclinical results because increased sensitivity in women is observed when estrogen levels are low, whereas estrogen has globally a pronociceptive effect in the rat. These data suggest that some of the effects of estradiol on visceral nociception

are species dependent. Moreover, estrogens may have differential effects depending on the organ considered. Indeed, results from experiments investigating the role of ovarian hormones on vaginal sensitivity in a rat model of ureteral stone suggested an anti-nociceptive role of estradiol. The study showed lower vaginal sensitivity during proestrus/estrus (high estradiol levels), than during met/diestrus[73]. These dual effects could result from different functions of ERα and ERβ.

On the fringe of research on the link between IBS and sex hormones, a few authors have raised the question of the possible protective effect of male sex hormones. In a study including 50 male IBS patients compared to 25 controls, serum and free testosterone were measured and correlated to sensitivity to CRD. Sensory thresholds for urgency and discomfort in patients were negatively correlated with testosterone levels, indicating a potential protecting effect of male hormones[106]. In animals, one study evaluated the effects of testosterone administration in a visceral pain model induced by artificial calculus. They concluded that testosterone had no effect on visceral pain because operated animals presented an increase in frequency and duration of ureteral crises independently from testosterone treatment[88].

Peripheral mechanisms underlying visceral hypersensitivity in IBS patients are widely unknown. Clinical trials using pharmacological drugs targeting the serotoninergic system in patients with visceral hypersensitivity have led to the proposal that gender differences may rely upon 5-HT-dependent mechanisms. In particular, the 5-HT3 antagonist alosetron provides abdominal pain relief with a greater effect in women than men[107]. This difference of efficacy could be explained by ovarian-hormone-driven 5-HT3 upregulation, making women more sensitive to this therapeutic agent. Nevertheless, studies are needed to understand the link between 5-HT receptors, estrogens and visceral hypersensitivity in IBS pathology.

Gender differences in central visceral pain processing and IBS pathophysiology: In IBS, the diminished thresholds of perception to visceral experimental stimulations in women compared to men can result not only from sex-related differences in peripheral encoding of the visceral pain message, but also from differences in spinal and supraspinal processing of this message, and/or from a greater reactivity of arousal (attention) and emotional (stress) circuits.

Gender differences in visceral pain perception in IBS have originally been attributed to hypervigilance, and it was suggested that this pronounced hypervigilance was primarily driven by greater anxiety in women[108]. Kilpatrick and colleagues confirmed the involvement of hypervigilance but only in naturally cycling female IBS patients[109], thus stressing the complex involvement of ovarian hormones in the process.

Gender differences in pain perception can also reflect structural and functional differences in central visceral nociceptive pathways. Indeed, women display differences in the processing of pain messages, such as enhanced "wind-up" in the spinal cord for instance[110,111] which corresponds to the progressive increase of the electrical response to repeated nociceptive stimulation in spinal cord neurons, possibly *via* the involvement of sex hormones and their spinal receptors[112].

Functionally, functional magnetic resonance imaging (fMRI) and positron emission tomography studies showed that colorectal stimulation and expectation of abdominal pain activate preferentially the arousal/emotional circuits (anterior cingulate cortex and amygdala) in female patients, while male patients present greater activation of the cortical regions (insula and dorsal prefrontal cortex)[113-118]. These patterns of activation were replicated with remarkable similarity in rodents using cerebral-blood-flow-related tissue autoradiography after aversive CRD[119,120]. The results indicate that sex-related differences in brain response to visceral stimulation are mainly due to alterations in the emotional arousal circuitry rather than visceral afferent processing circuits. However, in a task-free resting-state fMRI study, although disease-related differences (IBS *vs* healthy controls) were observed, no differences in emotional arousal circuit activation were evidenced between male and female IBS patients[121]. Moreover, the idea of a greater subjectivity and emotional response in women and of greater cortical control of limbic structures in men was recently challenged by an imaging study monitoring brain activity following the presentation of more male-oriented emotions to female and male patients. In this study, and contrary to previous reports, presentation of faces expressing fear and anger elicited greater activation of the emotional arousal circuits in men and greater activation of the cortical modulatory circuits in women[122]. Thus, the nature and the emotional valence of the stressor are critical to explain gender differences in brain activation patterns.

Structurally, there are widespread gray matter changes in IBS patients although the data is equivocal. Reductions have been reported in the insular and anterior cingulate cortices[123], while another study found increased gray matter thickness in viscera and somatosensory regions (insula and somatosensory cortex S2) with no change in the cingulate[124]. A recent study found no disease-related difference at all between patients and controls[125]. However, the same study reported a sex-related difference in patients, with IBS female subjects showing decreased cortical thickness in the anterior cingulate and insula, and increased cortical thickness in somatosensory and primary motor cortex when compared to female healthy controls. These alterations correlated with IBS symptom severity[125]. White matter and connectivity alterations have also been observed in IBS patients and are more marked in female than male subjects[126,127]. The data indicate that IBS patients have greater connectivity, with sex-specific patterns. Males have stronger connectivity between anterior cingulate subregions, amygdala, and insula, while females have stronger connectivity to and from the prefrontal modulatory regions[127].

If progress has been made in identifying circuits involved in gender-differences in IBS, little is known about their molecular determinants. One study evaluated the impact of serotonin transporter gene polymorphism on brain activation upon CRD and found that subjects with a weak function of the serotonin transporter (s/s genotype) respond with more activation in emotion-regulating brain regions such as the amygdala[128]. Similar alterations of amygdala reactivity were observed during emotional face processing[129]. Further support for the idea of sexual dimorphism of the serotoninergic system came from a study in rodents, which showed that in response to CRD, female but not male rats showed significant changes in cerebral blood flow in the raphe nucleus and in many target regions of its serotoninergic efferent projections[120]. Estrogens can act centrally as steroid neuromodulators to alter the function of glutamatergic or GABAergic systems and modulate synaptic plasticity[72,130]. Moreover, estrogens are known for their ability to shape dendritic spines and modify synaptic plasticity[131-133]. It is therefore likely that gender-related differences in these systems underlie gender-related differences in central processing of pain in IBS, especially during periods when estrogen levels are high. Further studies addressing these questions are warranted.

Key points: In IBS patients, heightened abdominal pain sensation is observed at the time of menses (low ovarian hormone concentrations), which suggests a protective role of estrogens; estrogens modulate peripheral and central nociceptive pathways and have a pro- or antinociceptive action on visceral sensation, depending on the model studied and the neurotransmitter system considered (MOR, NMDA, GABA, NK1); the 5-HT system may contribute to gender differences in pain-related IBS symptoms *via* modulation of gut sensitivity in the periphery and modulation of pain-associated emotional circuits in the brain**;** female IBS patients present higher activity of brain structures involved in emotional processing of pain sensation (*i.e.*, insula, cingulate cortex and amygdala) and greater connectivity between brain structures involved in cortical control (*i.e.*, prefrontal cortex).

Stress and ovarian hormone interactions in pathophysiology of IBS

Stress and female gender in IBS: Women are more vulnerable to life stress[134,135], are more prone to anxiety and depression[136], are exposed to life traumas more often than men[137], and present exacerbated IBS symptoms under stress[16], therefore, it was proposed that the interaction between ovarian and stress hormones may explain female predominance in IBS[138-142].

However, studies assessing gender difference in response to different types of stress failed to show a correlation between women's vulnerability to stressful adverse life events and neuroendocrine responses. In contrast, it was observed that male IBS patients have greater autonomic responses to a visceral stressor (rectosigmoid balloon distension)[143] and have greater autonomic responses and adrenocorticotropic hormone and cortisol release in response to stress[144,145] than female patients. These results suggest that gender differences in the effects of sex hormones on the autonomic systems and hypothalamo-pituitary axis (HPA) cannot account for differential responses to stress in IBS. However, the parameters of autonomic response assessed in these studies were indirect (*e.g.*, skin conductance and heart rate) and stress hormones levels were determined only in plasma and saliva but not in tissue. Also, methodological confounders such as menstrual phase of the cycle in female patients are not always factored in the data analyses or the clinical designs.

Hence, one cannot rule out the possibility of subtle hormone level changes in specific windows of time, or that molecular and cellular changes occur at the tissue level. Indeed, in rodent models, where the experimental designs can be controlled in a tighter manner and where invasive methodologies (*e.g.*, cerebral infusions of hormones) can be used, it was shown that there is a positive correlation between the levels of estradiol and progesterone and the levels of cortisol, corticotropin releasing hormone (CRH) and its receptors[146-149]. This is particularly true during the proestrus phase, where the levels of estradiol are at their nadir[150-152]. Consequently, these data indicate that ovarian hormones can increase HPA axis activation and that further studies in humans are required. Accordingly, the most recent research has been focusing on the link between ovarian hormones and gut response to stress (see below "Stress, ovarian hormones and IBS pathophysiology").

Finally, discrepant findings in humans can also be explained by the fact that ovarian hormones may influence stress responses through other ways than a direct action on the HPA axis, primarily by inducing changes in emotion regulation strategies through tuning up of limbic system reactivity[114,122,141]. In IBS patients, it was observed that women are psychologically more reactive to stress as showed by suggestive stress ratings[153]. A study showed that female IBS patients also had increased negative affect both at baseline and in response to a psychological stressor, even though this was not associated with any change in autonomic responses[154]. This altered emotional balance may explain IBS patients' somatic and psychiatric comorbidity, as well as heightened awareness of bodily sensations[155,156]. In line with the higher prevalence of stress and abuse history in women with IBS, it is thus possible to hypothesize that early traumatic experience can later influence vulnerability to stress mediated by altered limbic system activation[157].

Stress, ovarian hormones and IBS pathophysiology: Preclinical studies performed in rodents have shown that stress can have deleterious effects on gut physiology *via* mechanisms involving ovarian hormones. For instance, restraint stress can decrease colon motility and increase colon contractility in ovariectomized rats when compared to the sham group and these alterations are reversed by

administration of estradiol and progesterone[158]. The effects of ovarian hormones in this paradigm were mediated by the serotonin receptor 5-HT3R[158], the neurokinin receptor NK1[159] and the stress-related hormone thyrotropin-releasing hormone[160]. Most importantly, extensive work by Yvette Taché's group showed that the interplay between CRH and estrogen signaling pathways, both peripherally and centrally, modulates visceral hypersensitivity induced by colorectal distension in rodents[161-163]. These data suggest that overactivity of CRH signaling in the brain and the gut could explain the comorbidity of stress, depression and IBS in women[162].

In humans, it was shown by comparing healthy women with a life stress history and low-stressed women, equilibrated by menstrual phase, that life stress can confer a gut vulnerability[164]. In this study, women experiencing significant life events presented increased jejunal permeability and diminished secretory ability after being subjected to a mild cold pain stress. Interestingly, the abnormal jejunal epithelial response occurred independently of any autonomic activation or stress hormones release, and was not associated with changes in the levels of ovarian hormones. These findings suggest that a history of stress can induce maladaptive intestinal epithelial response to new stressors, even mild stressors, which do not induce autonomic or HPA activation[164].

A follow-up study by the same group addressed the question whether gender differences underlie maladaptive intestinal response to stress and showed that the mild cold pain stress specifically increased jejunal intestinal macromolecular permeability in women. No differences were detected in the autonomic, hormonal and psychological responses to acute stress parameters between men and women, hence suggesting that the maladaptive gut response is due to gender differences in the intestinal barrier[165]. The molecular mechanisms underlying such a vulnerability are still unclear but animal models suggest that CRH can enhance gut permeability by activating epithelial mast cells[166-170] and subsequent mast cell-dependent decrease of tight junction proteins expression[171,172].

Key points: IBS onset and symptoms severity are associated with acute or chronic stress; IBS female patients present emotional hyper reactivity to stress (limbic system hyper reactivity to stress); levels of estradiol, progesterone and stress hormones are positively correlated in rodent models of IBS; female intestinal barrier vulnerability in response to stress may underlie gender differences in IBS.

Effects of ovarian hormones on gut permeability and relevance to IBS

Estrogens and the gut barrier: ERα and ERβ have been identified in the GI tract, where ERβ is preferentially expressed on colon epithelial cells[173-177]. Estrogen functions in the gut encompass development and regulation of gut barrier, cell differentiation and proliferation, and architectural maintenance of the intestinal epithelium[177-179]. Studies on the role of estrogens on paracellular permeability and gut barrier integrity are scarce but there is now growing evidence that ERs play a key role in their regulation. Indeed, ERs have been implicated in chloride ion secretion[180] and nutrient transluminal transport[181,182]. Also, indirect evidence from contraceptive use, pregnancy, and hormone replacement therapy in patients with barrier-dysfunction-related pathologies such as inflammatory bowel diseases show that fluctuations in ovarian hormone levels influence the course of and the risk for these diseases[183-185]. Altogether, these observations suggest that ovarian hormones modulate gut permeability. Hence, they have prompted interest in studying the link between estrogens and gut barrier integrity, and especially paracellular permeability. Recent studies in female rodents have provided insights into how estrogens and ERβ signaling can modulate paracellular permeability. These pharmacological studies showed that distal colon epithelial paracellular permeability varies during the sexual cyclic fluctuations of estrogen levels due to variations in tight junction protein expression. Specifically, estrogen level peak during the follicular phase correlated with improved epithelial barrier as shown in the ileum of proestrus rats[186]. Decreased intestinal permeability in male rats was also found following estradiol supplementation[186]. Subsequent investigation of the link between estrogens and the regulation of paracellular spaces found that estradiol influence during the follicular phase resulted in decreased colonic permeability and ERβ-mediated upregulation of the tight junction proteins occludin and junctional adhesion molecule (JAM)-A in epithelial cells[187]. Similarly, the xenoestrogen bisphenol A dose-dependently decreased basal paracellular permeability through upregulation of JAM-A and occludin[188]. Interestingly, progesterone had no effect in these experimental designs. The effects of estrogens on tight junction protein upregulation were also observed in human colon cell line Caco-2[187,188]. In another study, ER expression and colon permeability were negatively correlated in mice and in human HT-29 and T84 colonic epithelial monolayers, thereby confirming a role for estrogens in the maintenance of epithelial permeability in the colon[189]. Finally, chronic oral administration of estrogen compounds (17β-estradiol benzoate and the phytoestrogen-rich soy germ fermented ingredient SG) was associated with less colon permeability during the follicular phase in female rats although the authors did not report any associated effect on occludin expression[190]. Overall, these studies support the idea that estrogens reinforce the gut epithelial barrier through ERβ-mediated upregulation of the tight junction proteins occludin and JAM-A. As such, it is reasonable to propose that estrogens can have a protective role on colon physiology and it has been shown that low ER levels are a vulnerability factor to colon inflammation[186,188,189].

Ovarian hormones, gut barrier and IBS: In the last couple of years, several studies have reported increased intestinal epithelial permeability in IBS patients (for re-

view, see Matricon *et al*[5]). Intestinal permeability is correlated with IBS symptom severity[191,192]. However, no study has evaluated the link between permeability, symptoms and ovarian hormones so far. Preclinical studies indicate that estrogens are protective but on the contrary, the clear IBS female predominance points to more complex mechanisms. Indeed, the scarce data on this topic suggests the existence of estrogen-dependent maladaptive intestinal epithelial responses to environmental factors such as stress. For instance, Alonso and coworkers specifically showed in healthy women (but not in men) that life or even acute stress could result in decreased jejunal secretory response and increased jejunal permeability[164,165]. Strikingly, these changes were independent of estrogen blood levels. These findings raise the interesting possibility that female over-susceptibility to IBS might be due to a dynamic estrogen-driven maladaptive response of the intestinal barrier to certain risk factors (*e.g.*, stress) rather than a phase-dependent intestinal hyper permeability.

Key points: A subset of IBS patients has increased intestinal permeability; estrogens reduce epithelial barrier permeability by upregulating tight junction proteins; in IBS patients, no correlation between ovarian hormones levels and intestinal permeability has been found.

Effects of ovarian hormones on immune activation and relevance to IBS

Effects of ovarian hormones on immune activation: Although immune response is a predominant function of the gut, little is known about the contribution of sex hormones to gut-associated lymphoid tissue activation and most of our knowledge comes from experiments performed in blood samples. A recent immunophenotypic study analyzed lymphocytes population and lymphocyte gene expression in both peripheral blood samples and small intestine biopsies of healthy men and women. This study demonstrated that immune activation and inflammation-associated gene expression were increased in women compared to men[193]. Gender-related differences in immune activation have been pinpointed by the observation of higher incidence of autoimmune diseases in women than in men and are mediated by both humoral and adaptative responses[194]. Gene expression analysis of isolated peripheral blood mononuclear cells recently showed a marked increase in the expression of inflammatory/cytotoxic effector genes, such as interferon, lymphotoxin and interleukin 12 receptor β2, in women compared to men[195]. The differential immune cells gene expression profile detected in women was indicative of enhanced cytotoxic T cell responses compared to men[195]. Sex hormones are thought to play a crucial role in the modulation of immune responses[196-198], possibly by direct action on immune cells. Indeed, a large number of these cells, including T cells and macrophages, expresses ovarian hormone receptors and in particular ERs[199-202]. Thus, estrogen signaling can modulate directly immune responses by regulating immune cell activation and proliferation and cytokine production[203-206]. T cell function is particularly affected by hormonal regulation since both estrogen and progesterone enhance their cytotoxic activity by stimulating reactive oxygen species production[207-209]. In mast cells, progesterone inhibits histamine secretion, which may explain the partial remission in certain inflammatory conditions during pregnancy when progesterone levels are high[210]. In contrast, mast cells express high affinity estrogen receptors and their activation by estradiol has been shown to potentiate histamine and serotonin secretions in mast cells pretreated with the mast cell secretagogue 48/80[211]. Only one study has considered the role of male hormones in immunity. Castration experiments in rats induced a significant decrease in $CD4^+$ and $CD25^+$ T cells and an alteration of $CD8^+$ T cell activation, which could be prevented by testosterone administration[212]. These results show that testosterone can modulate T-cell-mediated immunity and support the notion of gender-related differences in cell-mediated immune response depending on sex hormones.

Ovarian hormones, immune activation and IBS: To the best of our knowledge, only one study established a potential link between gender differences in IBS pathophysiology and immune activation. In this work, Cremon *et al*[213] performed quantitative immunohistochemistry of colonic immunocytes on endoscopic biopsies of IBS-D and IBS-C patients, and compared the results to patients suffering from microscopic colitis or ulcerative colitis and to healthy controls. IBS patients showed a significant increase in mucosal immune cell counts compared to healthy controls but the magnitude of the immune infiltration was significantly lower than in microscopic and ulcerative colitis. Further analyses concluded that immune activation in IBS patients was characterized by increased $CD3^+$, $CD4^+$, $CD8^+$ T cells and mast cells numbers. Mast cells were significantly increased in female IBS patients, whereas $CD3^+$ and $CD8^+$ T cells were decreased in male patients[213]. It is noteworthy that mast cells are involved in several diseases, among which, some are frequently associated with IBS and affect women particularly (painful bladder syndrome, chronic fatigue syndrome, and fibromyalgia)[214]. As described previously, mast cell degranulation is inhibited by progesterone and stimulated by estradiol[210,211]. An imbalance in ovarian hormone levels in IBS patients could, consequently, explain the mast-cell-mediated immune activation while increased T-cell-mediated activation in men could be due to testosterone modulation[212]. However, further studies are needed to elucidate the mechanisms of interaction between sex hormones and immune cells and to distinguish the differential effects of sex hormones. In this regard, studies assessing changes in GI immune activation along the menstrual cycle in correlation with ovarian hormones levels are warranted.

Key points: Recent findings suggest associations be-

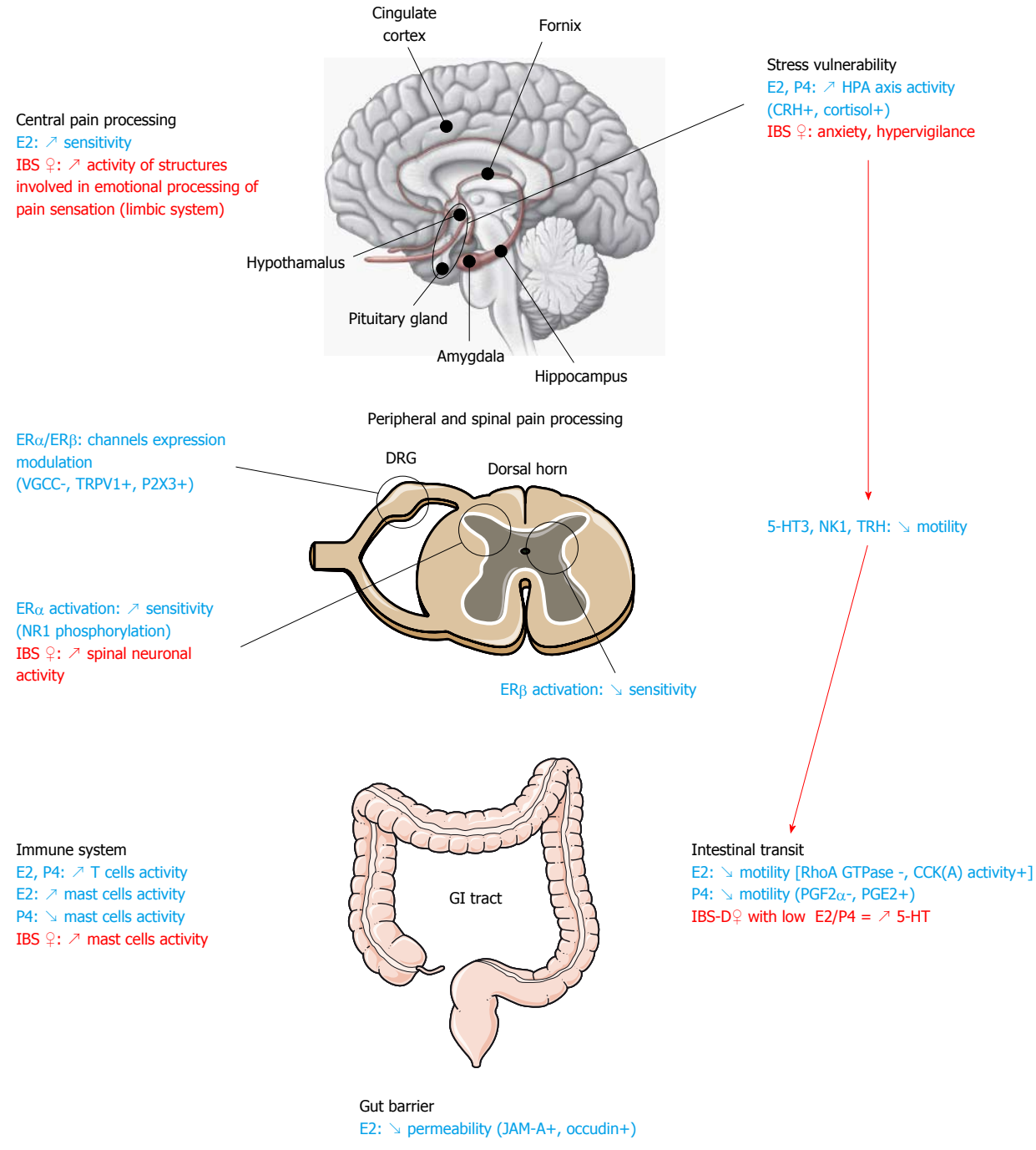

Figure 1 Possible mechanisms of action of ovarian hormones in the pathophysiology of irritable bowel syndrome. Arrows show increase or decrease. The symbol "+" after the name of a protein indicates upregulation of the protein. The symbol "-" indicates downregulation of the protein. E2: Estradiol; JAM: Junctional adhesion molecule; NK: Neurokinin; PG: Prostaglandin; P4: Progesterone; VGCC: Voltage-gated calcium channel; IBS: Irritable bowel syndrome; GI: Gastrointestinal.

tween immune activation and IBS, and especially, GI mucosal mast cell infiltration is consistently reported in IBS patients; estrogens promote mast cell activation; the only study assessing the effect of gender on mast cell infiltration in IBS found a specific increase of mucosal mast cell counts in the colon of female IBS patients.

CONCLUSION

Data summary

Sex ratio in IBS is highly skewed towards female gender. This suggests that sex hormones play a key role in IBS physiopathology as suggested by some findings reporting

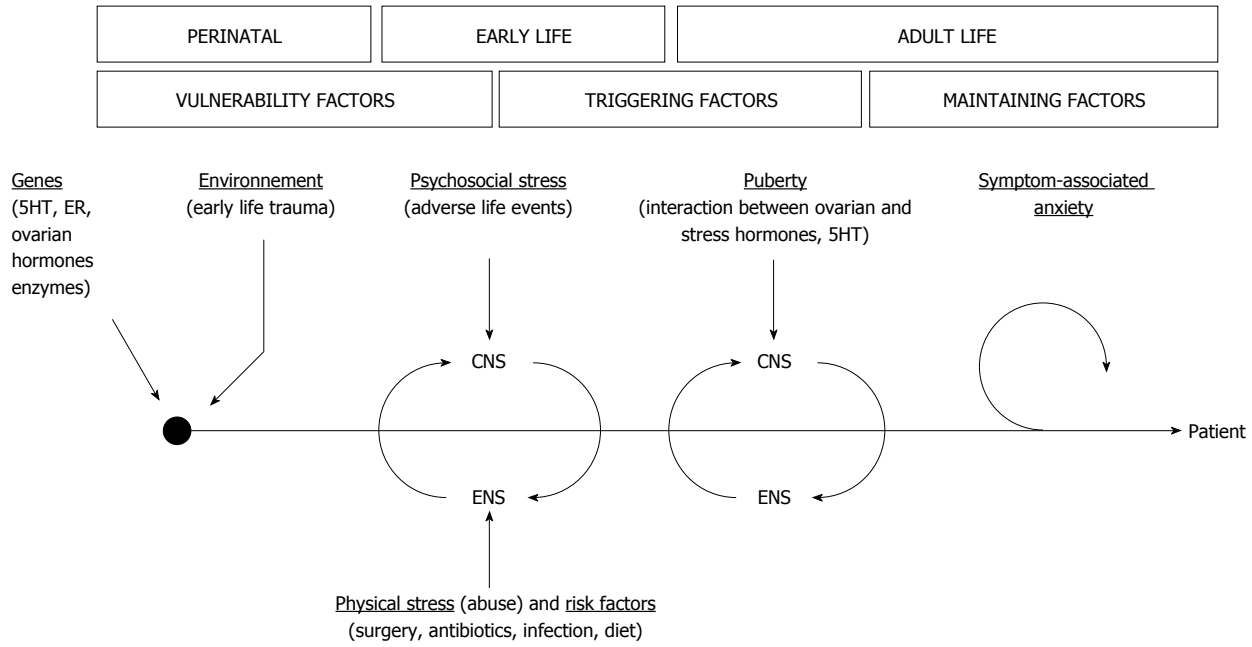

Figure 2 Integrative multiple hits model of irritable bowel syndrome pathophysiology. CNS: Central nervous system; ENS: Enteric nervous system; 5-HT: 5-hydroxytryptamine; ER: Estrogen receptor. Adapted from Mayer *et al*[139].

fluctuations in IBS symptoms during the menstrual cycle, especially exacerbation of abdominal pain at menses (*i.e.*, when ovarian hormones levels are low). However, the mechanisms underlying these gender-related differences are unclear. Clinical studies in the field remain scarce and the data obtained in humans are still weak and sometimes conflicting with findings from preclinical models. Moreover, our understanding is hampered by the fact that differences seen between male and female IBS patients are multifactorial and result from intrinsic differences in male and female hormonal systems, stress reactivity and emotionality, nociceptive system and inflammatory response, as well as sociocultural differences.

Nonetheless, the currently available data provide some insights into the potential mechanisms at play (Figure 1). In female IBS patients, estrogens seem to have a modulatory effect on GI pain by slowing down intestinal motor activity *via* mechanisms that likely involve the 5HT system. Interactions between the 5HT system and ovarian hormones have also been involved in differential peripheral pain processing. Moreover, in line with the frequent history of stress and adverse life events in female IBS patients, altered responsiveness of the HPA axis to stress and abnormal CRH signaling have been pinpointed as major factors responsible for IBS prevalence in women. It is not completely clear whether alterations of the emotional motor system[215] are a cause or consequence of the altered patterns of activity of the limbic system seen in female IBS patients, who display hyperactivity of emotional and attentional circuits (especially the amygdala, a key structure in the processing of aversive experiences). Reciprocal positive feedback in these systems could explain ongoing hyper-reactivity to stress in patients. Indeed, it has been shown in animal models of IBS that stress and hormones can have a synergistic action and result in changes in the periphery leading to IBS-like pathological features such as increased intestinal barrier vulnerability in response to stress or increased intestinal mast cell activation and infiltration.

Proposed integrative model

Given the diversity of pathophysiological mechanisms known at currently, it is essential to develop a disease model integrating the multifactorial aspects of gender-related differences in IBS. Such a model could be helpful to identify research avenues leading to a better understanding of IBS etiology. An integrative multiple hits model can thus be proposed to account for the numerous IBS risk factors, their possible interplay, and their time scale in the course of IBS development (Figure 2).

In this model, gender-based genetic differences (*e.g.*, polymorphisms in 5HT genes) confer a vulnerability to women to risk factors such as stress. Subsequent early life adverse events during childhood contribute to develop a hyper-reactive stress system, with hyper-responsiveness of HPA and emotional brain circuits, leading to heightened pain perception and cognition. In these predisposed patients, after puberty, ovarian hormones could act as triggering factors to sensitize further this priming, and ultimately contribute to the adult onset of IBS. Several mechanisms can be put forward to explain the transition to disease. Ovarian hormones may have synergistic actions with stress mediators and receptors to deleteriously impact nociceptive processes (*i.e.*, interaction with key neuromodulator systems such as 5HT and CRH, and changes in neuroplasticity) and gut function (*i.e.*, GI immune system, gut permeability and sensori-motricity). Interestingly, one can also integrate other IBS risk factors

in this scenario, and diet, GI infection or dysbiosis can all be considered as possible additional "hits" leading to IBS development. Finally, symptom-related anxiety and lifestyle can explain the lasting and intermittent course of IBS in the long run.

Future directions

This review highlights the complexity of the multiple actions of ovarian hormones in IBS and the key role played by stress. Consequently, it appears crucial to understand better the brain-gut interactions and how ovarian hormones modulate them. Animal models can be highly valuable to this end and could shed light on the role of genes and environment in gender-related differences in IBS. Another element stressed by the data reported herein is the scarcity of information regarding sex hormones involvement in patients. There is a need for powerful longitudinal studies taking into account phases of cycles and correlating symptoms and ovarian hormones levels. These studies should also use pair-matched cohorts and factor IBS subtypes in their analysis to minimize methodological confounders. Finally, assessing the role of male sex hormones has remained an unexplored research avenue even though such studies could prove very insightful.

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Cognitive behavioral approach to understanding irritable bowel syndrome

Goran Hauser, Sanda Pletikosic, Mladenka Tkalcic

Goran Hauser, Department of Internal Medicine, Division of Gastroenterology, Clinical Hospital Centre Rijeka, 51000 Rijeka, Croatia
Sanda Pletikosic, Mladenka Tkalcic, Department of Psychology, Faculty of Humanities and Social Sciences, University of Rijeka, 51000 Rijeka, Croatia
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Correspondence to: Dr. Goran Hauser, Department of Internal Medicine, Division of Gastroenterology, Clinical Hospital Centre Rijeka, Krešimirova 42, 51000 Rijeka, Croatia. goran.hauser@medri.uniri.hr
Telephone: +385-51-658122 Fax: +385-51-658122
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Abstract

Irritable bowel syndrome (IBS) is considered a biopsychosocial disorder, whose onset and precipitation are a consequence of interaction among multiple factors which include motility disturbances, abnormalities of gastrointestinal sensation, gut inflammation and infection, altered processing of afferent sensory information, psychological distress, and affective disturbances. Several models have been proposed in order to describe and explain IBS, each of them focusing on specific aspects or mechanisms of the disorder. This review attempts to present and discuss different determinants of IBS and its symptoms, from a cognitive behavioral therapy framework, distinguishing between the developmental predispositions and precipitants of the disorder, and its perpetuating cognitive, behavioral, affective and physiological factors. The main focus in understanding IBS will be placed on the numerous psychosocial factors, such as personality traits, early experiences, affective disturbances, altered attention and cognitions, avoidance behavior, stress, coping and social support. In conclusion, a symptom perpetuation model is proposed.

Key words: Anxiety; Attention; Irritable bowel syndrome; Neuroticism; Stress

Core tip: Irritable bowel syndrome is a complex, biopsychosocial disorder usually developing under stress, which builds upon hypersensitization, underlined by physiological specificities and heightened neuroticism. Symptom onset is followed by inappropriate cognitive interpretations that can be accompanied by affective disturbances. We consider increased attention to visceral sensation and different manifestations of anxiety to be key components that may lead to symptom exacerbation and perpetuation. This applies to patients who express higher trait neuroticism and are more prone to interpret even mild somatic changes as serious symptoms. An individualized approach is necessary for each patient to estimate current physical and psychological status.

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INTRODUCTION

One of the most common functional gastrointestinal disorders (FGIDs) is irritable bowel syndrome (IBS), broadly defined as a variable combination of chronic gas-

trointestinal (GI) symptoms, such as abdominal pain and discomfort and change in stool form and frequency, that are not explained by structural or biochemical abnormalities[1]. Additional characteristics include a high female predominance, heterogeneity of symptoms in relation to the predominating bowel habit (constipation-predominant, diarrhea-predominant or alternating symptoms) and common extraintestinal symptoms and comorbidity[2].

Despite a large body of research, IBS is still poorly understood and there is a need for a more comprehensive model of the disorder. It is currently accepted that symptom formation involves an interaction among multiple factors that include motility disturbances, abnormalities of GI sensation, GI inflammation and infection, altered processing of afferent sensory information, psychological distress and affective disturbances[1,3,4]. In other words, IBS is considered a biopsychosocial disorder[5].

This review addresses the biological and psychosocial factors that possibly contribute to the onset and perpetuation of IBS symptoms. In the first part of the review we describe previous findings on different components of the cognitive behavioral therapy (CBT) model that are relevant for understanding IBS. In the second part of the review we present a new model of IBS symptom perpetuation in which attention to visceral sensation and different manifestations of anxiety have a central role.

According to the biopsychosocial model[6] illness is viewed as a multifactorial entity resulting from the interactions between psychosocial and biological factors in the etiology and progression of the disease[7]. This model has become popular in explaining and clarifying the etiological mechanisms relevant for functional somatic syndromes in general and functional GI disorders in particular[8-11]. Several specific biopsychosocial models of IBS have been proposed, some emphasizing the contribution of biobehavioral factors[4,12-14] and others emphasizing the contribution of psychosocial factors[15-18].

There is a consensus that a CBT approach offers a generic framework for understanding functional somatic syndromes, such as IBS, while also providing effective treatment[5,11,19-24]. This approach is based on the classical CBT model of emotional distress proposed by Beck[25], which distinguishes between its developmental predispositions and precipitants, and its perpetuating cognitive, behavioral, affective and physiological factors. The CBT model has several advantages, for example, it uses operationally defined concepts resulting in a large body of supporting research and it is implemented in a wide range of illnesses, from heart disease to IBS[11,25].

The CBT model is built around three core concepts that help define the development and maintenance of IBS symptoms[11,20,21]. The first of these concepts incorporates the biopsychosocial assumption that biological (*e.g.*, bowel inflammation, hormonal changes, autonomic dysfunction, and abdominal pain), psychological (*e.g.*, altered attention, anxiety and depression, symptom interpretations, and illness behavior) and social (*e.g.*, environmental influences and social support) domains are equally important components in the understanding of illness. The second core concept is the differentiation among predisposing vulnerabilities for IBS (*e.g.*, genetics, early experiences, and neuroticism), those that precipitate the development of this condition (*e.g.*, adverse life events and everyday hassles), and those that maintain them once they have been established (*e.g.*, sensitization and selective attention). Finally, the third core concept of the model is the assumption that individuals can take control of the effect their illness has on their life by changing their cognition and behavior, which affects physiology and emotion, and vice versa.

In Figure 1 we illustrate the core concepts of the CBT model of IBS. This model is based on the existing data on IBS patients and it incorporates various aspects of the disorder, which have already been recognized as important by other researchers in previously published models[4,12-18].

Although the model separates predisposing, precipitating and perpetuating factors, one must keep in mind that they are continuously involved in bidirectional interactions, which means that in the context of mechanisms and processes they cannot be separated. Some components of the model can be considered either as predisposing, precipitating or perpetuating, depending on context, personal history or current illness status. For example, trait anxiety is related to neuroticism as a possible predisposing factor, state anxiety is a common reaction to various stressful situations (precipitating factor), but anxiety sensitivity also plays an important role in perpetuating the symptoms of the disorder.

Each of the model components will be described in detail in the context of IBS.

PREDISPOSING FACTORS

This component of the model relates to those factors that increase an individual's susceptibility to developing a wide range of functional disorders. Among them, genetics may play an important role but due to lack of obvious pathology (*e.g.*, specific biomarkers of disease) research for specific genes has been difficult. Genetic predispositions are shaped by early experiences that have been found to have significant influences on illness development. Finally, neuroticism as a personality trait has been repeatedly confirmed as a general predisposition for experiencing distress and has been recognized as a common trait underlying vulnerability to various illnesses[20,26,27].

Genetics

In spite of some inconsistent findings there is a general accordance with the hypothesis that IBS may be a complex genetic disorder. Family and twin studies have clearly established a genetic component in IBS and several studies point to genetic contributions to IBS[28,29]. Gut motility, visceral secretion, persistent pain, mood, sensation and inflammation are greatly influenced by genotype. There are several potential candidate genes that are asso-

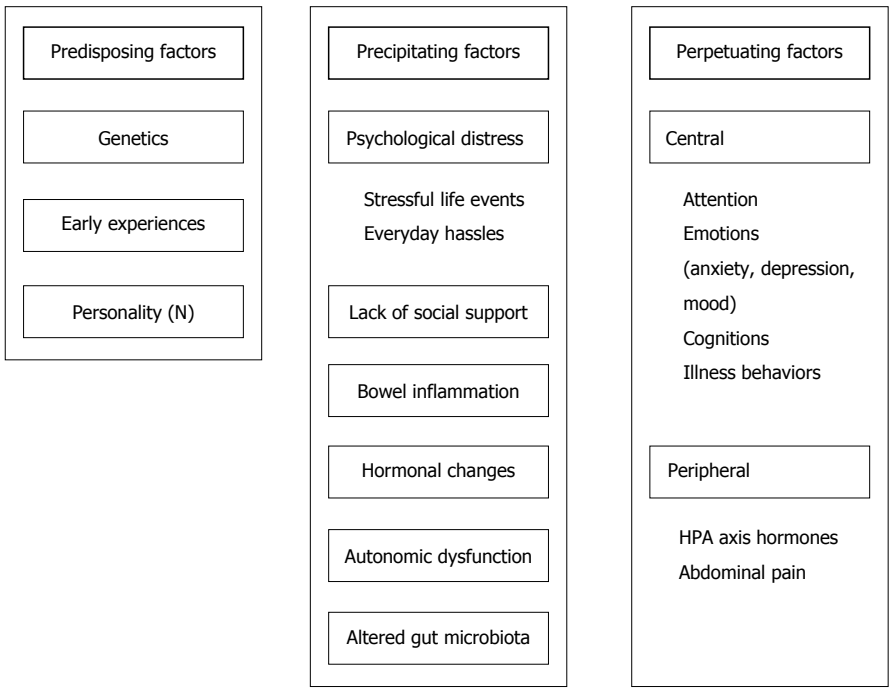

Figure 1 Cognitive behavioural therapy model of irritable bowel syndrome.

ciated with IBS. Research has focused on the relations of various gene polymorphisms with IBS symptom manifestations. Gene polymorphisms involve the serotonergic, adrenergic and opioidergic systems, and genes encoding proteins with immunomodulatory and/or neuromodulatory features[30]. A detailed review of genetic polymorphisms is beyond the scope of this paper, therefore, only a few are mentioned (for a full review, see Fukudo and Kanazawa[31]). One of the well-studied potential genetic groups involves the genes related to serotonergic mechanisms. Serotonin transporter or solute carrier 6A4 (SLC6A4) is the protein involved in serotonin reuptake after it has interacted with the downstream receptor. SLC6A4 is also expressed in the GI tract and there are conflicting data as to whether it is underexpressed in mucosal biopsies of IBS patients[32,33]. This may be partly because of small sample sizes and ethnic heterogeneity within the cohorts studied. Mutations in the serotonin receptor gene (5-HT2A, 5-HT3A and 5-HT3D) are another important mechanism that can cause disturbed serotonin (5-hydroxytryptamine; 5-HT) function. Higher expression can be associated with more severe pain in patients with IBS[34]. Furthermore, serotonin transporter polymorphism (5-HTT) can predispose towards stress hypersensitivity in patients with IBS[35]. Of course, a cautious approach is needed before making firm conclusions about the genetics of IBS. Some of the studies in this field have certain methodological issues, or are yet to be replicated.

Early experiences

Research indicates there is a relatively high (30%-56%) rate of abuse history among IBS patients[36], even when compared to patients with organic GI diseases[37]. A population-based survey has revealed that the prevalence of childhood abuse is significantly higher in persons with IBS compared to those without IBS[38]. In addition, there is a higher likelihood of GI symptoms in victims of abuse[39], which implies that there is a relationship between abuse and GI symptoms. Research on early adverse life events, which encompass a wider array of traumatic experiences than abuse alone, shows similar results. In a study by Chang[40], IBS was associated with a higher total early life trauma score, which included general trauma, as well as physical, emotional and sexual abuse under the age of 18 years. This relationship was significant even after controlling for anxiety and depression. In another study[41], female IBS patients reported a higher prevalence of general trauma, physical punishment, emotional abuse, and sexual events when compared to healthy controls. Emotional abuse was the strongest predictor of IBS. Although IBS treatment is primarily directed at precipitating factors while early experiences are a possible predisposing factor, addressing them during treatment might be beneficial for symptom reduction and quality of life improvement.

Neuroticism and other personality traits

Research in several countries has shown that IBS patients have higher levels of trait neuroticism than healthy persons have[42-49]. Neuroticism refers to a broad dimension of individual differences in the tendency to experience negative emotions and express associated behavioral and cognitive traits. Some of the traits that define neuroticism are fearfulness, social anxiety, helplessness, poor inhibition of impulses and irritability[50]. Even though

neuroticism scores vary slightly depending on age, sex and socioeconomic status, studies of the associations of neuroticism and health have controlled for these demographic factors and still found a significant negative association of neuroticism and health. Neuroticism has been linked to several mental disorders as well as physical health problems, even when depression is controlled for[26]. In IBS patients, high neuroticism has been linked to higher pain reports[46] and low response to treatment with antidepressants[51].

In addition to neuroticism, other fundamental personality traits included in the concept of the five factor personality model ("The Big Five": neuroticism, extraversion, agreeableness, openness, and conscientiousness) have been used to describe behavioral patterns of IBS patients. The findings related to other personality traits, however, are much less consistent than those found for neuroticism. Some studies have shown that IBS patients have lower agreeableness[44,49] and openness scores than healthy subjects[43,44,49], while others report no differences in agreeableness[42,43,47] or openness[42,47]. Extraversion is also sometimes reported as lower in IBS patients, for example, compared to peptic ulcer patients[52] or healthy controls[42,48], and other times no differences are found between IBS patients and healthy controls[43,44,47]. Studies have also reported higher conscientiousness scores in IBS patients than in healthy subjects[43,44], although there are reports of no differences[47,49] and even opposite results[42]. Personality traits may be important for IBS symptom expression, especially neuroticism, which has been established as a critical trait underlying general disease vulnerability.

PRECIPITATING AND PERPETUATING FACTORS

The second component of the CBT model of IBS refers to factors whose occurrence relatively closely precedes the onset of the illness, while the third component refers to factors that maintain and perpetuate the illness symptoms. As Deary *et al*[20] have appropriately illustrated, they present a unique autopoietic interaction of cognitive, behavioral and physiological factors for each individual. Both of these groups of factors are a result of specific psychological and physiological reactions that are determined by predisposing factors and could play a precipitating role in certain circumstances or a perpetuating role in others. For example, people with high levels of neuroticism are more likely to perceive minor physical symptoms or somatic changes as serious symptoms of possible disorder[23]. They are also more prone to interpreting events as negative, which could lead to higher levels of reported psychological distress, both in the context of major life events and everyday hassles, which may be related to specific avoidance behavior. Additionally, physiological factors such as mild bowel inflammation or alterations in the hypothalamic-pituitary-adrenal (HPA) axis hormones, which may be a result of previous infection, or any combination of predisposing factors (genetics, early experience, or personality traits), also play roles in IBS symptom generation and maintenance.

Psychosocial aspects of precipitating and perpetuating factors

Stressful life events and everyday hassles: Around three quarters of IBS patients report that stress causes them abdominal pain and bowel motility changes. In line with a history of early adverse experiences, IBS patients report a significantly higher number of stressful life events[53,54]. Research findings emphasize the importance of differentiating between a positive or negative assessment of a stressful event. Although patients with functional and organic GI diseases report similar numbers of stressful life events, patients with IBS and other functional diseases report more negative[51,55] and less positive stressful events[56]. It could be speculated that this is related to higher levels of neuroticism in the IBS population, making these patients more likely to interpret events in a negative manner. Research also shows a greater prevalence of IBS among male war veterans than the general population, which could be related to increased psychological stress, traumatic experiences and post-traumatic stress disorder[55]. Additionally, IBS prevalence is high (46%) among primary caregivers of chronically ill patients[57]. Taking all of these findings into consideration, there is strong evidence of a relationship between IBS and chronic or life stress.

Research on everyday hassles in IBS patients shows that daily levels of stress are related to symptom severity[58,59]. Even when neuroticism is controlled for, IBS patients report higher everyday stress levels than healthy subjects and subjects with digestion problems who do not meet the IBS criteria[59]. IBS patients perceive stressful events as more severe than inflammatory bowel disease (IBD) patients do, but considering that both patient groups have similar lowered levels of health related quality of life (HRQoL), it seems that both groups have similar levels of psychological stress[60].

Social support: Social support refers to any and all processes by which social relations can affect physical or psychological health[61,62]. Perceived social support is defined as a person's perception that other people will provide them with support when needed. Many studies indicate that perceived social support is more important for the person's health than objective indicators of received social support. It is believed that the cause of this finding could be the experience of acceptance and care of significant others[62]. From a cognitive perspective, social support acts by changing the cognitive appraisal of stressful situations. Perceived social support reduces the impact of stressful events on health either through supportive behavior of others or through the belief that support is available. It is presumed that supportive behavior improves coping, and that the perception of support availability leads to assessing the situation as less stressful[63].

Research shows that IBS patients report receiving significantly less interpersonal support than healthy persons do[64]. Higher support has been linked to lower symptom scores[65,66], but the question about the directionality of the relationship still remains. It is possible that support may lead to lower symptom scores, but it is also possible that patients with lower symptom scores elicit less anxiety and distress in significant others, making them more susceptible to provide help and support[65]. Additionally, it is possible that IBS patients with high symptom scores view their social support as less satisfactory due to their own psychological characteristics such as high neuroticism[42-49,66]. The effect of perceived social support on physical pain seems to be mediated by stress, more specifically - the higher the perceived social support, the lower the reported levels of stress and physical pain[66]. Findings also suggest that satisfaction with social support mediates the relationship between psychological distress and perceived stress. When perceived stress is low, satisfaction with social support does not affect the levels of psychological distress. However, when the levels of perceived stress are high, satisfaction with social support leads to the reduction of psychological distress[67].

Coping with stress is influenced by assessment of environmental and personal resources, hence social support can also be conceptualized as assistance with coping[62]. In line with the results of social support studies, research shows that IBS patients have lower coping capabilities than IBS non-patients, that is, persons without an IBS diagnosis who match IBS criteria but have not sought medical help[56]. When investigating the use of different coping strategies, research shows that IBS patients with a predominantly positive affect (low levels of anxiety, depression, negative mood and perceived stress) seek social support more often than IBD patients with a positive affect, IBS patients with negative affect and healthy controls[68]. Crane and Martin[69] found no differences between IBS and IBD patients in the use of passive coping strategies. Higher levels of anxiety and depression were associated with higher levels of behavioral passive coping in both groups, and with emotional passive coping in the IBS group alone. It seems that IBS patients use different coping mechanisms than healthy persons use, which could be a result of differences in personality characteristics. Research shows that in general, high extraversion and low neuroticism are predictors of high perceived social support[70]. IBS patients have higher levels of neuroticism[42-49], and perhaps lower levels of extraversion than healthy persons[42,48], thus, it is possible that their perceived social support is lower, which contributes to the negative impact of stress on their symptoms. Although research so far[66] has not established perceived social support as a predictor of HRQoL in IBS patients, further research is needed in this area.

Affective status: Research consistently shows that IBS patients present with higher levels of anxiety and depression than healthy persons[48,71-74]. Moreover, heightened levels of anxiety and depression were identified as predictors of IBS in a general population sample[75]. IBS non-patients also show higher anxiety levels than healthy persons show[45]. Pace *et al*[60] found that, although IBS and IBD patients have different levels of physical symptom severity, there are no differences in their psychological symptom severity, such as stress and anxiety. However, IBD patients with IBS-like symptoms report higher levels of anxiety and depression than IBD patients without IBS-like symptoms[76]. Furthermore, psychiatric comorbidity in the IBS population is common. A meta-analysis of 244 studies[77] showed that patients with FGIDs, including IBS patients, more frequently suffer from anxious and depressive disorders compared to healthy persons or patients with similar diseases of known organic pathology. IBS patients with comorbid psychiatric diagnoses report higher levels of anxiety, anxiety sensitivity, and worry[78].

Anxiety sensitivity is a stable personality trait distinguished from trait anxiety, which has also been found to be a risk marker for anxiety pathology. Unlike trait anxiety, which refers to the predisposition to respond anxiously to a wide range of stressors, anxiety sensitivity describes a more specific tendency to fearfully respond to one's own anxiety symptoms[79]. It is characterized by hypersensitivity to somatic sensations based on the belief that they have harmful physical, psychological, or social consequences[79,80]. An important construct that encompasses contextual cues or stressors, in addition to the interoceptive ones, is GI-specific anxiety. GI-specific anxiety can be defined as GI-related cognition, affect and behavior, which stem from fear of GI sensations, symptoms, and the context in which these sensations and symptoms occur[81]. GI-specific anxiety may be an especially important variable related to increased pain sensitivity, hypervigilance, and poor coping responses[45,81]. It could be hypothesized that other measures of psychological distress (neuroticism, anxiety sensitivity, and state anxiety) relate to IBS symptom severity through GI-specific anxiety[80].

Attention and perception: One of the possible mechanisms in IBS could be enhanced perception of, and selective attention to visceral stimuli, resulting from the dysfunction of the digestive system[82-84]. Conversely, central mechanisms that enhance responses to interoceptive information may be critical for maintaining and exacerbating symptoms[85]. The disruption of central brain control mechanisms that modulate the motility and sensation of the gut might be even more important than bowel dysfunction itself[86]. Research shows that patients with functional bowel disorders express attention-dependent alterations of central nervous system processing, as well as a generally negative emotional tendency in their cognitive processing strategies[87]. Models of attention to emotional material can be applied to the issue of attention-dependent alterations in IBS. Mogg and Bradley's[88] model of cognitive motivational analysis and Mathews

and Mackintosh's[89] model of a competitive activation network, point out that a valence or threat evaluation system enhances the activation of any items identified as potentially threatening thereby increasing automatic selective attention to such items. The first model[88] proposes two cognitive structures mediating attention-emotion interaction: (1) valence evaluation system (VES), which automatically evaluates threat posed by the stimulus; and (2) goal engagement system (GES), which controls current processing according to goals set by the individual. When VES is activated by the presence of a threatening stimulus it sends a signal to the GES, which interrupts current processing and orients attentional resources to the material signaled by the VES. Similarly, Mathews and Mackintosh's[89] model proposes that the threat-evaluation system enhances the representation of threatening stimuli in the competition for attentional resources. These models point to individual differences in attentional biases toward threatening stimuli[20,90].

Afzal *et al*[82] found evidence for selective processing of GI-symptom-related words compared with neutral words in IBS patients. A study by Kilpatrick *et al*[85] used the acoustic startle response to examine the early preattentive stages of information processing, and found that male IBS patients have a decreased ability to filter information, while female IBS patients have increased vigilance and greater attention to threat. Similarly, Martin and Chapman[91,92] used a modified exogenous cueing task on IBS patients and found signs of increased vigilance to GI symptoms. The authors showed that IBS patients have a faster orientation response to social threat and pain words than to neutral stimuli. In our recent study[93] we found evidence for Stroop facilitation to situational threat words. The facilitation index was positively associated with trait anxiety and GI-specific anxiety. Increased anxiety and worries about visceral symptoms led to a faster attentional engagement to situational threat words, which is consistent with the findings of Chapman and Martin.

Cognition: Within the cognitive-behavioral model of IBS the way in which the individual reacts cognitively to recurrent GI symptoms and life-events will in turn affect emotional responses, the severity of GI symptoms, and coping ability[94]. The importance of cognitive processes comes from the research showing that patients with IBS are characterized by biases in central processing of visceral stimuli[95]. As previously mentioned, IBS patients show enhanced perceptual responses to visceral sensations. They show a generally negative emotional tendency in their cognitive processing strategies[88] and their dysfunctional cognitions can maintain and exacerbate symptoms[94]. Three dysfunctional cognitions, within a cognitive behavioral framework, seem to be especially important in IBS patients[94]: hypervigilance, somatization and pain catastrophizing.

Hypervigilance relates to selective attention to information that matches the patient's set of beliefs about his/her disorder. For example, a patient can believe that his/her GI sensations are caused by an organic disease and he/she can consequently show hypervigilance to GI sensations. Bray *et al*[96] have specified that patients with IBS can express one of the three symptom attributional styles: somatizing attributional style (tendency to interpret symptoms as a physical disorder); psychologizing attributional style (tendency to interpret symptoms as an emotional response to stress); and normalizing attributional style (tendency to interpret symptoms as a normal experience). Contrary to common opinion, they have found that the normalizing style was predominant among IBS patients specifically in those attending general practice compared to patients referred to hospital clinics. The latter usually have more severe symptoms and could be less likely to accept psychological or normalizing explanations of their unexplained symptoms[96]. The concept of attributional style is closely related to the term "subjective theory of illness" or a system of illness-related ideas, convictions and appraisals[97]. The self-regulatory model of Leventhal[98] emphasizes the role of cognitive representations of illness and coping efforts on the patients' responses to health threats. This model suggests several important cognitive and affective facets of risk perceptions. Leventhal *et al*[98] have suggested that patients form ideas about their illness around five representation dimensions: identity, causal attributions, expectations of duration, consequences, and perceived control and curability. The search for the origin of the illness, as well as causal attributions, is a main focus of illness representations, which is associated with outcome measures.

Somatization is a widespread clinical phenomenon[99] and concerns the tendency to report multiple unexplained somatic complaints and physical illnesses whose expression can be influenced by stress and negative affect[94,100]. Spiegel *et al*[100] have emphasized that a significant number of IBS patients have a somatization trait exhibiting one or more related symptoms including functional chest pain, generalized weakness, numbness or tingling. This trait is expressed in a wide range of alterations, which include increased cognitive (*e.g.*, hypervigilance), affective (*e.g.*, fear), and behavioral (*e.g.*, health care seeking) responses to physical sensations[100].

Pain catastrophizing is the tendency to ruminate, magnify, or feel helpless about pain[94,101] and is conceptualized as a negative cognitive-affective response to anticipated or actual pain[102]. Catastrophic thinking specific to pain has been linked to GI symptom severity and has been identified as a key cognitive variable mediating the link between depression and the experience of pain in IBS patients[94,95].

The relationship between cognitions and pain is bidirectional; in other words, cognitive processes can influence pain and pain perception, but pain can disrupt cognitive processes as well[94,103]. Cognitive processes, such as appraisals, can play a significant role in the modulation of visceral pain[94], therefore, it is not surprising that CBT can have beneficial effects for patients with IBS.

Kennedy *et al*[94,103] postulated a hypothesis that IBS

is a disorder associated with cognitive impairment and revealed interesting findings about the impact of IBS on cognition. They assessed several cognitive domains including reversal learning and attentional flexibility, selective attention and response inhibition, working memory, and visuospatial episodic memory. The results showed that patients with IBS exhibit a deficit in visuospatial episodic memory functioning due to the negative impact of HPA axis dysregulation on hippocampus-mediated cognitive performance. The authors hypothesized that visuospatial memory impairment may be a common component of IBS. Of course, as the authors stated, there is a need for further investigation in order to identify the neurobiological mechanisms influencing cognitive performance in this group of patients.

Illness behavior: Cognitive interpretations of symptoms influenced by stress and social support availability, may lead to alterations in patient behavior. The behavior in which patients engage to decrease or avoid symptoms includes health care seeking, avoidance behavior, and maladaptive behavioral coping strategies. The use of coping strategies has already been described in relation to social support and is not repeated here. Health care seeking behavior refers to physician visits and frequent somatic complaints. IBS patients have more frequent visits to the physician and are more likely to consult for minor illnesses than patients with peptic ulcer[104]. In a population sample, persons with bowel dysfunction have reported more frequent visits to the physician and more non-GI symptoms than persons without bowel dysfunction. They are also more likely to visit a physician for those non-GI symptoms, and have reported that they view their colds and influenza-like illnesses as more serious than those of other people[105]. IBS patients who frequently consult a physician do not differ from IBS non-consulters in levels of mental distress or chronic stress, however they report significantly higher GI symptom intensity[106]. Even children of IBS patients have more physician visits than children of healthy persons, both for GI and non-GI symptoms. Although frequent GI complaints in children whose parents have IBS are not explained by the parent' s biased perceptions, it seems that parental IBS status and their solicitous responses have independent effects on the child's symptom complaints[107].

Avoidance behavior has not been measured directly in IBS patients, but data from treatment studies suggest targeting cognitive and behavioral factors can improve IBS symptoms[108,109]. Based on clinical practice experiences we can list common behavior reported by IBS patients, which can roughly be divided into avoidance and control behavior. Avoidance behavior includes activities such as avoiding exercise, food, sex, work and social situations, while control behavior includes checking for blood in stools, wearing baggy clothes when bloated, having medications on hand, and similar types of behavior[109]. The patients use control and avoidance behavior in an attempt to gain control over their symptoms, reduce their intensity, or avoid possible embarrassing consequences that they might have. Unfortunately, this behavior perpetuates the symptoms and overall, they are not beneficial for the patient[108].

Biological aspects of precipitating and perpetuating factors

Bowel inflammation: Numerous studies have investigated the presence of inflammatory cell activity in IBS patients. There is evidence to support the existence of mild inflammation, at least in a subgroup of IBS patients. For instance, there is evidence of elevated blood levels of T lymphocyte activity markers, increased counts of intraepithelial lymphocytes in some areas of the colon, increased T lymphocyte counts in the lamina propria of the rectum[110], as well as elevated counts and activation of mast cells in the jejunum[111], with indications that mast cell activation near nerve endings seems to be correlated with the severity and frequency of abdominal pain[112].

One of the most frequently used markers of bowel inflammation is fecal calprotectin. In general, patients with IBS express negative or clinically insignificant levels of calprotectin, which makes this marker a valuable tool for differentiating between IBD and IBS. Research shows that compared to IBD patients, IBS patients have significantly lower levels of calprotectin[113-115] while no differences in calprotectin are found when comparing them to healthy persons[113,115,116]. In a study investigating the differences between IBD patients in remission with and without IBS-like symptoms, fecal calprotectin was significantly higher in those with IBS-like symptoms[117], connecting IBS-like symptoms to microscopic inflammation in the absence of structural disease[118]. Also, there are indications that calprotectin is a good predictor of the physical component of HRQoL in IBS patients[119], with patients of low HRQoL showing higher calprotectin levels although no patients had clinically significant values of calprotectin.

HPA axis and hormonal changes: Neurobiological models of IBS point out alterations in neuroendocrine circuits as well as autonomic nervous system, which result in maladaptive responsiveness to various stressors[120]. Stress reactions include the activation of the HPA axis. In short, the perception of physical or psychological stress results in HPA axis activation, leading to increased secretion of corticotropin-releasing hormone (CRH), which stimulates the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary, resulting in increased cortisol secretion from the adrenal cortex. Some studies support the concept of a dysregulated HPA axis system in IBS patients[95,120]. For example, Hellhamer and Hellhamer[35] have listed IBS among disorders with a hypoactive HPA axis. Such hypocortisolemic disorders are characterized by a triad of symptoms: fatigue, pain and stress sensitivity. Hellhamer and Wade[121] have postulated a two-stage model of such disorders: first, chronic stress results in prolonged hypercortisolism that later develops into

hypocortisolism. In such circumstances the adrenal glands remain hypertrophic but secrete lower levels of cortisol.

The connection between low cortisol levels and symptoms of pain is the impact of cortisol on prostaglandins, important mediators in pain perception. They sensitize peripheral and central pain receptors, while cortisol has a suppressive effect on their secretion. Prostaglandins also contribute to illness response characterized by elevated body temperature, altered mood, fatigue and hyperalgesia[35].

Generally, several different hypotheses have been proposed about the relationship between HPA axis dysfunction and IBS. One of them is that elevated immune activity and resulting elevated cytokine levels stimulate the HPA axis, resulting in its hypersensitivity. However, although some studies have reported elevated basal plasma cortisol levels in patients with IBS compared to healthy persons[122], others show lower salivary and plasma cortisol levels[123] pointing to a decreased HPA axis reactivity[124]. A possible explanation for such contradictory results is the possibility that the type of hormonal dysregulation (reduced or elevated cortisol levels) depends on the predominant symptoms a patient is experiencing. It seems that functional pain symptoms are related to reduced cortisol secretion while depressive symptoms are related to elevated cortisol secretion[124]. In support of this assumption, Ehlert *et al*[124] found elevated cortisol levels and a hypersensitive HPA axis in a group of patients with FGIDs who expressed higher depression and anxiety, and found a hypofunctional HPA axis in a group of patients with high somatization levels. It would also seem that patients with a faster resolution of cortisol to basal values express milder symptoms and higher QoL scores[125]. Some studies have indicated that individuals who experience early adverse life events have higher cortisol levels after exposure to a visceral stressor. However, HPA axis hypersensitivity as a reaction to a visceral stressor is under the influence of early stressful experiences rather than IBS diagnosis *per se*[125].

In addition to cortisol, sex hormones can also modify the perception of visceral sensations and pain, but considering the many interactions female sex hormones have with pain pathways, the underlying mechanisms are difficult to define. Women with IBS have a lower pain threshold during rectal distension[124,126-128] compared to men with IBS and healthy women, while there are no sex differences among healthy subjects or among men with and without IBS[122]. When rectal sensitivity is compared across the phases of the menstrual cycle, results indicate that women with IBS are more sensitive during menstruation, which is not the case in healthy women[129,130]. Nevertheless, upon repeated noxious stimuli (rectosigmoid distension), even healthy women show visceral sensitization or heightened perceptual responses[122]. These findings indicate that there is a possible role of female sex hormones in increased pain perception. Many women with IBS report symptom flare-ups in the perimenstrual and perimenopausal phases and symptom fluctuations during their menstrual cycles[131-133]. Some studies have shown that the time of menses is associated with looser stools compared with follicular and luteal phases[133,134]. Additionally, IBS is diagnosed more often in women with dysmenorrhea than in those with a normal cycle[135]. It is possible that hormonal disparities and fluctuations may be responsible for differences in IBS prevalence and symptom presentation among women and between women and men[136-138].

Research on sex differences in HPA axis reactivity shows that even though men express higher cortisol and ACTH levels after psychological distress, women express higher cortisol level as a reaction to opioid antagonist administration on CRH neurons. It is possible that sex hormones influence opioid regulation of the HPA axis. CRH neurons receive inhibitory information from neurons producing β endorphins through μ-opioid receptors. The expression of μ-opioid receptors in the rat brain is modulated by steroid hormones while estrogen stimulates opioid secretion. If we apply these findings to human physiology, then we can explain the absence of differences in cortisol levels after stress exposure between men and women in the luteal phase as well as the findings about the hypoactivity of the HPA axis in women in the follicular phase compared to men[139]. Despite the inconsistencies in research findings, sex differences in HPA axis sensitivity are another possible reason for a higher prevalence of women in the IBS population.

To conclude, results assessing neuroendocrine responses in IBS patients are mixed, depending on the patient's life history and affective status[120].

Autonomic dysfunction: It is believed that the variability of the dynamic balance of the sympathetic and the parasympathetic systems is the basis for the preservation of stability and health, in other words, that the variable conditions in the environment favor patterns of variability instead of static levels. In line with this assumption, an autonomic imbalance in which one system dominates over the other leads to a decrease in dynamic flexibility and negative health outcomes[140]. The hypothesis about the relationship of parasympathetic hypoactivity and FGIDs has been present for a long time. Even Tougas[141] has suggested that low vagal tone along with sympathetic hyperactivity alters visceral perception. Although research has generally shown altered autonomic function in IBS patients, the findings have been inconsistent. Some researchers[69] have observed increased sympathetic and decreased parasympathetic activity in IBS patients, while others[142,143] have found this pattern to be typical for constipation-predominant IBS (IBS-C) patients, and the opposite pattern typical in diarrhea-predominant IBS (IBS-D) patients[142]. A study by Elsenbruch and Orr[144] found differences in postprandial autonomic activity depending on IBS subtype. IBS-D patients show a postprandial sympathetic dominance, which is the result of parasympathetic depression rather than sympathetic activation. The change in parasympathetic activity is related to reports of postprandial symptom exacerbation. These

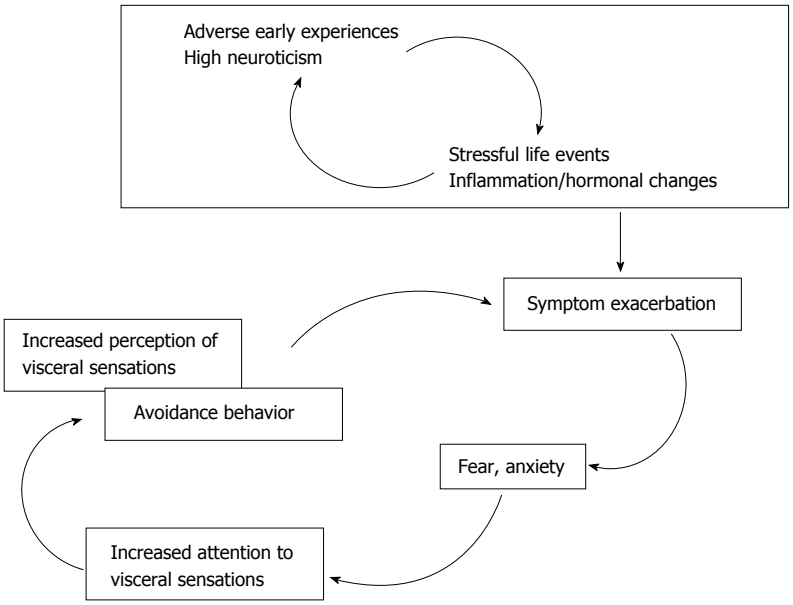

Figure 2 Irritable bowel syndrome symptom perpetuation model.

alterations are not found in IBS-C patients or healthy subjects, although IBS-C patients also report postprandial symptom exacerbation. These results have to be regarded with caution, as pointed out in a meta-analysis performed by Tak *et al*[145]. The authors reviewed 14 studies of autonomic activation in functional bowel disease patients and found that research shows they have lower parasympathetic activation compared to healthy subjects, but also that IBS subtypes cannot be differentiated based on that activation. The included studies, however, varied greatly in quality, so the authors concluded that autonomic dysfunction in IBS patients at this moment cannot be confirmed or disputed[145].

Altered gut microbiota: Considering that the human microbiota in adults is stable over time it is interesting to note that IBS patients show higher variability and quantitative composition of the microbiota compared to healthy persons[146,147]. Research on fecal microbiota in IBS patients indicates reduced levels of fecal lactobacilli[148] and bifidobacteria[148-151]. Some research[152] has shown decreased levels of lactobacilli in IBS-D patients, while other studies[153] have found increased levels of lactobacilli in that subgroup of IBS patients. This inconsistency can be the result of different molecular methods used, as well as the result of unstable symptoms in patients samples, diet or the high variability of microbiota in IBS patients[154].

Abdominal pain: Abdominal pain is a common and most disturbing symptom in FGID such as IBS. It seems that the evaluation of abdominal pain is altered in IBS and this may be attributable to affective disturbances, negative emotions and cognitions in anticipation of or during visceral stimulation and altered pain-related expectations[2]. Dissection of pathways linking higher cortical function with emotional, attentional and perceptual factors to the final expression of symptoms is the key to our understanding of pain mechanism underlying IBS[155].

Dorn *et al*[156] have reported that IBS patients show an increased tendency to report pain, but similar neurosensory sensitivity compared to controls, and this tendency is correlated with psychological distress.

THE IBS SYMPTOM PERPETUATION MODEL

The CBT model of IBS also serves as a framework for describing the vicious circle of symptom generation and perpetuation, based upon the feedback loops between the underlying processes.

Therefore, we propose the IBS symptom perpetuation model (Figure 2) attempting to explain the factors contributing to symptom maintenance. The novel aspect introduced by this model is the identification of key elements which drive the vicious circle of symptom exacerbation. These key elements (attention to visceral sensation, trait anxiety, visceral anxiety and anxiety sensitivity) should be the target of psychosocial interventions.

Building upon genetic predispositions that include several possible gene polymorphisms and personality traits, primarily neuroticism, early adverse events create a baseline sensitive to further adversities. This baseline is heterogeneous among IBS patients, namely because the personal histories of patients in combination with their genetic makeup create individual patterns with a wide range of variation. What the patients do share is the hypersensitivity resulting from their altered psychophysiological baseline. Hypersensitivity to stress manifested by increased limbic system reactivity can be directly associated with increased perception of and responses to

visceral sensations via inappropriate upregulation of pain facilitation systems[18,22].

Precipitating events, usually stressful life events, trigger the onset of symptoms that are then a subject of cognitive interpretations resulting in affective disturbances, primarily anxiety, visceral anxiety and anxiety sensitivity. These disturbances alter cognitive interpretations increasing the perception of the symptoms themselves, and alter illness beliefs that lead to avoidance behavior. Such cognitive alterations can lead to symptom exacerbation and long-term symptom perpetuation.

We consider increased attention to visceral sensation and different manifestations of anxiety to be key components that could lead to symptom exacerbation (abdominal pain and associated bowel disturbances) as illustrated in Figure 2. Anxiety can serve as a mediator between symptom maintenance and altered attention that finally leads to increased perception of visceral sensation and avoidance behavior. Should we conclude that this explanation of symptom maintenance applies to all IBS patients? Of course not. Those patients who express higher trait neuroticism are more prone to interpret even mild somatic changes as serious symptoms and to respond anxiously in such situations. This heightened anxiety can lead to increased attention to and perception of visceral sensations and consequently to symptom exacerbation. The patients aim to reduce discomfort and accompanying negative feelings by avoiding the situations they perceive as threatening. As a result of such individual differences, related to the contribution of predisposing and precipitating factors to symptom maintenance, an integrative analysis of psychological, biological and symptom measures should be performed for each patient as a part of a systematic clinical translational approach, as suggested by Hellhamer and Hellhamer[35]. This is a way to get an estimate of each patient's status by assuming a concurrent effect of biological, psychological and social factors on disorder expression and persistence.

Based on the described components of the self-maintaining circle it can be assumed that by changing behavior and/or cognition through various psychotherapeutic approaches, primarily the cognitive-behavioral, it is possible to change affective states, which in turn might reduce symptoms and improve the patients' overall QoL. This assumption is based on the core premise of the CBT approach that physiological, cognitive/affective and behavioral responses are interdependent and responsible for maintaining the disorder[108]. For this reason, changing cognitions (*e.g.*, reinterpreting symptoms or redirecting attention), behavior (*e.g.*, exposure to threatening stimuli or situations followed by a positive outcome), or both may indirectly reduce anxiety and lead to an improvement in symptoms. David and Szentagotai[157] have proposed a cognitive framework that incorporates the constructs of cognitive psychology into the CBT approach. It is a general model that can be adapted for specific clinical problems, such as IBS. There are seven steps one should take into account when approaching the key elements of the self-maintaining circle of IBS: (1) the covert stimulus (*e.g.*, bodily sensations, such as GI symptoms, emotional arousal, memories and anticipation); (2) input and selection (*e.g.*, patients tend to selectively attend to GI stimuli); (3) perception and symbolic representation of the stimulus (definitions and descriptions of the stimulus, *e.g.*, they may interpret symptoms as a physical disorder or as an emotional response to stress); (4) nonevaluative interpretation of the symbolic representation of the stimuli (inferences about unobserved aspects of the perceived stimulus; *e.g.*, the patient could ruminate "I worry that symptoms could appear suddenly"); (5) evaluative interpretations of processed stimuli (non-neutral appraisals of stimuli or images that can be conscious or unconscious; *e.g.*, "If my symptoms appeared suddenly, I could be embarrassed"); (6) emotional response to processed stimuli (arousal, worry and anxiety); and (7) coping mechanisms to feelings arising from response to stimuli (*e.g.*, patients could try to eliminate what they evaluate negatively, either by avoidance or escape). Psychotherapists can intervene at any point based on each patient's status.

In conclusion, the CBT model of IBS provides not only a useful overview of the interactions between psychological and biological processes underlying IBS symptoms but also gives an effective treatment for alleviating these symptoms.

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Irritable bowel syndrome: Pathogenesis, diagnosis, treatment, and evidence-based medicine

Lekha Saha

Lekha Saha, Department of Pharmacology, Post Graduate Institute of Medical Education and Research, Chandigarh 160012, India
Author contributions: Saha L solely contributed to this paper.
Correspondence to: Lekha Saha, Assistant Professor, Department of Pharmacology, Post Graduate Institute of Medical Education and Research, Sector 12, Chandigarh 160012, India. lekhasaha@rediffmail.com
Telephone: +91-172-2755253 Fax: +91-172-2755253
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Abstract

Irritable bowel syndrome (IBS) is a chronic and debilitating functional gastrointestinal disorder that affects 9%-23% of the population across the world. The percentage of patients seeking health care related to IBS approaches 12% in primary care practices and is by far the largest subgroup seen in gastroenterology clinics. It has been well documented that these patients exhibit a poorer quality of life and utilize the health care system to a greater degree than patients without this diagnosis. The pathophysiology of IBS is not clear. Many theories have been put forward, but the exact cause of IBS is still uncertain. According to the updated ROME Ⅲ criteria, IBS is a clinical diagnosis and presents as one of the three predominant subtypes: (1) IBS with constipation (IBS-C); (2) IBS with diarrhea (IBS-D); and (3) mixed IBS (IBS-M); former ROME definitions refer to IBS-M as alternating IBS (IBS-A). Across the IBS subtypes, the presentation of symptoms may vary among patients and change over time. Patients report the most distressing symptoms to be abdominal pain, straining, myalgias, urgency, bloating and feelings of serious illness. The complexity and diversity of IBS presentation makes treatment difficult. Although there are reviews and guidelines for treating IBS, they focus on the efficacy of medications for IBS symptoms using high-priority endpoints, leaving those of lower priority largely unreported. Therefore, the aim of this review is to provide a comprehensive evidence-based review of the diagnosis, pathogenesis and treatment to guide clinicians diagnosing and treating their patients.

Key words: Irritable bowel syndrome; Pathogenesis; Diagnosis; Treatment; Evidence-based medicine

Core tip: Irritable bowel syndrome (IBS) has been well documented; these patients exhibit a poorer quality of life and utilize the health care system to a greater degree than patients without this diagnosis. The pathophysiology of IBS is not clear. Many theories have been put forward, but the exact cause of IBS is still uncertain. The complexity and diversity of IBS presentation makes treatment difficult. Although there are reviews and guidelines for treating IBS, they focus on the efficacy of medications for IBS symptoms. Therefore, the aim of this review is to provide a comprehensive evidence-based review of the diagnosis, pathogenesis, prevention and treatment to guide clinicians diagnosing and treating their patients.

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INTRODUCTION

Irritable bowel syndrome (IBS) is a gastrointestinal (GI) disorder characterized by altered bowel habits in association with abdominal discomfort or pain in the absence of detectable structural and biochemical abnormalities[1].

The understanding of IBS has undergone a rapid evolution with scientific advancement, but historically it was recognized over 150 years ago. In 1849, Cumming reported, "The bowels are at one time constipated, another lax, in the same person. How the disease has two such different symptoms I do not profess to explain"[2]. IBS is a common functional bowel disorder that generates a significant health care burden and can severely impair quality of life and is the most commonly diagnosed gastrointestinal condition. The etiology is poorly understood and many factors are involved. Understanding the pathogenesis of IBS is important because today's newer pharmacotherapy agents are beginning to target the known pathophysiologic mechanisms of IBS[3]. Altered gastrointestinal motility, visceral hypersensitivity, post infectious reactivity, brain-gut interactions, alteration in fecal micro flora, bacterial overgrowth, food sensitivity, carbohydrate malabsorption, and intestinal inflammation all have been implicated in the pathogenesis of IBS[3]. However, the perceived symptoms from these mechanisms consist of abdominal pain or discomfort, bloating, diarrhea, and constipation. Not all symptoms are gastrointestinal, for instance, fatigue is very common. Historically, medical management has focused on symptomatic treatment of these individual complaints[3]. Serotonin is largely present in the enterochromaffin cells in the gut and is a major regulator of the peristaltic reflex and sensory relays in the gut[4]. There are two lines of evidence supporting the view that serotonin regulation is abnormal in IBS. The release of serotonin in plasma appears to be reduced in those with constipation-predominant IBS (IBS-C) and increased in diarrhea-predominant IBS (IBS-D)[5]. A defect in serotonin signaling was noted in both IBS and ulcerative colitis, with a reduction in normal mucosal serotonin and serotonin transporter immunoreactivity in both diseases[6].

Studies have also begun to focus on the molecular level with serotonin receptor agonists and antagonists. The role of psychosocial factors in IBS also must be considered because these factors influence treatment options and patients' expectations. According to an American Gastroenterology Association (AGA) technical review[7], research into this area has yielded four general observations. First, psychological stress exacerbates gastrointestinal symptoms magnifying the severity of diarrhea, abdominal discomfort, and so on.

Next, psychological and psychiatric co morbidity is often represented among IBS patients. These psychosocial factors influence the illness experience, patient expectations, and treatment outcome of IBS patients. Lastly, the AGA emphasizes that these factors also dictate which patients consult physicians. All these considerations must be kept in mind when considering long-term treatment goals *via* pharmacotherapy or psychological management.

Functional GI disorders (FGID), most notoriously functional dyspepsia (FD) and IBS, take a prominent place within the "functional somatic syndromes", together with chronic fatigue syndrome and fibromyalgia, with which they frequently overlap[8]. FGID are frequent disorders of which the pathophysiology is incompletely understood. Psychosocial factors are believed to influence GI sensorimotor function and/or symptom generation in FGID as predisposing, precipitating or perpetuating factors; comorbidity with psychiatric disorders, mostly mood or anxiety disorders is frequent[8]. Modern epidemiological, psychophysiological and functional brain imaging research has partially clarified the mechanisms through which these psychosocial factors may act on GI function or symptomatology[8], although the exact nature of their relationship remains a matter of controversy. The "brain-gut axis" can be conceptualized as the bidirectional connection system between the GI tract (with its enteric nervous system) and the brain (central nervous system) through (autonomic) neural, neuroimmune and neuroendocrine pathways. Thus, when gut function is disturbed, the cause of this disturbance can be found in the GI tract itself or in the modulatory input from the central nervous system *via* the brain-gut axis[8]. The percentage of patients seeking health care related to IBS approaches 12% in primary care practices and is by far the largest subgroup seen in gastroenterology clinics[7]. It has been well documented that these patients exhibit a poorer quality of life and utilize the health care system to a greater degree than patients without this diagnosis but have other FGID[9,10]. Patients with IBS visit the doctor more frequently, use more diagnostic tests, consume more medications, miss more workdays, have lower work productivity, are hospitalized more frequently, and consume more overall direct costs than patients without IBS. In this review, an evidence based diagnosis, pathogenesis, and treatment will be presented, to guide clinicians diagnosing and treating their patients.

DEFINITION AND EPIDEMIOLOGY

IBS is a chronic and debilitating functional gastrointestinal disorder that affects 9%-23% of the population across the world (World Gastroenterology Organization, 2009)[11]. Over the past 20 years, the definition of IBS has evolved, driven largely by expert opinion and based on studies that have identified symptoms that discriminate those labeled as IBS from organic disease, as well as factor analyses that have identified clear symptom clusters. Classically, IBS presents with abdominal pain or discomfort that is relieved by defecation or is associated at its onset with a change in stool frequency (either an increase or decrease) or a change in the appearance of the stool (to either loose or hard). The absence of red flag (alarm) symptoms such as gastrointestinal bleeding, weight loss, fever, anemia or an abdominal mass support such a symptom complex as IBS rather than as structural disease[12]. A number of other comorbid conditions may occur more often than expected by chance in those with IBS, including gastro-esophageal reflux, genito-urinary symptoms, fibromyalgia, headache, backache and psychological symptoms[13]. Hence, IBS can present to a number

of different subspecialists and is often initially misdiagnosed[13].

IBS can be subdivided into those who tend to have predominant diarrhea or predominant constipation[1,13,14]. There is also a group of IBS patients who have mixed constipation and diarrhea. To complicate matters, those with one predominant bowel pattern can alternate with the other. Highly variable bowel symptoms support a diagnosis of IBS, but the coexistence of abdominal pain and disturbed defecation remains a sine qua non for diagnosis. According to WHO DMS-IV code classification for IBS and its subcategories, IBS can be classified as either diarrhea-predominant (IBS-D), constipation-predominant (IBS-C), or with alternating stool pattern (IBS-A) or pain-predominant. In some individuals, IBS may have an acute onset and develop after an infectious illness characterized by two or more of the following: fever, vomiting, diarrhea, or positive stool culture. This post-infective syndrome has consequently been termed "post-infectious IBS" (IBS-PI)[15].

IBS is a remarkably common condition according to population-based studies[13,14,16]. In Western countries, including the United States and Australia, approximately 10% of the general population fulfills the Rome Ⅲ criteria for IBS, although many do not ever consult for the problem. IBS overlaps with a number of other unexplained gastrointestinal symptom complexes, including chronic constipation and dyspepsia, suggesting that these conditions may not be discrete entities, but represent disorders with a common aetiopathogenesis[17]. In the West, there tends to be a female predominance but this is not seen in the East. It has been postulated that IBS is under diagnosed in Asia and the condition will increase in prevalence because of changes in diet and infectious risk factors[18].

PATHOPHYSIOLOGY

Traditionally, IBS has been conceptualized as a condition of visceral hypersensitivity (leading to abdominal discomfort or pain) and gastrointestinal motor disturbances (leading to diarrhea or constipation)[7,14]. The gastrointestinal motor disturbances identified, including changes in intestinal transit, do not easily explain mixed or alternating IBS[14]. Some have suggested that these abnormalities are secondary to psychological disturbances rather than being of primary relevance. However, not all patients with IBS have significant psychological overlay and referral bias may partly account for the psychological associations[7,14]. Hints as to why visceral hypersensitivity and gastrointestinal motor disturbances may arise are emerging. There is increasing evidence that organic disease of the gastrointestinal tract can be identified in subsets of patients who fulfill the Rome criteria for IBS. Evidence for subtle inflammatory bowel disease, serotonin dysregulation, bacterial overgrowth and central dysregulation continue to accumulate. The underlying causes of IBS remain to be adequately identified, but IBS-PI is a clear-cut entity. Furthermore, a genetic contribution to IBS also seems likely[13].

Infection and Immune activation in IBS

There is increasing evidence regarding the role of immune activation in the etiology of IBS, which has mainly been shown in studies investigating mechanisms of IBS-PI[19]. Approximately 1 in ten patients with IBS believe their IBS began with an infectious illness. Prospective studies have shown that 3%-36% of enteric infections lead to persistent new IBS symptoms; the precise incidence depends on the infecting organism. Whereas viral gastroenteritis seems to have only short-term effects, bacterial enteritis and protozoan and helminth infections are followed by prolonged IBS-PI. Risk factors for developing IBS-PI include, in order of importance, prolonged duration of initial illness, toxicity of infecting bacterial strain, smoking, mucosal markers of inflammation, female gender, depression, hypochondriasis, and adverse life events in the preceding 3 mo. Age older than 60 years might protect against IBS-PI, whereas treatment with antibiotics has been associated with increased risk. The mechanisms that cause IBS-PI are unknown but could include residual inflammation or persistent changes in mucosal immunocytes, enterochromaffin and mast cells, enteric nerves, and the gastrointestinal microbiota[20]. Exposure to intestinal infection induces persistent low-grade systemic and mucosal inflammation, which is characterized by an altered population of circulating cells, mucosal infiltration of immune cells and increased production of various cytokines in IBS patients. Recent studies have also indicated an increased innate immune response in these patients by evaluating expression and activation of Toll-like receptors[21]. 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 largely dependent on infectious gastroenteritis and/or psychological stress. Additional studies are necessary to understand the precise mechanism of immune activation and its relationship to the development of IBS[22].

Serotonin dysregulation

Serotonin (5-HT), acting particularly through the 5-HT$_3$ and 5-HT$_4$ receptors, plays a significant role in the control of gastrointestinal motility, sensation, and secretion[23-25]. Furthermore, observations that plasma 5-HT concentrations are reduced in IBS patients with constipation[25,26], but raised in those with diarrhea[26,27], especially those showing postprandial symptoms[27], provide further support for its involvement in the motor and sensory dysfunction associated with this condition. Thus there has been considerable interest in these receptors as possible therapeutic targets for IBS, with agonists at the 5-HT$_4$ receptor predicted to enhance gastrointestinal propulsion (that is, to be prokinetics)[28-30] and antagonists at the

5-HT$_3$ receptor to slow gastrointestinal transit and reduce visceral sensation[28,31-33].

Bacterial overgrowth

Studies indicate that small intestinal bacterial overgrowth (SIBO) is prevalent in IBS, it remains unclear whether SIBO causes IBS[34]. Although, the bacterial overgrowth hypothesis of IBS may be biologically plausible, there is also a strong rationale for competing hypotheses. It is unlikely that SIBO is the predominant cause of IBS in all comers, because competing explanations are sensible and defensible. Moreover, data indicate that the test used to promulgate the SIBO hypothesis - the lactulose hydrogen breath test - may not have measured SIBO in the first place[34]. We do not have evidence of SIBO being absent before IBS symptoms, and present after IBS emerges. There is not a dose-response relationship between small intestinal microbiota and IBS symptoms. The relationship between SIBO and IBS is highly inconsistent among studies. Many effective IBS therapies do not address SIBO at all, yet have a more favorable "number needed to treat" than antibiotics. IBS does not behave like a traditional infectious disease, suggesting that microbes may not principally cause the syndrome. Other factors may confound the relationship between SIBO and IBS, including proton pump inhibitors. Whereas the brain-gut hypothesis is evolutionarily sensible, the bacterial hypothesis is harder to defend from an evolutionary perspective. So it can be said that bacteria may contribute to some IBS symptoms, but that bacteria cannot be the only explanation, and a causal link between SIBO and IBS is not secure[34].

Central dysregulation and brain-gut interaction

Psychosocial factors appear to be important in IBS, although whether these factors directly alter gastrointestinal function remains uncertain. It is also possible that gastrointestinal dysfunction modulates central processes too. For example, there is good evidence now that abuse in childhood or adulthood is associated with IBS, although whether it is of etiological importance remains in dispute[35]. Anxiety and depression are also common in IBS[7,14]. Some have conceptualized IBS as a somatization disorder, but the clear evidence for an organic pathophysiology in some cases of IBS makes this unlikely[14,35].

The central nervous system modulates various functions such as secretion, motility, and blood flow[36]. Signals from the gut, in turn, are involved in regulating reflexes. Perception of events in the gut involves activation of afferent pathways, with information being modulated at different levels, peripheral as well as central[37]. A major advance in our understanding of brain-gut interaction and its alteration in IBS occurred with the introduction of functional magnetic resonance imaging. This technique allowed assessment of the difference in cortical function in response to gut stimulation between healthy subjects and IBS patients[38], opening the door for potential pharmacologic and behavioral interventions. There are differences in brain responses in patients with IBS that have been documented. For example, measures of regional cerebral blood flow during rectal distention have shown that IBS patients have greater activation of the anterior cingulate cortex, amygdala and dorsomedial frontal cortex, in contrast to patients with ulcerative colitis and controls[39]. It has been postulated that the brains of people without IBS are better able to activate endogenous pain inhibition areas. This could represent a genetic predisposition to IBS. The antidepressant amitriptyline has been shown to reduce rectal pain and this has been correlated to activation of the right prefrontal cortex, right insula and perigenual anterior cingulate cortex[40]. Such central changes might explain the potential benefit of antidepressants in IBS.

Genetics

Studies have suggested that there is a genetic contribution to IBS, although the importance of this remains in dispute[41]. A search for candidate genes continues, with the working hypothesis that environmental factors likely play an important role in the pathogenesis in the genetically primed individual.

DIAGNOSIS AND CLINICAL MANIFESTATIONS

Diagnostic criteria have evolved since 1979 when Manning *et al*[42] first published their criteria. The changes have included the Rome I criteria, which were revised to the Rome II guidelines[13], and now to the most recent Rome III criteria to allow for ease of diagnosis. The Rome II criteria state that a patient must have abdominal pain or discomfort for at least 12 wk, which need not be consecutive, during the past 12 mo. This pain or discomfort must have at least two of the following three features: relief with defecation, association with a change in stool frequency, or association with a change in stool consistency. The Rome III diagnostic criteria simply state that a patient must have recurrent abdominal pain or discomfort at least 3 d/mo in the last 3 mo associated with two or more of the following features: improvement with defecation, onset associated with a change in stool frequency, or onset associated with a change in stool consistency[3]. A 2009 position statement issued by the American College of Gastroenterology (ACG) states that no symptom-based criteria have ideal accuracy for diagnosing IBS[43]. Therefore, the ACG Task Force defines IBS as abdominal pain or discomfort that occurs in association with altered bowel habits over a period of at least 3 mo. Understanding the pathogenesis of IBS is important because today's newer pharmacotherapy agents are beginning to target the known pathophysiologic mechanisms of IBS. Altered gastrointestinal motility, visceral hypersensitivity, post infectious reactivity, brain-gut interactions, alteration in fecal micro flora, bacterial overgrowth, food sensitivity, carbohydrate malabsorption, and intestinal inflammation all have been implicated in the

Table 1 Summary of diagnostic criteria used to define irritable bowel syndrome

Diagnostic criteria	Symptoms, signs, and laboratory investigations included in criteria
Manning (1978)	IBS is defined as the symptoms given below with no duration of symptoms described. The number of symptoms that need to be present to diagnose IBS is not reported in the paper, but a threshold of three positive is the most commonly used: Abdominal pain relieved by defecation More frequent stools with onset of pain Looser stools with onset of pain Mucus per rectum Feeling of incomplete emptying Patient-reported visible abdominal distension
Kruis (1984)	IBS is defined by a logistic regression model that describes the probability of IBS. Symptoms need to be present for more than two years. Symptoms: Abdominal pain, flatulence, or bowel irregularity Description of character and severity of abdominal pain Alternating constipation and diarrhea Signs that exclude IBS (each determined by the physician): Abnormal physical findings and/or history pathognomonic for any diagnosis other than IBS Erythrocyte sedimentation rate > 20 mm/2 h Leukocytosis > 10000/cc Anemia (Hemoglobin < 12 for women or < 14 for men) Impression by the physician that the patient has rectal bleeding
Rome Ⅰ (1990)	Abdominal pain or discomfort relieved with defecation, or associated with a change in stool frequency or consistency, PLUS two or more of the following on at least 25% of occasions or days for 3 mo: Altered stool frequency Altered stool form Altered stool passage Passage of mucus Bloating or distension
Rome Ⅱ (1999)	Abdominal discomfort or pain that has two of three features for 12 wk (need not be consecutive) in the last one year: Relieved with defecation Onset associated with a change in frequency of stool Onset associated with a change in form of stool
Rome Ⅲ (2006)	Recurrent abdominal pain or discomfort three days per month in the last 3 mo associated with two or more of: Improvement with defecation Onset associated with a change in frequency of stool Onset associated with a change in form of stool

IBS: Irritable bowel syndrome; 5-HT: 5-hydroxytryptamine.

pathogenesis of IBS. However, the perceived symptoms from these mechanisms consist of abdominal pain or discomfort, bloating, diarrhea, and constipation. Historically, medical management has focused on symptomatic treatment of these individual complaints. In addition, our current pharmaceutical repertoire is usually limited to treatment for only one symptom.

As individual symptoms are not completely accurate in diagnosing IBS, criteria have been developed to identify a combination of symptoms to diagnose the condition. Manning *et al*[42] promulgated the original account of this approach. Two of four studies that have evaluated the accuracy of the Manning criteria suggested they perform well, with a sensitivity of 78% and specificity of 72%. Kruis *et al*[44] developed another set of criteria; three of four studies that examined the accuracy of the Kruis symptom score suggested it provides an excellent positive predictive value with a high sensitivity (77%) and specificity (89%). The Rome criteria subsequently were developed and have undergone three iterations. One study has evaluated the accuracy of Rome I criteria, and determined it had a sensitivity of 71% and specificity of 85%. Studies have demonstrated that there are no consistent differences in sensitivity or specificity between Manning, Rome I , and Rome Ⅱ and support the validity of symptom-based IBS criteria[45]. A cross sectional study by Engsbro *et al*[46] exploring the sensitivity of Rome Ⅲ criteria in primary care in patients suspected of irritable bowel syndrome. In this study, a total of 604 patients were referred and 499 were included (32.8 ± 9.5 years, 75% were female). The Rome Ⅲ criteria were fulfilled by 376 patients (sensitivity, 0.75; 95%CI: 71%-79%). Rome Ⅲ-positive patients more frequently reported disturbed defecation, had a higher symptom burden, and lower disease-specific health-related quality of life compared with Rome Ⅲ-negative patients. The various symptom-based criteria identified slightly different subpopulations with the highest agreement between the Rome Ⅱ and Ⅲ criteria[46] (Table 1).

TREATMENTS

Before discussing treatment options with patients suspected of IBS, the physician should carefully perform a detailed history and physical to exclude other diagnoses with symptoms similar to those of IBS. The American

College of Gastroenterology Functional GI Disorders Task Force stated that the current data do not support extensive testing in IBS patients[25]. IBS patients do not appear to have a higher prevalence of organic disease than the general population. If no alarming findings exist such as weight loss, hematochezia, iron deficiency, and symptoms that are typical of IBS, routine diagnostic testing is not recommended. If symptoms are not typical or alarm features are present, testing should include complete blood cell count, comprehensive metabolic profile, an inflammatory marker such as erythrocyte sedimentation rate or C-reactive protein, and thyroid stimulating hormone level. If diarrhea is predominating, fecal leukocytes and stool analysis for *Clostridium difficile* when appropriate (such as patients with antibiotic use within 3 mo or recent chemotherapy) should be obtained. Travel and social history may make stool tests for Giardia and Cryptosporidium antigens appropriate. Serology for celiac disease, preferably the tissue transglutaminase or TTG-IgA, should be performed as part of the workup for all patients suspected of having IBS associated with diarrhea or mixed subtype. Sanders *et al*[47] demonstrated that a higher prevalence of celiac disease exists in IBS patients (4.67%) compared with the general population (< 1%). However, a recently published study found that 1.7% of IBS patients were positive for TTG, and this was not different from the placebo group[48]. Nonetheless, testing for celiac disease does seem reasonable in non constipating IBS. Colonoscopy is acceptable in patients with a family history of inflammatory bowel disease; colon cancer; alarm symptoms, such as hematochezia, nocturnal or progressive abdominal pain, weight loss, anemia, elevated inflammatory markers, or electrolyte disturbances; or in patients over 50. When a colonoscopy is performed in patients with IBS-D, random biopsies should be performed to rule out microscopic colitis. These are general suggestions, as each individual patient will present with unique characteristics. The physician must realize that a strong physician-patient relationship will be the foundation for effective treatment and realistic expectations[3]. Many patients with IBS have been bounced around the medical field for many years with varying diagnoses because of the lack of interest or profound frustration by the physician in treating IBS, possible stigma of this disease as being a psychiatric entity, or lack of clinical, physical, or laboratory diagnostic criteria. The medical literature supports gaining the confidence of the patient on the first clinical interview through attentive listening, and detailed explanations of the pathophysiology, natural history, management, and prognosis of IBS[49,50]. Responding to all the patient's concerns and questions and spending time in the initial visit validates their problem. This reassurance aids in the patient's attempts to understand and accept his or her affliction. Setting appropriate goals and limits gives patients a more structured environment and a sense of purpose and allows them to participate in their own health care strategy[3]. Once a rapport with the patient has been established, long-term goals for this chronic illness are easier to obtain as evident by a decrease in the number of health care visits, reduction in symptoms, and improved patient satisfaction[3]. The physician should also emphasize the chronic nature of this syndrome because nearly 75% of patients continue to have a diagnosis of IBS 5 years later[51].

Non-pharmacological therapies

A complementary and alternate medicine (CAM) is often used for chronic medical conditions, health promotion, and/or disease prevention[52]. Currently available systematic reviews provide conflicting findings about the effectiveness of CAM therapies for IBS. The American College of Gastroenterology Task Force on IBS[3] reported that CAM therapies have not demonstrated any strong evidence-based support for positive outcomes. Other systematic reviews, however, indicate evidence of effectiveness[53]. Among mind-body therapies, hypnotherapy and cognitive-behavioral therapy seem to be the most widely accepted by IBS patients. Relaxation techniques have been studied for their potential role in alleviating IBS symptoms. Multiple studies have indicated positive correlations among psychological distress, daily stress, and GI symptom aggravation[54-57] that triggered IBS symptoms[58]. Women with IBS tend to report a higher amount of psychological distress and lifetime psychopathology than those with no GI symptoms[58]. Relaxation training may be beneficial for symptom improvement and appears to be at least as effective as standard pharmacological treatment. Acupuncture can cause physiological changes that affect various endogenous neurotransmitter systems. Of specific interest to the treatment of IBS is the influence of acupuncture and moxibustion on the serotonergic and cholinergic neurotransmission of the brain-gut axis. Both animal and human trials indicate specific targets for acupuncture on serotonergic, cholinergic, and glutamatergic pathways as well as reductions in blood cortisol levels[59-63].

EXERCISE

Exercise can help maintain GI function and reduce stress, which can help relieve some IBS symptoms. Studies of IBS indicate positive relationships between physical activity and symptom relief[64]. Physical activity, such as pedaling a bicycle, protects against GI symptom aggravation and alleviates gas in several studies[64-66]. The practice of yoga has also demonstrated reduction of IBS symptoms in both adult and adolescent populations[67,68]. Pranayama yoga has been identified as an exercise regimen that increases sympathetic tone, which is decreased in IBS-D patients[69]. In a two-month study, a yoga intervention group practiced twice daily, while the conventional treatment group received 2-6 mg loperamide daily. Results indicated that yoga demonstrated improvement of IBS symptoms equivalent to conventional treatment[69].

DIET MODIFICATION

A primary goal of all IBS interventions is to provide the

patient with relief of symptoms and improve the quality of life. Although the data from clinical trials may in some cases not provide strong evidence for benefits of dietary modification, it remains the primary non-pharmacological clinical intervention for IBS patients; exclusion diets are successfully used by many clinical practitioners[3]. Food intolerances or allergies are strong contributors to the exacerbation of IBS symptoms. Individuals with IBS often discover that certain foods aggravate symptoms[70-72], while others have found relief from IBS symptoms by modifying their daily diet and increasing exercise activities[73-75]. Symptoms of IBS may be associated with visceral hyperactivity, GI motility disturbances, sugar malabsorption, gas-handling disturbances, and abnormal intestinal permeability[1,76]. Elimination diets are often employed that remove the most common allergens from the diet[77]. Although some patients reported that removing wheat, dairy products, eggs, coffee, yeast, potatoes, and citrus fruits from their diets is helpful, such restrictions may be difficult to follow[72]. Dietary restrictions may provide patients with relief of IBS symptoms over time, while entirely skipping meals has been found to worsen IBS symptoms[65,72].

MACRONUTRIENTS: FAT, SUGAR, AND SUGAR ALCOHOLS

IBS studies indicate a positive relationship between fat intake and increased stool number and diarrhea. Intake of carbohydrates can also aggravate IBS symptoms[72]. Offending carbohydrates include fermentable oligo-, di-, and monosaccharides and polyols (FODMAPs). This group includes fructans, galactans, lactose, fructose, sorbitol, xylitol, and mannitol[78]. Sorbitol and other sugar-alcohols found in most sugar-free or reduced-sugar products are poorly absorbed in the GI tract and may cause increased flatulence, abdominal discomfort. Other types of sugar-alcohols proposed to aggravate IBS symptoms include mannitol, xylitol, erythritol, lactitol, maltitol, and isomalt[71]. Due to the multitude of variables related to IBS symptoms, study results are difficult to validate and challenging to interpret.

FIBER

Fiber intake from fruits and vegetables is inversely correlated to bloating[74]. The addition of psyllium fiber, especially for persons with IBS-C, reduced IBS symptoms in some people[71,79,80] while either wheat bran or a low-fiber diet was found to be an ineffective management measure as evaluated by two meta-analyses of a total of 30 studies[80]. Because most of the evaluated studies had small sample sizes, the results are highly variable. Other widely variable factors included the amount of soluble (5-30 g) and insoluble (4.1-36 g) fiber added to the diet and the duration of study intervention (3-16 wk). Overall, consumption of soluble fiber resulted in a decrease in global IBS symptoms and constipation, whereas insoluble fiber demonstrated a less significant effect. Neither intervention, however, decreased abdominal pain in IBS patients. Due to its moderate effectiveness, additional intake of soluble fiber may be recommended for IBS-C patients. Studies also revealed that pain relief was not associated with increased fiber intake and that the addition of insoluble fiber such as nuts or whole grains to the diet had either no effect or exacerbated IBS symptoms[79].

LACTOSE INTOLERANCE

Patients with IBS were found to have significantly more subjective lactose intolerance complaints (bloating, distention, and diarrhea) than those without IBS and to have increased likelihood of lactose malabsorption than the general population[81]. Thus, decreased intake of lactose can benefit some IBS patients[82]. It is hypothesized that, following ingestion of lactose, hydrogen gas is produced and gut distention is promoted due to bacterial fermentation of the unabsorbed lactose. Interestingly, the majority of IBS sufferers, however, failed to test positive for hydrogen breath tests that indicate lactose intolerance[82].

PHARMACOTHERAPY

In the past patients with IBS were treated by giving medicines targeting individual symptoms of IBS such as bloating, abdominal pain, diarrhea, and constipation. However, newer medications are beginning to focus on the molecular level like serotonin receptor agonists and antagonists and drugs that act locally on chloride channels (Lubiprostone) and guanylate cyclase receptors (linaclotide) in the gastrointestinal tract[83]. The problem is that no one drug fits all, meaning that the IBS population is very diverse with each individual presenting with different prevailing complaints. The heterogeneity of the IBS population exists because of the wide range of complaints and the varying degree of symptom severity. Because of poorly designed studies and ill-defined outcomes, the medical literature regarding IBS therapy is generally inconsistent[84,85]. The placebo response in IBS patients is quite significant with short-term trials reporting a 30%-80% response[86]. One can imagine the difficulty of treating a syndrome that is heterogeneous in its presentation, lacks in significant supporting medical literature, and has a remarkably high placebo response rate. Even though patients' symptoms overlap, addressing them individually allows the physician to simplify and organize the appropriate medical therapy.

ABDOMINAL PAIN

The major contributing factor in abdominal pain experienced by IBS patients is visceral hypersensitivity. The management of abdominal pain in IBS has changed very little over the past few decades: antispasmodics remain a cornerstone of therapy. Antispasmodic agents work by anticholinergic properties like dicyclomine and hyoscya-

mine. The evidence of the effectiveness of these agents is not compelling, as even the meta-analyses for smooth muscle relaxants are conflicting. One meta-analysis demonstrated an advantage over placebo for antispasmodics in terms of abdominal pain and distention[87]. Brandt *et al*[43] examined 18 randomized controlled trials, of which only three included dicyclomine and hyoscyamine, but concluded the trials were of suboptimal quality based on study design with inadequate duration of treatment. With only one of those previously mentioned three studies demonstrating a statistically significant improvement in global IBS symptoms and abdominal pain[88] and more frequent anticholinergic side effects versus placebo (69% *vs* 16%), it is easy to understand why insufficient data exist about antispasmodics. Even though the antispasmodic medications have not demonstrated an overwhelming statistically significant advantage[84], it is common practice in the United States to utilize these agents. The anticholinergic effects, including constipation, dry mouth, visual disturbances, and urinary retention, can lead to discontinuation of these medications. These medications can be given as an oral formulation or a sublingual tablet, and be dosed on an as-needed or regular basis. Many patients benefit by taking the medication before meals. If known exacerbating factors such as a particular diet or stress are anticipated, these medications can be given as a prophylactic measure. It has also been noted that medicines such as dicyclomine can lose effectiveness with chronic use; therefore, it may be best employed on an as-needed basis[7]. Given the potential side effect of constipation, these medications should be used cautiously in IBS with constipation predominating[43].

EFFECTIVENESS OF ANTIDEPRESSANT AGENTS IN THE MANAGEMENT OF IRRITABLE BOWEL SYNDROME

Tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs) are more effective than placebo at relieving global IBS symptoms, and appear to reduce abdominal pain. There are limited data on the safety and tolerability of these agents in patients with IBS. Nine trials were identified that tested TCAs in various doses for IBS. TCAs clearly were superior to placebo (NNT = 4, 95%CI: 3-6)[43]. There is no convincing evidence that the dose needed has to be in the antidepressant range, and most trials tested low-dose TCAs. In two of the trials, abdominal pain was the primary endpoint and a significant benefit was observed. Five trials that assessed SSRIs also showed a benefit in IBS over placebo (NNT = 3.5)[43]. Theoretically, SSRIs should be of most benefit for IBS-C, whereas TCAs should be of greatest benefit for IBS-D because of their differential effects on intestinal transit time, but there is a lack of available data from the clinical trials to assess this clinical impression. The safety of using antidepressants in IBS remains poorly documented, although data suggest that the SSRIs are tolerated better than the TCAs. No data on the efficacy of SSRIs or other new antidepressant drug classes are available in the literature[43].

When addressing abdominal pain in the IBS patient, it is helpful to distinguish whether the pain is constant/chronic versus intermittent with known exacerbating factors. The latter has better results when treated with the antispasmodics, whereas the former may have a better response from low-dose TCAs or SSRIs[3]. Antidepressants in IBS patients can facilitate endogenous endorphin release, blockade of norepinephrine leading to enhancement of descending inhibitory pain pathways, and blockade of the pain neuromodulator, serotonin[89,90]. TCAs, *via* their anticholinergic properties, also slow intestinal transit time, which may provide benefit in diarrhea-predominant IBS[91]. The goal is to reduce the visceral hypersensitivity, allowing for better management of chronic pain. Reducing abdominal pain allows for reduced anxiety and a distraction from these patients' IBS complaints[43]. A 2009 meta-analysis concluded that antidepressants were significantly more effective than placebo for the relief of pain and global symptoms. The treatment effects were similar for SSRIs and TCAs[92]. Some patients will hesitate to use antidepressants because of the associated stigma of these medications; therefore, the management of chronic pain should be emphasized. Counseling the patient regarding the potential side effects of constipation and sedation is essential, and caution should be used when prescribing these medications in constipation predominant IBS[43]. Treatment with TCAs generally starts with a very low dose given before bedtime and even with gradual increases never reaches the same doses that are used to treat depression. Often only 25-50 mg of amitriptyline can be utilized with success, although one can start with a very low dose of 10 mg daily. Currently, the evidence for using SSRIs is limited and inconsistent. These agents may be more beneficial in treating patients with concomitant anxiety and constipation-predominating IBS; generally, there are fewer side effects.

Bloating

Bloating is unfortunately a very subjective complaint among IBS patients and remains extremely difficult to treat. The majority of the medications designed for this indication have not been helpful. Simethicone and activated charcoal theoretically should aid in alleviating bloating, but have not demonstrated a true clinical or even statistical benefit. The role of prokinetic agents has yet to be defined and further well-designed studies are needed[86]. Because even IBS treatments such as dietary fiber supplementation can actually worsen bloating secondary to colonic metabolism of non digestible fiber, care must be taken in prescribing fiber in patients with a significant bloating problem[86,93]. Non absorbable sugars like lactulose potentially used for constipation predominating patients can exacerbate gaseous distention. The physician should instruct the patient to be mindful of gaseous food (*i.e.*, beans, carbonated beverages, *etc.*) and attempt to elicit any aerophagia symptoms.

Constipation

When treating mild to moderate symptoms of constipation-predominant IBS, dietary and lifestyle modifications should be the initial management tools. Patients should increase their consumption of fiber-enriched foods, and the physician needs to encourage fluid intake to prevent stool dehydration. Teaching the patient to schedule times for bowel evacuations with the aid of stimulating substances such as coffee or prunes allows for a regimental routine, thus eliminating previously unrecognizable bad habits. Bulking agents (corn fiber, bran, psyllium, polycarbophil, ispaghula husk, and methylcellulose) are a simple and inexpensive next-treatment option. In theory, adding these to the diet increases luminal water, which adds bulk to the stool and allows easier stool passage. One meta-analysis of 13 trials using bulking agents concluded that evidence was lacking to firmly demonstrate an advantage with only polycarbophil and ispaghula husk in three trials exhibiting improvement in constipation[84]. Not surprisingly, no benefit was seen with abdominal pain or bloating. Furthermore, a systematic review summarized that all 13 trials were flawed in methodology and fiber was no more effective than placebo[94]. A randomized placebo controlled trial compared the effectiveness of increasing dietary content of soluble fiber (psyllium) or insoluble fiber (bran) in patients with IBS[95]. It was concluded that those patients taking psyllium had a significant improvement in relief of symptoms and overall reduction in severity of symptoms. However, bran showed no clinical benefit and actually caused worsening of symptoms in many cases[93]. Given that these agents possess a relatively safe profile, it is reasonable to prescribe a trial as initial management for constipation with the understanding that these agents can worsen bloating and abdominal discomfort. Currently, there are no randomized controlled trials examining laxatives in IBS patients[43]. However, polyethylene glycol can be considered for refractory cases as it was shown to improve stool frequency but not abdominal pain[86].

Lubiprostone is a locally acting chloride channel activator that enhances chloride-rich intestinal fluid secretion. It was initially approved for use in chronic idiopathic constipation, but later received approval for use in women with constipation-predominant IBS. Two placebo-controlled trials as well as an open-label study showed significant overall response to the medication[96]. The approved dose for IBS is 8 μg twice daily, and 24 μg dosing can be used for constipation. There seem to be no short-term safety issues and the main side effect is nausea. However, long-term safety remains to be established. Further studies will need to be performed to determine its role in treatment of male IBS patients. Currently, it is best reserved for women with IBS and severe constipation that has been refractory to other treatments.

Diarrhea

When considering treatment for IBS-D, the physician should attempt to elicit any particular stressors that might initiate the patient's exaggerated gastro colic reflex. The anecdotal event could include eating, walking, traveling with the fear of not being near a restroom, or stressful encounters in a social setting or even at work. As previously mentioned, keeping a diary of not only foods but also events or situations that correlate with the onset of diarrhea can help the patient in recognizing these stressors and allow the physician to better coordinate therapy. Once these predictable episodes of diarrhea are known, the physician can begin to utilize conservative, first-line treatment with anti diarrhea agents. Of the two most commonly used anti diarrhea agents, loperamide and diphenoxylate HCl-atropine, loperamide is the only one to have been studied for IBS-D. These medications increase gastrointestinal transit time by interacting with the GI musculature, thus allowing for more water absorption[86]. Of the few randomized controlled trials, the data indicated a decrease in diarrhea without any effect on global IBS symptoms or abdominal pain[94]. The physician should instruct the patient to discontinue these medications once the diarrhea has subsided to prevent constipation. Because of this side effect, the physician should have a higher threshold in prescribing these agents in IBS patients with alternating diarrhea and constipation[43]. Although opioid medications can decrease diarrhea, they should be used with extreme caution because of the possibility of severe constipation and obviously for the addiction potential. As a result, most physicians avoid using these agents. Cholestyramine may have a role in the treatment of diarrhea-predominant IBS, but further evidence is needed to better elucidate the role of bile acid malabsorption and its treatment in IBS[86]. Cholestyramines' side effect of constipation should be remembered. As mentioned above, patients with multiple IBS symptoms that include abdominal pain and diarrhea may benefit from low dose TCAs, which decrease the frequency of bowel movements and treat the visceral hypersensitivity. Alosetron is a 5-hydroxytryptamine (serotonin) 3-receptor antagonist, which modulates visceral afferent activity from the gastrointestinal tract[96]. A meta-analysis that included multiple randomized controlled trials demonstrated its efficacy in relieving global IBS symptoms. These trials demonstrated effectiveness versus placebo for improvement of abdominal discomfort, stool frequency, consistency, and urgency[10,97]. It has been found to be most effective in women with IBS-D. Constipation was reported in approximately one third of patients using alosetron[10,97]. Severe constipation and ischemic colitis were rarely reported as well as some potential drug-related fatalities[86,97]. After being withdrawn from the market, it was reapproved by the United States Food Drug Administration with restrictive guidelines[7], and is currently available under a specific prescribing protocol, with a starting dose of 1 mg daily.

MISCELLANEOUS TREATMENT STRATEGIES

One of the interesting approaches is the utilization of

antibiotics in IBS patients with SIBO. A study by Pimentel *et al*[98] found that out of 202 IBS patients, 157 or 75% had abnormal lactulose hydrogen breath test results signifying bacterial overgrowth. However, the study did show that patients with successful eradication had statistically significant improvement in abdominal pain and diarrhea. The same author subsequently published a double-blinded randomized controlled trial substantiating that the normalization of the lactulose breath test with antibiotics in IBS patients led to a significant reduction of IBS symptoms[99]. In the TARGET 1 and TARGET 2 trials, patients with IBS and without constipation were randomly assigned to receive either rifaximin 550 mg three times a day or a placebo for 2 wk. In this study, results showed that those patients that received rifaximin were more likely to report relief of global IBS symptoms than those that received a placebo[100]. These were large studies enrolling over 1200 patients with greater than 70% completing the study which followed the patients for 12 wk after treatment. Like most IBS studies, there was a predictable response in the placebo group. Currently, there are insufficient data to recommend breath testing for SIBO in all IBS patients as the optimal test is unclear. It is also not clear why antibiotics are effective-are they treating small bowel bacterial overgrowth or altering the colonic flora? The benefit from treatment appears to be transient. Therefore, the routine use of antibiotics in all IBS patients is not recommended. However, it is reasonable to try a 2-wk trial of rifaximin in those patients with IBS without constipation and with moderate to severe symptoms, especially bloating, who have failed other therapies. In prior studies, there were no significant side effects of rifaximin compared with placebo, but currently its cost can be a prohibitive factor.

ALTERNATIVE THERAPIES FOR IRRITABLE BOWEL SYNDROME

Many IBS patients turn to herbal preparations because of a widespread perception that they are safe and effective for a variety of ailments. Although many patients utilize herbal and alternative approaches, they usually do not volunteer this information during the physician interview, so it is important to specifically ask about these agents in a nonjudgmental fashion. An excellent review by Spanier *et al*[101] examined these alternative therapies. Though unstudied in IBS, aloe has been frequently used in treating constipation-predominant IBS. Peppermint oil, which has antispasmodic properties by relaxing smooth muscle, demonstrated efficacy in terms of abdominal discomfort and pain and abdominal distention in IBS patients in three randomized trials when compared with placebo[102,103]. The American College of Gastroenterology Task Force on IBS determined that antispasmodics, such as peppermint oil, may provide short-term relief, but evidence for long-term efficacy is not available and evidence for safety and tolerability is limited[43]. Perhaps the most common strategy employed by patients is to alter the native flora of the colon with "probiotics" such as the commercially available preparations of the Lactobacillus species[84,101]. Patients have often tried these preparations even before seeking medical care due to widespread marketing techniques and availability. Trials to date remain conflicting and no clear benefit has yet to be established for lactobacilli. However, Bifidobacteria, Saccharomyces boulardii and other combinations of probiotics demonstrate some efficacy. The probiotic strain Bifido bacterium infantis 35624 (one capsule per day) has been shown to reduce pain, bloating, and defecatory difficulty and to normalize stool habit in IBS patients, regardless of predominant bowel habit[104]. The probiotic strain Bifido bacteriumlactis DN-173 010 has been shown to accelerate gastrointestinal transit and to increase stool frequency among IBS patients with constipation[53]. However, a systematic review of randomized clinical trials evaluating the efficacy, safety, and tolerability of probiotics in IBS determined that only Bifido bacterium infantis 35624 showed significant improvement in global and specific IBS symptoms in appropriately designed studies[104]. The theory behind the mechanism for improvement appeared to be downregulation of a proinflammatory state. No other probiotic showed significant improvement in IBS symptoms in an appropriately designed study[104]. The best clinical evidence for probiotic efficacy is in protection against infection, especially in neonatal and elderly groups. The role of probiotics in IBS remains uncertain given the limited clinical studies[104]. The role of psychological therapies has been analyzed in multiple studies[105]. The methodological design of most of these studies was inadequate; therefore, unequivocal evidence is lacking. However, the ACG Task Force concluded that cognitive therapy, dynamic psychotherapy, and hypnotherapy are more effective than usual care in relieving global symptoms of IBS[43]. Along the lines of alternative therapy, many patients will seek methods considered nontraditional in Western medicine. This is not surprising given the frustration of the symptoms. Individual patients may obtain relief from acupuncture, meditation, and relaxation techniques. There has been a recent study showing the effectiveness of mindfulness-based stress reduction in a small number of patients[105].

TREATMENT OF NONGASTROINTESTINAL SYMPTOMS

The IBS patient population has a wide variety of other symptoms. A study by Gralnek *et al*[106] of the health-related quality of life (HRQOL) of IBS showed significant other symptomatology. Patients with IBS had lower scores on the SF 36[107], a QOL scale. This was specifically noted in areas such as bodily pain, emotional well-being, fatigue, and poor social functioning. It is recommended that clinicians perform routine screening for diminished HRQOL in their IBS patients[94]. Bringing a treatment strategy into play that addresses these other mental and physical symptoms is difficult; again, the rela-

Table 2 Emerging therapies for irritable bowel syndrome

Agent	Mechanism of action	Targeted disorder	Clinical status
Peripheral acting agents			
Crofelemer	CFTR inhibitor	IBS-D	Phase 2b complete
Linaclotide (MD-1100)	Guanylatecyclase-c agonist	IBS-C	Approved by US FDA in 2012, 30th August
Arverapamil (AGI-003)	Calcium channel blocker	IBS-D	Phase 3
Verapamil	Kappa opioid agonist	IBS	Phase 2b complete
Mitemcinal	Motilin receptor agonist	IBS-C	Phase 2
Peripheral and central acting agents			
Ramosetron	5-HT 3 antagonist	IBS-D	Phase 3
TD-5108	5-HT 4 agonist	IBS-C	Phase 2
DDP-773	5-HT 3 agonist	IBS-C	Phase 2
BMS-562086	Corticotropin-releasing hormone antagonist	IBS-D	Phase 2
GW876008	(319) Corticotropin-releasing hormone antagonist	IBS	Phase 2
DDP-225	5-HT 3 antagonist and NE reuptake inhibition	IBS-D	Phase 2
GTP-010	Glucagon-like peptide	IBS pain	Phase 2
AGN-203818	Alpha receptor agonist	IBS pain	Phase 2
Solabegron	Beta-3 receptor agonist	IBS	Phase 2
Espindolol (AGI-011)	Beta receptor antagonist	IBS (all subtypes)	Phase 2
Dextofisopam	2,3 benzodiazepine receptors	IBS-D and IBS-M	Phase 3

IBS-C: Irritable bowel syndrome with constipation; IBS-D: Irritable bowel syndrome with diarrhea; IBS-M: Mixed irritable bowel syndrome; CFTR: Cystic fibrosis transmembrane conductance regulator; US FDA: United States Food and Drug Administration.

tionship and rapport between the physician and patient is very important.

EMERGING THERAPIES FOR IBS

Our current knowledge on the pathogenesis of IBS has led to the identification of a wide variety of novel agents targeting various mechanisms, now in various stages of development. This discussion will focus on drugs that have progressed beyond the proof of concept stage of development and will consider agents with predominantly peripheral effects, as well as those with both peripheral and central effects. Table 2 summarizes the status of various centrally and peripherally acting agents which are under various stages of clinical trial (Table 2).

CONCLUSION

IBS is a common disorder characterized by abdominal pain and altered bowel habit for at least 3 mo. A 2009 position statement issued by the ACG states that no symptom-based criteria have ideal accuracy for diagnosing IBS. Therefore, the ACG Task Force defines IBS as abdominal pain or discomfort that occurs in association with altered bowel habits over a period of at least 3 mo. The Task Force recommends that further investigations are unnecessary in young patients without alarming features with the exception of celiac sprue serology, which may be of benefit in some patients. Further investigation such as colonoscopy is recommended in those over 50 years of age and in patients with alarming features. Trials suggest psyllium fiber, certain antispasmodics, and peppermint oil are effective in IBS patients although the quality of the evidence is poor. Evidence suggests that some probiotics may be effective in reducing overall IBS symptoms but more data are needed. Anti diarrheals reduce the frequency of stools but do not affect the overall symptoms of IBS. 5HT 3 antagonists are efficacious in IBS patients with diarrhea and the quality of evidence is good. Patients need to be carefully selected, however, because of the risk of ischemic colitis. 5HT 4 agonists are modestly effective in IBS patients with constipation and the quality of evidence is good although the possible risk of cardiovascular events associated with these agents may limit their utility. Tricyclic antidepressants and selective serotonin reuptake inhibitors have been shown to be effective in IBS patients of all subtypes. The trials generally are of good quality but the limited number of patients included in trials implies that further evidence could change the confidence in the estimate of effect and therefore the quality of evidence was graded as moderate. Non absorbable antibiotics are effective particularly in IBS-D and selective C-2 chloride channel activators are efficacious in IBS-C with a moderate quality of evidence. Psychological therapies may also provide benefit to IBS patients although the quality of evidence is poor. Patients with IBS often seek CAM therapies, including cognitive-behavioral therapy, herbal therapies, probiotics, mind-body therapies, acupuncture, dietary changes, and exercise. Although most CAM therapies seem to provide some benefit in alleviating IBS, it is apparent that the duration, dosages, and specifics of the intervention greatly affect the outcomes. More studies need to be conducted to establish the subtle nuances associated with these treatments (*e.g.*, specific probiotics, standardization of herbal extracts, yoga style, *etc.*) to provide the most significant benefit for IBS. A wide variety of novel agents targeting various mechanisms of IBS are now in various stages of drug development.

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Role of negative affects in pathophysiology and clinical expression of irritable bowel syndrome

Maria Rosaria A Muscatello, Antonio Bruno, Giuseppe Scimeca, Gianluca Pandolfo, Rocco A Zoccali

Maria Rosaria A Muscatello, Antonio Bruno, Giuseppe Scimeca, Gianluca Pandolfo, Rocco A Zoccali, Psychiatry Unit, Department of Neurosciences, University of Messina, 98125 Messina, Italy
Author contributions: Muscatello MRA contributed to the literature search, manuscript writing, and final revision; Bruno A suggested topics to be included and contributed to the writing of the manuscript; Scimeca G managed the literature search and contributed to the writing; Pandolfo G contributed to the literature search and writing of the manuscript; Zoccali RA contributed to the study idea, manuscript writing and final revision of the article.
Correspondence to: Rocco A Zoccali, Professor, Psychiatry Unit, Department of Neurosciences, University of Messina, Via Consolare Pompea, 1, 98125 Messina, Italy. rocco.zoccali@unime.it
Telephone: +39-90-2212092 Fax: +39-90-695136
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Abstract

Irritable bowel syndrome (IBS) is regarded as a multifactorial disease in which alterations in the brain-gut axis signaling play a major role. The biopsychosocial model applied to the understanding of IBS pathophysiology assumes that psychosocial factors, interacting with peripheral/central neuroendocrine and immune changes, may induce symptoms of IBS, modulate symptom severity, influence illness experience and quality of life, and affect outcome. The present review focuses on the role of negative affects, including depression, anxiety, and anger, on pathogenesis and clinical expression of IBS. The potential role of the autonomic nervous system, stress-hormone system, and immune system in the pathophysiology of both negative affects and IBS are taken into account. Psychiatric comorbidity and subclinical variations in levels of depression, anxiety, and anger are further discussed in relation to the main pathophysiological and symptomatic correlates of IBS, such as sensorimotor functions, gut microbiota, inflammation/immunity, and symptom reporting.

Key words: Depression; Anxiety; Anger; Mood disorders; Irritable bowel syndrome; Neuroticism; Stress; Brain-gut axis; Microbiota; Hypothalamic-pituitary-adrenal axis

Core tip: This review deals with the role of negative affects in pathophysiology and clinical expression of irritable bowel syndrome (IBS). Depression, anxiety, and anger play a key role in dysregulation of the brain-gut axis, contributing to the majority of pathophysiological and symptomatic correlates of IBS. Research efforts to integrate different knowledge provide further insight into the pathways linking negative psychological states to health and disease, leading to identification of individual vulnerability and susceptibility factors, including subsyndromal conditions, which should be addressed to promote better health in the population and more effective and efficient prevention and treatment of IBS.

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INTRODUCTION

Irritable bowel syndrome (IBS) is a disorder of the lower gastrointestinal tract characterized by a set of gastrointestinal (GI) symptoms such as abdominal discomfort or pain[1], bloating or feelings of abdominal distension,

alterations in bowel habits (constipation and/or diarrhea), and altered stool passage (urgency or feeling of incomplete evacuation)[2]. Diagnosis of IBS depends on symptom-based criteria such as the Rome criteria, after excluding the presence of any organic GI diseases[3]; beyond diagnostic criteria, clinical routine biopsies and examinations have shown that the number of lymphocytes, mast cells, enterochromaffin cells, and nerve fibers are, at least in IBS subtypes, increased[4-7] and an increased amount of nerve-activating mediators is released from the colonic mucosa in IBS patients[8]. Estimated prevalence of IBS in the general population varies from 5.8% to 26.1%[9] and much of the variability in prevalence rates is probably due to different symptom-based diagnostic criteria, sample selection, access to health care, and/or cultural factors. In clinical samples, IBS accounts for approximation 3% of all general practice[10] and up to 70% of referrals to gastroenterology clinics[11].

Regarding sex differences, a higher prevalence is found in women, with a female-to-male ratio close to 2[12]. As predominant bowel pattern, women more commonly report functional constipation, defined as persistent symptoms of difficult, infrequent, or seemingly incomplete defecation[13], with no other apparent etiology, and abdominal pain[14]. Furthermore, women are more likely than men to seek medical assistance[15]. The onset of IBS usually occurs between the ages of 15 and 65 years, and the mean age at presentation is the mid 30s, although in a number of subjects, symptoms may date back to childhood. The course of the illness is chronic, fluctuating, and relapsing; in some cases, symptoms can spontaneously resolve and the syndrome may have a good prognosis, as documented by long-term, follow-up studies[16,17].

The etiopathogenesis of IBS is complex and not yet completely understood. IBS may be better conceptualized as the resultant of the complex interactions of a number of factors such as abnormal colonic motility, visceral hypersensitivity, enhanced pain perception[18,19], low-grade inflammation involving mast cells[20], dietary intolerance[21], alteration of microbiota, the intestinal microbial community[22,23], abnormalities in the autonomic nervous system[24,25], and stress[26,27].

A major contribution to the understanding of the pathogenesis of IBS has come from the hypothesis of the hyper-reactivity of the brain-gut axis; a model describing bidirectional pathways among central nervous system (CNS), autonomic nervous system (ANS), and enteric nervous system (ENS), thus linking emotional and cognitive areas in the CNS with visceral afferent sensation and intestinal function[28,29]. A growing body of evidence indicates that IBS is viewed as being caused by dysregulation of the brain-gut axis, involving neural, endocrine and neuroimmune pathways that are affected and often disrupted by psychosocial and environmental stressors, including life events, or by physical stressors (infection/inflammation)[30,31].

The biopsychosocial model of illness and disease[32] has provided a conceptual framework that is helpful for understanding the bidirectional relationship between mind and body, integrating biological science with individual features. According to this model, illness and disease result from simultaneously interacting systems at multiple levels (cellular, tissutal, organismal, interpersonal, and environmental), and research in this field is oriented to determine the ways in which biological, psychological, and environmental factors interact to explain illness onset, course, and outcome[33]. The biopsychosocial model applied to the understanding of IBS pathophysiology assumes that psychosocial factors, interacting with biological mechanisms such as peripheral/central neuroendocrine and immune changes, may induce and aggravate symptoms of IBS, modulate symptom severity and persistence, influence illness experience and quality of life, and affect treatment response and outcome[34]. However, it remains unclear which psychological factors are the most relevant in explaining these outcomes.

The natural history and risk factors of IBS suggest that the disease may begin in early life. The quality of the early family environment can provide a main source of strength or vulnerability in later life[35]. Mothers represent a major source of interaction and regulation of physiological and psychological processes, therefore, maternal care influences the physiological and psychological development of the child, whereas inadequate maternal care is related to developmental problems both in human infants and in rats[36]. In rodent models it has been shown that the early postnatal stage is a crucial period for the healthy development of pups; alterations in early experiences are thought to have long-lasting consequences for stress responsiveness and emotions[37]. An extension of this model postulates that adverse events in early life, experimentally reproduced with the use of the maternal separation procedure, are associated with maladaptive behavioral and hormonal responses to stress and may contribute to increase vulnerability to disease in later life. Maternal separation (MS), a model of early life stress, has been used in rodents for understanding the effects of early life stress on the development of the CNS and across a variety of systems including the brain-gut axis[38].

Thus, the multiple alterations across the regulation of the brain-gut axis animal models provide strong evidence for a deep understanding of stress-related GI disorders also in humans. It has been suggested that problems during the perinatal period may contribute to the susceptibility to develop IBS in humans; events such as prenatal undernutrition and painful experiences might interfere with GI physiological maturation[39].

Over the past decade the significant interplay among psychopathological factors, psychiatric comorbidity, chronic emotional distress as possible associated features or co-factors in IBS onset, course, and clinical expression has gained attention by research strategies pursued by different specialties, such as psychiatry, psychology, neurobiology, and pain.

In this review we focus on the role of negative affects, including depression, anxiety and anger on the pathophysiology and clinical expression of IBS, taking into account that association between emotions and GI health is bidirectional and effects can accumulate over a long period of time. The focus on affective traits is congruent with the line of research whose effort is to target those basic dispositions relevant to psychopathology that may affect the development, course and outcome of IBS, possibly influencing the search for individualized and integrated treatment strategies oriented to improve individual outcomes.

AFFECTIVE STYLES: POSITIVE *VS* NEGATIVE AFFECTS

The term affective style refers to a range of individual differences in different parameters of emotional reactivity, involving valence-specific features of emotional reactivity and affective processing and regulation. In keeping with its main role in defining mental health and quality of life, affective style has been studied in detail. From a psychophysiological point of view, several parameters of affective style can be objectively measured including: (1) threshold to response; (2) magnitude of response; (3) rise time to peak of response; (4) recovery function of the response; and (5) duration of response, where the last three parameters refer to different aspects of affective chronometry or the time course of emotional responding[40].

Evidence from both animal and human neurobiological and brain imaging studies shows that the key brain regions contributing to the supposed emotion processing circuit are the prefrontal cortex (PFC), amygdala and extended amygdala/ventral striatum, including nucleus accumbens, hypothalamus and anterior cingulated cortex (ACC)[41].

Beyond detection and evaluation of emotionally salient stimuli, emotion processing also involves the experience, regulation and expression of the affective response; the capacity to regulate effectively negative emotions and to decrease the duration of aversive affects is considered one of the key components of affective style[42].

A large amount of research shows that emotional experience is dominated by two core and broad dimensions accounting for the variability in individual levels of psychological well being and distress: positive affect (PA) and negative affect (NA)[43,44]. PA can be defined as an affective construct that reflects a level of pleasurable engagement with the environment and that involves both emotional and cognitive components, such as joy, enthusiasm, happiness, high energy levels, interest, motivation, and mental alertness[45]; low levels of PA are characterized by poor energy and fatigue.

In contrast, NA is a dimension of subjective distress including a range of aversive mood states, such as sadness, anger, disgust, guilt, fearfulness, and depression. Both PA and NA can be conceptualized either as a transient state or as a trait, defined as individual differences in general affective level. Affective traits are stable dispositions to experience the corresponding mood factors (positive or aversive emotions). Hence, high-trait NA subjects are more likely to experience pervasive and intense states of negative affect, whereas high trait PA individuals report generally higher levels of positive affect, maintaining a generally high activity level. High NA individuals usually experience significant levels of distress and dissatisfaction across time and in everyday life situations, even in the absence of objective stressors; they are also more introspective, suffer from poor self-esteem and tend to focus on the negative side of self, others, and the world in general. Moreover, high NA individual tend to be hyper-reactive in front of stressful events[46].

More recently, the constructs of PA and NA, along with a dimension defined as physiological hyperarousal (HA), have been included in the tripartite model, which aims to assess distinctive and overlapping features of depression and anxiety. The core assumption of the model is that both disorders share a general distress factor; however, depression should be characterized by a mixed state of high NA and low PA, whereas anxiety should be a state of high NA and HA[47]. Accordingly, NA is thought to be the underlying construct for both depressive and anxiety disorders.

Whether PA and NA should constitute the extreme and opposite poles of the mood dimension has been a matter of debate in the emotion literature. Although their names seems to suggest that they are opposite poles of the same continuum, PA and NA appear to be highly distinct dimensions; as stated by Zautra *et al*[48] "most of us believe that positive feelings are the opposite of negative feelings, and that a person who is unhappy is also sad. These statements are truisms in the language of feelings, affects, and emotions. Most of the time, positive and negative feeling states are independent of one another". This issue has not only theoretical implications, but also impacts on clinical research; as highlighted in a review on the influence of PA on health: "If PA and NA are bipolar ends of the same construct, benefits of PA may merely reflect the absence of NA rather than the presence of positive feelings. Alternatively, should the two be mutually independent, PA could provide benefits independent of NA levels"[49].

The conceptualization of PA and NA as independent dimensions has received support from neurobiological and brain-imaging studies that have demonstrated the existence of separate cerebral circuits and anatomical systems mediating positive and negative affectivity[40] and, further, that the propensity toward one or the other type of affect reflects individual dispositions probably grounded in neurobiological differences. In terms of the brain centers involved, the frontal lobes of the right hemisphere have been implicated in NA and behavioral inhibition (withdrawal tendencies), whereas the frontal

lobes of the left hemisphere have been implicated in PA and approach behaviors[40]. The existence of two separate systems and brain circuits for PA and NA has powerful implications for clinical work, because therapeutic approaches may focus on decreasing negative affective experiences or increasing positive ones.

From a personality perspective, NA as a trait is the core component of two similar personality constructs, neuroticism, and type D (distressed) personality. Neuroticism, defined as "a broad dimension of individual differences in the tendency to experience negative, distressing emotions and to possess associated behavioral and cognitive traits"[50], is characterized by overstated reactivity to physiological changes, by emotional instability with overwhelmingly negative emotions. Neuroticism has been related with a broad range of physical health problems, included IBS[51,52], even when depression and other risk factors are controlled. Type D personality, a concept derived from empirical and theoretical research, is regarded as consisting of two stable broad traits: NA, and social inhibition (SI), which refers to a tendency to suppress and inhibit the expression of emotions[53,54]. Type D personality seems to confer a specific vulnerability to chronic stress, and it has been associated with high rates of medical comorbidity, unfavorable clinical courses, both medical and psychological, and low subjective and physician-assessed health ratings[55].

Overall, affective styles, also conceived as personality traits in different theoretical models of personality, represent stable and chronic attributes of individuals and thus they may have predictive value; in this sense, NA is related to long-term emotional distress, general maladjustment, and with a broad range of subjective complaints and reported physical symptoms.

ROOTS OF NA: TEMPERAMENT, EPIGENETICS, AND CHILDHOOD ANTECEDENTS

Temperament has been defined as biologically based individual differences in reactivity and regulation that are almost stable over time; probably in part heritable, it shapes the propensity to approach or withdrawal from novel and unfamiliar situations and, ultimately, the way individuals adapt[56]. Such individual differences appear early in life, and have been biologically connected with differences in emotional responsivity, as approach tendencies/behavioral activation are related to PA, whereas withdrawal tendencies/behavioral inhibition are linked to NA. Accordingly, infants and children characterized by behavioral inhibition show high NA, and low levels of PA, social interactions, and approach behaviors; moreover, they are fearful in unfamiliar contexts[57]. Temperament dimensions are normally distributed in the general population, but it is widely accepted that certain temperamental features, such as NA and behavioral inhibition, may have a role in the development of later psychopathology and affective disorders. As suggested by recent research, behavioral inhibition is recognized as an early temperamental precursor of later anxiety disorders[58], whereas low PA is an acknowledged risk factor for depression[59].

It has been hypothesized that, on an inherited temperamental basis that predisposes vulnerability to withdrawal tendencies/behavioral inhibition, the expression of an affective style characterized by preponderant NA is ultimately determined by environment. A number of environmental factors possibly playing a role in the formation of a negative affective style have been identified: negative parenting styles, insecure parent-child attachment, parental anxiety, and adverse life events may interact with behavioral inhibition in maintaining the pathways toward psychopathology and mental distress[58].

On a neurobiological level, it has been suggested that adverse events in early childhood affect experience-depending maturation of structures on those brain systems underlying emotional functioning[60].

A growing line of research is converging on epigenetics, the study of heritable changes in gene expression that occur without changes in DNA sequence. Epigenetic mechanisms can change genome function under exogenous influence; moreover, epigenetic changes allow for the stable propagation of gene activity states for generations afterwards[61].

From an epigenetic point of view, among environmental factors, early life experiences are associated with different outcomes in life-long health and behavioral pathways both in animals and in humans[62]. It has been demonstrated that differences in maternal care in rats during the first weeks of life are associated with long-term effects on behavior and brain function persisting into adulthood, mainly *via* alterations in the stress response[63]. Similarly, in humans, childhood maltreatment is significantly associated with psychopathological outcomes in adult life[64].

Moreover, maternal emotional states, such as anxiety, psychological distress, and depression, are associated with child health, and parental functioning problems, such as restricted emotional expression, poor emotional supportiveness, and role conflicts, are associated with poorer psychological and physical well being of children with chronic illnesses[65].

NEGATIVE AFFECTS AND IBS: POTENTIAL LINKS

The effect of negative affects and emotions on health has been long recognized; particularly, the physiological consequences associated with affect and emotion arousal provide one potential mechanism by which emotional states may influence physical health, leading to increased vulnerability to illness[66]. In general, negative affects are related to unhealthy patterns of physiological functioning, while positive affects are associated with better

health, although the role of other potential mediators of this association, such as healthy lifestyles, stronger social networks, more positive social interactions, frequency of stressful events, and a putative role of PA as stress buffer need to be further investigated[49].

From a neurobiological point of view, CNS pathways (outflows from cortico-limbic-pontine networks) to gut form the emotional motor system (EMS) that can be viewed as an extension of the limbic system into the gut, involved in the modulation of autonomic and neuroendocrine pathways, and pain[67]. The tonic modulation exerted by the EMS include serotonergic, noradrenergic and opioidergic descending spinal pathways. The most studied pathways in IBS is the serotonin (5-HT) neurotransmission system, and it has been hypothesized that the co-occurrence of NA and IBS may be related to serotonergic hypofunction both in the ENS and CNS. Thus, affective and emotional symptoms should be considered as specific and integral to the illness, rather than causal factors or consequences of IBS[68]. We discuss here further mechanisms that may subtend the link between NA and IBS, and in particular the potential roles of autonomic nervous system, stress-hormone system and immune system.

ANS

The ANS can be considered the neural interface conveying top-down and bottom-up signals. The same cerebral regions (amygdala, hippocampus, and prefrontal cortex) implicated in the modulation of gut function are also involved in the regulation of affectivity, mood, and emotions, and, thus, in the development of social behavior and coping strategies[69]. At the ANS level, a relative hyperfunction of the sympathetic activity along with a relative hypofunction of the parasympathetic activity (low vagal tone) has been described in mood and anxiety disorders[70]; similar findings have been described in IBS, and increased sympathetic tone has been shown to enhance visceral hypersensitivity[71]. The processing of sensory information, mainly of noxious stimuli, strongly relies on emotional, motivational, and cognitive components, all of which are likely affected by affective styles. ANS output can be activate by ascending interoceptive signals from the gut, by descending cognitive or emotional influences, or in response to external or internal demands. Depending on the class of stimuli (threats to body homeostasis, severe environmental stressors, as well as strong emotions such as anger, fear, and sadness), ANS output can override local ENS function[72]. The effect of sympathetic outflow to the gut is inhibitory, thus it slows GI transit and secretion, whereas parasympathetic activity on the gut is excitatory, and also mediates the release of 5-HT from enterochromaffin cells[73]. Autonomic dysfunction, mainly lower parasympathetic activity, may account for the burden of extraintestinal symptoms and overlapping chronic pain disorders in IBS, such as chronic pelvic pain, headache, fibromyalgia, and psychiatric disorders[28,30]. An intriguing hypothesis is that top-down sympathetic and parasympathetic activity from brain to gut is thought to mediate affect-related patterns of regional changes in motor, secretory and perhaps immune activity in the GI tract, and these changes are similar to the changes in facial expressions and body postures induced by emotional states[72]. Accordingly, long-lasting alterations in ANS output to the gut may induce changes in peripheral target cells (*e.g.*, adrenergic and serotonergic receptors) with subsequent feedback from gut to brain and possible effects on those brain regions that receive this prolonged input, as highlighted by neuroimaging studies[69]. Thus, the hypothesis of ANS dysfunction may provide a stimulating theoretical frame for better understanding the association between changes in brain-gut signaling and NA.

Stress-hormone system: hypothalamic-pituitary-adrenal axis

With the term "stress", Selye defined the physiological adaptive responses of the organism to emotional or physical threats ("stressors"), whether real or perceived[74]. Stress evokes adaptive "fight or flight" responses aimed to maintain the stability of the internal environment and to ensure survival; these responses are driven by the activation of the hypothalamic-pituitary-adrenal (HPA) axis. Corticotropin-releasing factor (CRF) is a crucial neuropeptide in the regulation of the HPA axis, and the final common pathway in the stress response. It has several central effects, including cardiovascular by sympathetic activation leading to increased arousal, alertness, rapid heart rate and respiration, cerebral blood flow regulation, and stress-induced analgesia[75]. Glucocorticoids and catecholamines secreted in response to stressor represent the primary mediators in the chain of hormonal events triggered in response to stress; cortisol, the hormonal end-product of the HPA axis, is one of the most potent endogenous feedback compound on the pro-inflammatory signal transduction machinery[76]. Negative feedback involving the activation of neural, neuroendocrine and immune mechanisms limits the stress response, leading the organism back to a state of homeostasis; thus, the "ideal" stress response should be time limited. However, if the stressor persists and becomes chronic, the adaptive system becomes defective or excessive, and the prolonged activation of the HPA axis and ANS lead to alterations in mood and affects, a loss of cognitive and affective flexibility, and inhibition of those vegetative processes that are likely to impede survival of the organism during a life-threatening situation, like appetite control, digestion, sleep, sexual activity, and endocrine programs for growth and reproduction[75]. Stress is considered a significant factor involved in illness and well-being; stress-induced disorders affect the whole body, including the GI tract, which is a sensitive target[77], and is strongly related to psychiatric disorders. Regarding depression, research has focused on a central role of a pathologically dysregulated HPA axis; stress-induced hypercortisolemia leads to the central downregulation of glucocorticoid receptors, im-

pairing negative feedback of cortisol and enhancing levels of CRF and adrenocorticotrophic hormone[78]. Early stressors are thought to predispose individuals to adult-onset depression *via* a stable hyperactivity of the HPA system. It has been demonstrated that individuals with both depression and antecedents of early-life trauma exhibit structural reductions in regions involved in HPA axis regulation, whereas individuals with depression alone do not. A recent study has shown that smaller hippocampal volume is observed before the manifestation of clinical symptoms of depression in at-risk adolescents; particularly in those who experienced high levels of adversity during childhood[79]. The authors suggested that smaller hippocampal size may be an inherited trait predisposing individuals towards the development of those psychiatric disorders that are triggered or worsened by stress, thus partially mediating the effect of early-life adversity on depression during longitudinal follow-up. Increased HPA-axis activity, documented by cortisol awakening response, is also found in anxious people[80] and, in healthy adults, it has been associated with changes in experimentally induced NA[81].

Regarding IBS, a major role of stress in the pathophysiology, symptoms severity, visceral pain, and treatment outcome has been documented[82]. Similar to that found in depression, epidemiological data have shown that a history of early trauma (sexual or physical abuse, and neglect) in genetically predisposed subjects can be a key predisposing factor for the development of IBS in adulthood; however, it occurs in only a minority of all IBS patients. The mechanisms by which early trauma can affect susceptibility to stress-related disorders are the induction of persistent changes in the responsiveness of HPA axis to further stressors[83], and possibly, epigenetic changes in glucocorticoid receptor expression[84]. Important insights on the role of early stress on later development of GI symptoms come from animal models. The impact of maternal separation on the brain-gut axis and on GI function has been extensively studied: separated rats show alterations in both central and peripheral neurotransmitter systems, mainly 5-HT and glutamate, activation of the HPA axis with changes in the CRF system; alterations in pain pathways and visceral hypersensitivity with stress-induced hyperalgesia and increased colonic motility; GI immune changes, with aberrant activation of both colon- and systemic-mediated immunity; intestinal barrier dysfunction, with increased permeability and inflammatory cell infiltration in the lamina propria; and disrupted microbiota persisting into adulthood[85]. Based on the literature, it appears difficult to determine at which level of the HPA axis the primary dysfunction is located; nevertheless, there is sufficient evidence that, in susceptible individuals, alterations in the central stress circuits mediated by early-life adversity and NA play a major role in the pathogenesis and clinical expression of IBS.

Immune system

The immune system is highly connected with other physiological systems and susceptible to the effects of hormones, neurotransmitters, and other mediators. Direct anatomical and functional links between the CNS and the immune system provide a biological pathway by which affects, emotions, and psychological distress may influence immunity. Chronic stress is associated with altered immunity and an increased susceptibility to a number of diseases[86], and there is emerging evidence that positive affects can moderate the negative impact of stress on immunity. PA as a trait has been associated with increased cellular immune competence, greater NK cell cytotoxicity, and increases in secretory IgA responses to antigen challenge, increased number of helper T cells, and antibody levels[87]. Conversely, a growing body of research has found that negative affects and emotions are associated with immunological dysregulation in experimental paradigms aimed to evoke transient mood changes. NA as a stable personality dimension with related coping styles such as repression, rejection, and sensitivity, has been associated with altered leukocyte counts in peripheral blood and dysregulated cellular immune function[88]. Individuals high in hostility, a stable personality disposition derived from anger, exhibit significant increases in NK cell cytotoxity when compared with those who are low on hostility[89]. The relationship between depression and immunity is complex, because depression has been alternatively associated with both immune suppression and immune activation. A recent study has shown that depression and melancholia are associated with activation of cell mediated immunity (CMI); the linked CMI and inflammatory responses are also associated with the onset of depression and with the melancholic cluster symptoms of depression. Exposure to previous depressive episodes seems to amplify the size of CMI responses, possibly increasing the recurrence of depressive episodes[90]. However, markers of impaired cellular immunity, mainly diminished NK cell cytotoxicity, have also been associated with depression[91]. Nevertheless, the significant associations of findings of both immune suppression and immune activation with depression raise serious questions concerning the validity of the construct of depression as a homogeneous disorder.

The associations between immunity and GI function are unequivocal, because up to 80% of the immune cells of the whole organism are contained within the gut mucosa and the gut-associated lymphoid tissue[78]. Accordingly, the gut represents a key structure in maintaining the balance between tolerance and immunity[78], but it also is a target for unbalanced immune processes. There is increasing evidence suggesting a role for immune activation in IBS[92].

Findings from a study aimed to examine associations between depression, fatigue, and immunity in IBS patients *vs* healthy controls demonstrate that IBS samples are characterized by higher numbers of mast cells in the gut lamina propria compared to controls; moreover, fatigue and depression ratings are correlated with mast cell counts[93]. Evidence for a low-grade inflammatory state

in both IBS, depression, and anxiety is discussed in the section related to inflammation as a feature of IBS.

Based on the literature, it is clear that dysfunctional affective and behavioral patterns are main factors in the activation of immune pathways linking to IBS, taking into account that communication between the brain, immune system and gut is bidirectional.

PSYCHIATRIC COMORBIDITY, NEGATIVE AFFECTS AND IBS: WHAT IS THE EVIDENCE?

The issue of comorbidity among psychiatric disorders and IBS is well documented. The prevalence of psychiatric disorders in IBS patients is high, with up to 60% of patients diagnosed as having a comorbid psychiatric disorder[94]. Results from a prospective, randomized controlled trial aimed to investigate the relationship between bowel symptoms, psychological variables and rectal sensitivity in severe IBS, showed that 44% of IBS patients had psychiatric comorbidity[95]. The higher rates of comorbidity refer to patients seen in referral centers, and it remains unknown whether those rates of psychiatric disorders are as high in the community of non-consulters, or whether they apply exclusively to those subjects who have sought treatment[2]. Other authors have suggested that excess comorbidity characterizes only a subset of IBS patients who show a tendency to noticing somatic sensations and a lower threshold for consulting a physician; although, psychopathological factors in IBS have been considered to be related to inducing illness behavior rather than being causally related to the condition itself[96]. Nevertheless, there is a growing body of evidence supporting an association between psychological distress, environmental stress, and IBS.

A population-based nested case-control study[97] demonstrated that psychosocial distress was significantly associated with the presence of a functional GI disorder, although it was not explained by health-care utilization. Studies conducted on university students have highlighted the role of depression and anxiety in predicting IBS status[98], and in community subjects, depression, anxiety, sleep disorders, and somatic symptoms such as headache, and backache were more frequently associated with IBS independent of other variables such as age, sex, education level, marital status, smoking, alcohol use, and BMI[99].

No specific psychiatric disorder has been associated with IBS, but mood disorders (major depression and dysthymia), anxiety, and somatoform disorders have been the most frequently diagnosed conditions[100]. In some authors' opinion, high rates of psychiatric disorders in IBS probably reflects referral bias[96], although recent evidence has shown that psychiatric comorbidity is not only present in tertiary care, but also in primary care[101] and in non-help-seeking community-based patients[102]. A random community-based telephone survey aimed to investigate the prevalence, comorbidity and risk correlates of IBS and generalized anxiety disorder (GAD) in a general population (n = 2005) showed that about one in six people with IBS in the community had comorbid GAD that added to the severity and impairment of IBS, also associated with core depressive symptoms[103]. It is also recognized that patients with IBS or other functional GI disorders tend to have additional functional disorders in various other organ systems (*e.g.*, unexplained pain, weakness, and sexual complaints), and complaints of multiple drug sensitivities and allergies. Moreover, they make twice as many health-care visits per year as age-matched controls, undergo excessive diagnostic tests and surgical procedures, use many over-the-counter and prescription medications, and become refractory to treatment[104]. Thus, the implications for health economics are significant: IBS patients incur substantially more direct health-care costs than non-IBS patients, and 66% of these excess costs are for non-GI indications, and mainly for psychiatric comorbidity[105].

It must be stressed that the comorbidity between IBS and psychiatric disorders seems to be bidirectional; also, the prevalence of IBS in psychiatric patients who seek treatment is high, with a prevalence of 19% in schizophrenia, 29% in major depression, and 46% in panic disorder among other disorders[106]. The prevalence rate of IBS is particularly high in patients diagnosed with double depression (major depressive episode plus dysthymia, a chronic depressive condition), and 57.6% of patients with double depression met the criteria for IBS when compared with controls[107]. These patients are also more likely to complain of symptoms of weakness, nocturnal bowel movements, and GI symptoms related to stress[107]. Furthermore, in 50% of patients, the psychiatric disease preceded the onset of the bowel disease, whereas in 37.5% of patients the bowel symptoms appeared first; significantly more patients with double depression also reported a family history (61.5% *vs* 22.5%) of IBS than controls did[107]. Anxiety sensitivity and illness attitudes and intrusiveness are predictive of elevated IBS symptoms[108].

Maladaptive affective experiences are distinguished from their adaptive counterpart by several indexes, such as frequency, intensity, duration, and inappropriateness[109]. Although they represent a main part of psychiatric disorders (*e.g.*, depressed mood is fundamental to clinical depression, as well as anxiety, and is the main symptom of GAD), they should be not be considered identical[109].

As underlined in the previous sections, symptoms of depression and anxiety, related emotional disorders, individual differences in the tendency to experience negative affect, and related personality traits may reflect a chronic psychological characteristic rather than a discrete, identifiable psychiatric condition. This distinction is important because diagnosable psychiatric disorders, characterized by full, severe symptoms, occur occasionally in the population, whereas subclinical affective symptoms and personality traits involving depression and anxiety,

besides being more common, may be associated with worse health outcomes, and with increased susceptibility to illness just as the full-blown affective disorders[110].

Here, we summarize research that has evaluated negative affects or affective symptoms in IBS, specifically addressing depression, anxiety and anger. These constructs overlap substantially with more general constructs such as psychological distress and with trait features of negative affect.

Although it may seem rather obscure, from a psychopathological point of view, depressive and anxious affects are included in the same section, because the vast majority of clinical-based research and review papers have not investigated these two affective dimensions separately. Plausible explanations are that depression and anxiety are the most common mental disorders in the general population, often comorbid and overlapping conditions, and mainly, they are categorical psychiatric disorders with a precise set of diagnostic criteria. In contrast, anger, despite its wide diffusion in the general population, has received little attention from psychiatry and clinical psychology, and anger has not been studied in as much depth in its connection to IBS.

ROLE OF DEPRESSION, ANXIETY AND ANGER IN IBS

In the following sections we review the research that has examined the negative affects depression, anxiety and anger in relation to the main pathophysiological and symptomatic correlates of IBS such as sensorimotor functions, gut microbiota, inflammation and immunity, and symptoms reporting.

Visceral and pain hypersensitivity and abnormal colonic motility

Perceptual hypersensitivity to experimental and physiological gut signals in IBS patients has been systematically demonstrated since the first studies on this issue[111,112], and it has been proposed as a reliable biological marker of the illness. Nevertheless, it is not clear whether this may reflect a true hypersensitivity or rather a central affective alteration of the processing of normal or even reduced gut signals[72].

Recent studies have shown that the hypersensitivity for rectal distension in IBS is largely determined by hypervigilance for visceral stimuli, anticipation, and a marked tendency to label visceral sensations as painful rather than being a true hypersensitivity; namely an enhanced detection of visceral stimuli[113]. Recent studies on IBS patients have directly assessed hypervigilance by the use of the emotional Stroop test - a measure of emotional interference and attentional bias - and results have shown that IBS patients present attentional biases to GI pain-related, and social threat words[114]. A good deal of research has increased knowledge about the strong influence of negative affects, cognitive processes, arousal, and conditioning on visceral hypersensitivity and pain sensitivity, not only in subjects with IBS, but also in those with subtle GI symptoms within the general population. Anxiety and depression scores are elevated in subjects who report abdominal pain and are positively correlated with pain duration[115]. Moreover, subjects with abnormal anxiety and depression scores have generally a higher burden of abdominal pain, and this association is most prominent in women[115]. The influence of depressive symptoms on rectal sensitivity in different subtypes of IBS patients (diarrhea/constipation-predominant *vs* alternating subtypes) has also been investigated. Depression levels are negatively correlated with rectal pain threshold, but only in the alternating subtype of IBS, suggesting that depression might affect pain thresholds differently according to the subtype of IBS[116]. Other authors have not found a relationship between rectal sensitivity and negative affects, anxiety, depression, and neuroticism[117].

Attempting to elucidate to what extent affective disturbances contribute to disturbed neural responses to visceral stimuli, brain imaging findings in healthy subjects have documented increased brain activity during negative affects in posterior cingulate cortex, thalamus and somatosensory cortex in the rectal distension paradigm[118]. It has indeed become clearer that the emotional context affects the neural processing of visceral stimuli even in healthy subjects[19]. Brain imaging studies[119] have shown that IBS patients, in response to visceral pain stimulation, present differential brain activations in ACC and insular regions, and in prefrontal and limbic regions, such as the amygdala and hippocampus; brain regions that play a major role in the central pain matrix, besides being involved in the affective component of pain. A functional magnetic resonance imaging study[120] has demonstrated that patients with IBS experience significantly more pain and discomfort upon rectal distension, despite unaltered rectal sensory thresholds. Depression and anxiety scores are associated with subjective stimulus ratings, but not with rectal sensory thresholds. Furthermore, depression symptoms are correlated with activation of the PFC and cerebellar areas, whereas anxiety scores are significantly associated with pain-induced activation of the anterior mid-cingulate cortex and pregenual anterior cingulate cortex. The above brain regions are involved in the processing of visceral afferent information, in emotional arousal and in cortical modulation. This study was particularly interesting because the examined sample of IBS patients was characterized by relatively minor psychopathological symptoms, although intriguing implications about the role of negative affects rather than that of psychiatric disorders can be inferred. Depression, anxiety, stress, anticipation, and the recall of painful memories can enhance the perception of visceral pain, whereas relaxation and distraction can decrease it[29].

Intestinal motility patterns, such as enhanced gastrocolic reflex, altered gastric emptying, increased small

intestinal transit, and small bowel contractions following stressful events are present in 25%-75% of patients with IBS[100]. Patients with diarrhea-predominant IBS (IBS-D) have shorter small-bowel and colonic transits than those with constipation-predominant IBS (IBS-C), who exhibit slowed whole-gut transit. Psychological distress can elicit an enhancement of colonic motility and diarrhea[15]. This effect is particularly pronounced in IBS patients, who tend to have a greater colonic motor response to both psychological and physical stress in comparison to healthy controls. In a large sample (n = 1021) of university students, participants with IBS were more likely to have clinical anxiety and reported higher levels of anxiety sensitivity, neuroticism, and trait worry than their asymptomatic counterparts[121]. In this group, anxiety was specific to visceral sensations, and this measure was the strongest predictor of IBS diagnostic status[121]. The authors have suggested that IBS may reflect a vicious cycle of anxiety response in which fears and negative beliefs about GI sensations lead to increased vigilance toward bodily sensations, and in turn, the visceral sensations themselves may further increase as a result of anxiety. Taken together, these results suggest that while predisposing vulnerability factors such as NA or neuroticism and enhanced stress responsiveness contribute to the development of anxiety problems, they may also predispose the individual to anxiety-related visceral sensations, thereby increasing IBS risk. A recent study comparing the distribution of symptoms and psychological parameters in adult patients with different functional GI disorders has demonstrated that depression and anxiety are related to the anatomical extent of the problem, such as the number of sites of complaint[122]. Moreover, sex differences were found: in male subjects, the extent of sites of functional GI disorder is strongly related to trait anxiety, whereas in female subjects it is strongly related to depression[122].

Evidence of the association of anger and IBS symptoms is incomplete, although this negative affect seems to have a role in altering colonic motility. IBS patients exposed to experimentally induced anger-provoking conditions became angrier than healthy controls and have increased colonic motor activity[123]. A further study by the same research group has shown that, during anger-provoking paradigms, IBS patients show a decrease of antral motor activity, whereas in controls the same activity is increased[124]. Suppression of anger, namely the cognitive and behavioral efforts to restrain angry feelings, is associated with prolonged gastric emptying and delayed gut transit in IBS patients[125]. Moreover, there is an association between anger control or repression and IBS symptoms such as abdominal pain and increased postprandial colonic motility[126]. A study by our research group has shown no significant differences in anger and defense mechanisms between IBS patients and controls, and thus we suggest that anger experience and expression, as well as defensiveness, do not seem to play a critical role in IBS patients who do not manifest a concurrent psychiatric disorder or emotional disturbance[127]. Nevertheless, a subsequent study of depression, anxiety and anger in subtypes of IBS subjects has shown that patients with IBS-C compared with IBS-D are characterized by higher levels of trait anger; a stable dispositional feature that includes a general tendency to experience and express anger, along with higher levels of depression and anxiety[68]. Findings from a study aimed to compare levels of self-reported trait and suppressed anger and recalled childhood abuse in patients with IBS (n = 75) or Crohn's disease (n = 76) showed that IBS patients were more prone to experience and suppress anger in everyday situations than patients with Crohn's disease[128]. However, only trait anger remained elevated in IBS patients after controlling for other psychological variables; moreover, childhood sexual abuse was more prevalent in IBS than Crohn's disease patients but it was unrelated to trait anger[128].

Together, these studies highlight the contributioin of negative affects in mediating the pain experience and altered bowel motility in IBS.

Abnormal gut microbiota

The GI microbiota is a rich microbial ecosystem crucial to health and disease; it colonizes the human gut immediately after birth, and plays a key role in maintaining physiological functions of the host, such as digestion and metabolism, trophism, and the development of the mucosal and systemic immune systems[129]. A link between the intestinal microbiota and IBS has been suggested; the composition (reduction in lactobacilli and bifidobacteria) and temporal stability of the GI microbial community is altered in IBS patients compared with controls[130]. Furthermore, several IBS symptoms, such as abdominal pain and bloating may be related to excessive production of gas by bacterial fermentation in the distal bowel and colon. In previous studies, small intestinal bacterial overgrowth (SIBO), characterized by the presence of an abnormally high number of bacteria in the small bowel, has been associated with IBS, although with conflicting results. In a sample of IBS patients scoring significantly higher on psychological distress variables including depression, anxiety, and somatization, there were no differences in psychological distress between $SIBO^+$ and $SIBO^-$ patients; the authors concluded that a possible influence of psychological distress on the association between SIBO and bowel symptoms was unlikely[131].

Recently, the construct of the brain-gut axis has been recently expanded to include gut microbiota in a bidirectional way[132], and knowledge in this field is rapidly expanding.

In rodents, a putative role of the gut microbiota in emotional behavior has been hypothesized, even though little is known about the possible mediating signaling mechanisms. The induction of depression- and anxiety-like behaviors influences the composition of the microbiota by enhanced activation of the stress response and disruption of the microbial habitat *via* alterations in

colonic motility[133]. Although there appears to be some conflicting findings, preclinical data indicate that an altered or absent microbiota can influence the development and functioning of the CNS, and modulate behavior. Beyond the evidence, it seems premature to translate preclinical work on animal models to the clinic. A recent study[134] on a large cohort of IBS subjects showed that the majority of IBS patients presented changes in microbiota composition when compared with controls; however, some of the IBS samples had no microbial abnormalities. This latter subsample showed a prevalence of 40% depression, which was significantly higher than the overall rate of 22% found in the study. Moreover, the depression rate in the IBS cohort with altered microbiota was comparable to that of the general population. The hypothesis of the possible association between altered microbiota composition and NA actually relies on animal models, and further clinical studies will allow us to better understand the putative role of microbiota in the gut-brain axis in humans.

Inflammation and immunity

In physiological conditions, the gut is in a chronic state of inflammation, with a balance between commensal microbes and the immune system. Although colonic biopsy specimens are usually normal in IBS patients, direct evidence for a role of inflammation and immune activation in IBS has been provided[3]. It is estimated that postinfectious IBS may represent up to 30% of all IBS cases[135], and sustained immune activation has been found in this sub-group of patients[9]. Furthermore, low-grade immune activation in IBS patients in the absence of an episode of acute gastroenteritis is reported[136].

Inflammatory mediators, including cytokines, prostaglandins, bradykinins, nerve growth factors, adenosine, and serotonin (5-hydroxytryptamine), are all substances involved in visceral hypersensitivity, alterations in motility, increased gut secretion, and possibly contributing to diarrhea[2]. Studies focusing on cytokine measurements have shown increased levels of the proinflammatory cytokines interleukin (IL)-6 and IL-8 in IBS patients, whereas the presence of extraintestinal comorbidity, including depressive states, is associated with increased levels of IL-1β and tumor necrosis factor-α[137]. A recent study of the correlation between anxiety-depression status and cytokines in a small sample of patients with IBS-D has shown that IBS-D patients who meet the anxiety-depression criteria have increased IL-1β levels, and decreased levels of IL-10, an anti-inflammatory cytokine, when compared with controls[138]. The authors have suggested that anxiety-depression status may cause changes in the IL-1β and IL-10 levels of IBS patients, resulting in an imbalance of the proinflammatory and anti-inflammatory cytokines, thus leading to aggravation of IBS[138]. The sample size (IBS-D group: n = 12; anxiety-depression IBS-D group: n = 16; control group: n = 15) does not permit us to make robust inferences from the findings, which need to be replicated in larger samples. Nevertheless, this study is particularly interesting because it was the first to report the influence of negative affects (depression and anxiety) on inflammatory parameters in IBS-D.

Negative affects have recognized common pathways with inflammatory conditions and confer increased risk for disorders with an inflammatory etiology[139]. Depressed mood has been linked with increased levels of proinflammatory cytokines and other inflammatory mediators, such as acute phase proteins, cellular adhesion molecules, and chemokines[140]. Cytokine production is associated with the so-called sickness behavior, due to the impact of proinflammatory cytokines on the brain, and characterized by a number of behavioral symptoms such as weakness, depressed mood, anhedonia, lethargy, loss of appetite, feelings of depression, and cognitive deficits. The strict similarity between sickness behavior and depression has led to the cytokine hypothesis of depression, whose main assumption is that both psychosocial and internal stressors may trigger depression *via* inflammatory processes *via* changes in neuroendocrine and neurotransmitter systems[91].

In contrast with the large body of research examining the association between depression and inflammation, less is known about the relationship between anxiety, anger and inflammation. Anxious subjects have higher levels of the proinflammatory cytokine IL-6[141]. Higher levels of IL-6 and C-reactive protein (CRP) among individuals with high anger and lower levels of the same inflammation markers among those with high anger control have been found[142]. Moreover, trait anger and hostility vary positively with levels of CRP and IL-6[143], suggesting that angry tendencies may confer much of the variability in inflammation associated with negative emotions.

Health care-seeking behavior

From an epidemiological point of view, GI symptoms are remarkably frequent in the general population, with up to 70% of individuals reporting one or more symptoms[15]. Moreover, only a percentage of individuals affected by IBS in the general population ever seek the attention of the health care system for their symptoms. Part of the variation in rates of health care-seeking behavior is possibly attributable to characteristics associated with the health care system of a particular country or region, and another explanation might be due to social learning in early childhood[2]. In psychological terms, social learning of illness behavior takes place through reinforcement, when parents respond to abdominal complaints of their children with increased attention, or through modeling, when parents affected by IBS behave in a way that reveals a concern with illness. The effects of social learning on illness behavior in IBS have been highlighted: children of mothers with IBS have more non-GI as well as GI symptoms, more school absence, and attend more medical examinations for GI symptoms than children of non-IBS mothers[144]. Beyond

accessibility to the health care system, and social learning, psychological distress, psychiatric comorbidity, and NA have been indicated as significant factors in use of health care by IBS adults. It has been shown that in adult IBS patients, health-care-seeking behavior and treatment outcome are adversely associated with anxiety, chronic social stress, acute stressors, and maladaptive coping style[145]. The majority of IBS patients who seek medical advice for GI problems have an associated affective disorder. Anxiety in particular has been designated as an important determinant of medical consultation among patients with IBS[146]. In contrast, it has been suggested that negative emotions may not always facilitate care-seeking. The co-occurrence of nonspecific physical symptoms and stressful life events may lead to the misattribution of symptoms to the stressor, and thus, to delay the decision to seek medical attention[147]. A recent study on a community sample of university students with IBS-like symptoms compared with a non-IBS reference group has shown that health care utilization is mainly associated with IBS symptom severity, and not with emotional distress[148]. As reviewed in previous sections, rates of negative affects and psychiatric comorbidity in IBS patients are higher than in the general population[94]. Moreover, these high rates are not encountered only in health-care-seeking IBS patient samples, but also in community-based samples, suggesting that affective disturbances in IBS patients are involved in the pathophysiology of the illness rather than merely a determinant of health care use[99]. Furthermore, the timing of onset of psychological factors, the shared assumption that NA encompasses temperamental features and stable personality traits, and the bio-psychosocial frame in which IBS is actually viewed, further suggest the influence of NA in IBS development that goes beyond a possible role only in the use of health services[149]. It seems plausible that health-care-seeking behavior in IBS patients might be influenced by many factors; among these, symptom severity, especially pain, duration of illness, and somatization may have some role[150].

INTERPLAY BETWEEN GUT AND BRAIN: TREATMENT IMPLICATIONS

Current treatments for IBS are based on symptom representation and comorbid conditions such as lifestyle, diet and stress disorders. Dietary changes, such as a stepwise food exclusion approach, as well as fiber and/or probiotic supplementation, have been proposed when symptoms are mild. For moderate symptoms, pharmacological treatments, including antispasmodics, prokinetics (5-HT3 antagonists and/or 5-HT4 agonists, dopamine antagonists), antibiotics, and bulking agents, can be considered. Medications are mainly symptom directed, because the pathophysiological mechanisms for development of IBS are complex and multifactorial.

It is well known from the literature that the efficacy of pharmacotherapy for IBS is weak. Despite the wide range of available drugs and the high prevalence of the disease, to date, no single drug or particular combination strategy has been shown to be superior to the others[151].

Severe IBS, characterized by high levels of pain, diminished quality of life, and/or comorbidity with mood and anxiety disorders is frequently resistant to first- and second-line therapies[152].

Within the frame of the bio-psychosocial model of IBS, and taking into account the bidirectional communication between the brain and the gut, centrally acting treatments, such as psychopharmacological or psychological treatments, can be used to reduce the impact of the GI symptoms.

Psychotropic drugs, mainly antidepressants, are commonly prescribed for IBS.

The rationale for prescribing tricyclic antidepressants (TCAs) is that these drugs have demonstrated efficacy in modulating pain perception *via* central regulatory mechanisms, and for this effect they are also used to treat chronic neuropathic pain and other chronic pain conditions[153]. Selective serotonin reuptake inhibitors (SSRIs) have a lower side effect profile than TCAs, although their efficacy as antinociceptive agents is questionable[2]. Moreover, the prokinetic effect of SSRIs (*e.g.*, stimulation of small-intestinal motility and induction of nausea) can intensify certain IBS symptoms[154].

Anxiolytic agents, particularly the benzodiazepines (BZDs), combined with antispasmodics, have been one of the most common treatment strategies for IBS patients. However, such therapies have not received supportive evidence from high-quality clinical trials[155], and the potential role of BZDs in the treatment of IBS needs further evaluation[156], taking into account the risk of tolerance, the potential for dependency, and rebound after withdrawal.

Considering the partial benefits of pharmacotherapy, a significant amount of research has evaluated psychological and psychosocial treatments for IBS. The effect of various techniques of psychotherapy, such as cognitive behavioral therapy, interpersonal psychotherapy, hypnotherapy, and relaxation/stress management on IBS has been evaluated. Nevertheless, as demonstrated by a recent meta-analysis[157], psychological interventions are not superior to placebo, and there is no convincing evidence that treatment effects are sustained in long-term follow-up. IBS treatment thus requires complex and multidisciplinary management, and the establishment of a positive and empathic doctor-patient relationship to provide individualized treatment and the education and support needed to help patients to deal with what is often a lifelong disorder.

CONCLUSION

Previous research has suggested that negative emotions and attitudes have detrimental effects on health, and a number of behavioral and biological mechanisms could underlie these associations.

Growing evidence supports the hypothesis that negative affects are a major component of the biopsychosocial model of IBS, because they play a key role in dysregulation of the brain-gut axis, contributing to the majority of pathophysiological and symptomatic correlates of IBS. Beyond temperament and affective dispositions, there is strong support, both from preclinical and clinical studies, for the psychosocial role of the environment in altering behavior and influencing nervous, autonomic and immune functions. Furthermore, early life adversity produces epigenetic changes in those brain regions that influence behavior, affective attitudes, and process information about potentially threatening or harmful environments.

As highlighted in the present review, major contributions to advancing our understanding of the role of NA in IBS have come from different research disciplines, such as gastroenterology, stress medicine, psychiatry, psychology, and neurobiology. Future focus in this research field should move towards the development of integrative research strategies involving these apparently divergent, yet complementary disciplines. In our opinion, efforts aimed to integrate different fields of knowledge will provide further insight into the pathophysiological pathways linking negative psychological states to health and disease, leading to the identification of individual vulnerability and of those susceptibility factors, including subsyndromal conditions, that should be addressed to promote better health in the population and more effective and efficient efforts at prevention and treatment of IBS.

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Breath tests and irritable bowel syndrome

Satya Vati Rana, Aastha Malik

Satya Vati Rana, Aastha Malik, Department of Super Specialty Gastroenterology, Post Graduate Institute of Medical Education and Research, Sector 12, Chandigarh 160012, India
Author contributions: Both authors contributed to the manuscript.
Correspondence to: Satya Vati Rana, Professor, Department of Super Specialty Gastroenterology, Post Graduate Institute of Medical Education and Research, House No 137, Sector 15-A, Chandigarh 160012, India. svrana25@hotmail.com
Telephone: +91-987-6139933 Fax: +91-172-2744401
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Abstract

Breath tests are non-invasive tests and can detect H_2 and CH_4 gases which are produced by bacterial fermentation of unabsorbed intestinal carbohydrate and are excreted in the breath. These tests are used in the diagnosis of carbohydrate malabsorption, small intestinal bacterial overgrowth, and for measuring the orocecal transit time. Malabsorption of carbohydrates is a key trigger of irritable bowel syndrome (IBS)-type symptoms such as diarrhea and/or constipation, bloating, excess flatulence, headaches and lack of energy. Abdominal bloating is a common nonspecific symptom which can negatively impact quality of life. It may reflect dietary imbalance, such as excess fiber intake, or may be a manifestation of IBS. However, bloating may also represent small intestinal bacterial overgrowth. Patients with persistent symptoms of abdominal bloating and distension despite dietary interventions should be referred for H_2 breath testing to determine the presence or absence of bacterial overgrowth. If bacterial overgrowth is identified, patients are typically treated with antibiotics. Evaluation of IBS generally includes testing of other disorders that cause similar symptoms. Carbohydrate malabsorption (lactose, fructose, sorbitol) can cause abdominal fullness, bloating, nausea, abdominal pain, flatulence, and diarrhea, which are similar to the symptoms of IBS. However, it is unclear if these digestive disorders contribute to or cause the symptoms of IBS. Research studies show that a proper diagnosis and effective dietary intervention significantly reduces the severity and frequency of gastrointestinal symptoms in IBS. Thus, diagnosis of malabsorption of these carbohydrates in IBS using a breath test is very important to guide the clinician in the proper treatment of IBS patients.

Key words: Bacterial overgrowth; Breath test; Carbohydrate malabsorption; Irritable bowel syndrome; Lactulose breath test; Small intestine; Sorbitol breath test

Core tip: Bloating and distention are often attributed to dietary factors by patients with irritable bowel syndrome (IBS). Recently, small intestinal bacterial overgrowth (SIBO) has been advocated as a pathogenetic factor of IBS. Sugar malabsorption in the bowel can lead to bloating, cramps, diarrhea and other symptoms of IBS as well as affecting absorption of other nutrients. The breath test is now a well-established noninvasive test for assessing malabsorption of sugars in the small intestine. The glucose breath test has been reported as a better diagnostic method for determination of SIBO. Therefore, this review highlights the role of breath tests in diagnosis and management of IBS.

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INTRODUCTION

Breath tests are inexpensive, simple and non-invasive, inexpensive tests which can be used for (1) detection of excess bacteria in the small intestine; (2) evaluation of carbohydrate maldigestion; and (3) estimation of intestinal

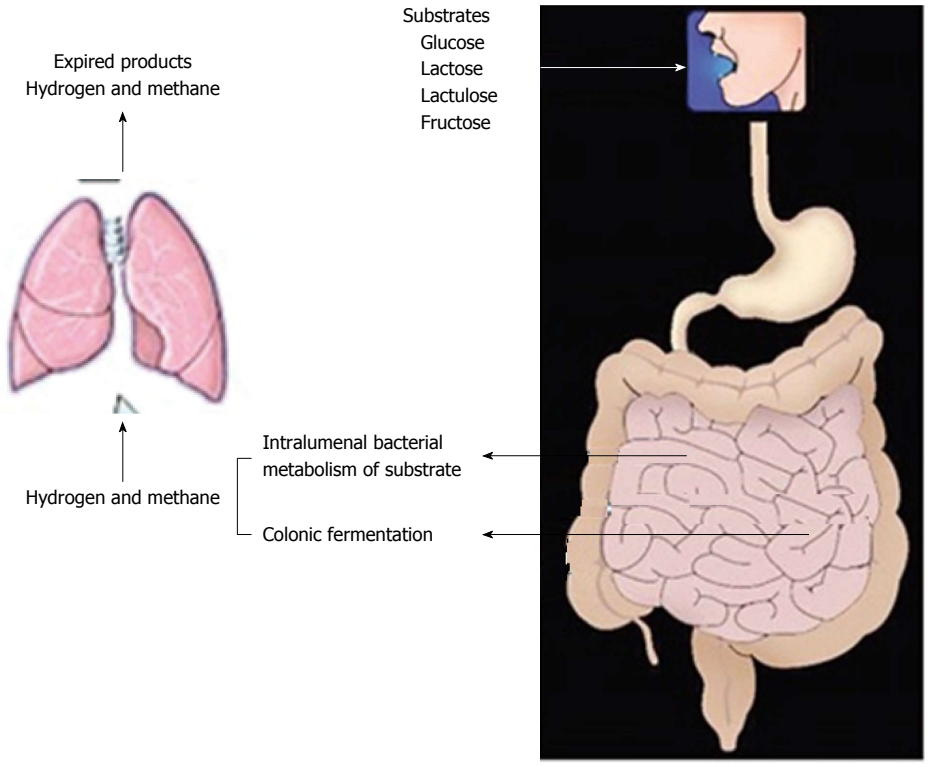

Figure 1 Principle of breath testing.

transit time. In order to diagnose irritable bowel syndrome (IBS), all the above parameters should be ruled out.

In 1970s, breath hydrogen (H_2) was used to estimate lactose malabsorption. Lactose malabsorption was also studied by Newcomer and associates[1] using $^{14}CO_2$-labeled lactose, breath H_2 and blood sugar changes. In 1978, it was observed that not all disaccharides were hydrolyzed and absorbed in the small intestine during the digestion of foods with help of breath H_2[2].

Breath testing consists of measurement of H_2/methane (CH_4) produced by bacterial fermentation of unabsorbed carbohydrate that is ingested by subjects (Figure 1). Subsequent breath samples are collected atspecific time intervals (*i.e.*, every 15 or 30 min) for 2-5 h. These breath samples are analyzed using the SC Microlyser (Figure 2) to measure amount of exhaled H_2 and CH_4. H_2 and CH_4 gases exhaled in the breath are generally the end result of fermentation of carbohydrate ingested by bacteria in intestine[3]. CO_2 is produced by all cells during metabolism, but only bacteria produce H_2 and CH_4 as metabolic by-products. Thus, if either H_2 and/or CH_4 are produced in body, this proves that a substrate has been exposed to intestinal bacteria with leading tobacterial fermentation[4].

TYPES OF BREATH TESTS

Breath tests are most frequently used for diagnosis of lactose, sorbitol and fructose malabsorption, the glucose breath test (GBT) for small intestinal bacterial overgrowth (SIBO) and the lactulose breath test for orocecal transit time.

GLUCOSE BREATH TEST

Under physiological conditions, glucose is straight away absorbed in the small intestine[5]. However, if there is bacterial overgrowth in small intestine, bacterial fermentation of glucose leading to production of H_2 can take place prior to the absorption of glucose, which is measured by increase in H_2/CH_4 concentration. Thus, any increase $\geqslant$ 10 ppm in H_2/CH_4 concentration in two consecutive readings above the basal value is to be considered as significant and indicates about SIBO.

LACTULOSE BREATH TEST

Lactulose is a simple disaccharide. Generally, there is no lactulase enzyme in the small intestine to hydrolyze this sugar, therefore it is transported intact to the colon where it is metabolized by colonic bacteria. End products of its metabolism include H_2 and CH_4. The time interval between ingestion of lactulose and rise in breath H_2/CH_4 concentration $\geqslant$ 10 ppm in two consecutive readings above the basal value is measure of orocecal transit time.

LACTOSE BREATH TEST

Lactose intolerance is prevailing throughout the world. Subjects generally avoid milk and other dairy products to improve their symptoms. For effective utilization, lactose requires hydrolysis by the enzyme lactase. An increase in H_2/CH_4 concentration $\geqslant$ 20 ppm in two consecutive readings above the basal value is considered lactose in-

Figure 2 Gases released can be detected by Breath analyzer.

tolerance. The breath test is now being considered to be the most practical and dependable method to diagnose malabsorption of lactose.

FRUCTOSE BREATH TEST

This test can help to determine if individual has any problem in fructose digestion. Individuals with fructose intolerance may show symptoms like gas, diarrhea, gas, bloating and cramping. Fructose occurs as simple sugar in fruits, vegetables, and honey. When fructose comes in contact with normal bacteria in the intestine, H_2 and/or CH_4 gas is expired. Usually, a dose of 25 g of fructose is used. An increase in $H_2/CH_4 \geqslant 20$ ppm in two consecutive readings above the basal value indicates fructose intolerance.

SORBITOL BREATH TEST

Sorbitol is found in stone fruits, and also used as an artificial sweetener in sugar-free gum and mints. It is poorly absorbed in small intestine. Sorbitol breath test determines if an individual can absorb small amount of sorbitol. This can help to decide if dietary restriction of sorbitol can lead to improvement in gastrointestinal symptoms.

The various types of breath tests for H_2/CH_4 measurement are shown in Figure 3.

ROLE OF BREATH TESTS IN IBS

IBS is incessant condition of intestine. According to the Rome Ⅲ criteria[6], it is defined as recurrent abdominal pain or discomfort at least 3 d/mo in last 3 mo associated with two or more of the following: (1) improvement in abdominal pain with defecation; (2) onset associated with a change in frequency of defecation; and (3) onset associated with a change in form (appearance) of stools.

IBS is characterized by impaired defecation, abdominal discomfort and bloating. IBS is functional gastrointestinal disorders in which a variety of factors, including abnormal visceral sensation, psychosocial factors and altered motility interact to cause symptoms. Although mechanisms underlying IBS are not fully known, a best possible explanation of symptoms may be that 92% of IBS patients suffer from bloating[7]. Some investigators have reported increased H_2 gas production following administration of fermentable substrates in subjects with IBS compared with healthy controls[8]. A possible explanation for these observations has been that certain individuals who meet diagnostic criteria for IBS may actually have SIBO, due to colonization of the proximal small bowel with fermenting bacteria or intolerance to a carbohydrate.

SIBO IN IBS PATIENTS

Exact prevalence of SIBO in newly diagnosed IBS is not known. Variable data are reported in the literature which reflect different sensitivity and specificity of methods, either biochemical or microbiological, used for diagnosis of SIBO. An exact estimation of SIBO prevalence should have important therapeutic implications as SIBO and symptoms related to it (*i.e.*, abdominal bloating) can be successfully treated bynon-absorbable antibiotics[9,10]. Breath tests are not only easy to perform but are also non-invasive compared with jejunal aspiration. These also give quicker information in comparison to jejunal aspiration[11]. SIBO occurs in wavering frequencies in IBS[12,13]. It varies according criteria used to measure SIBO and

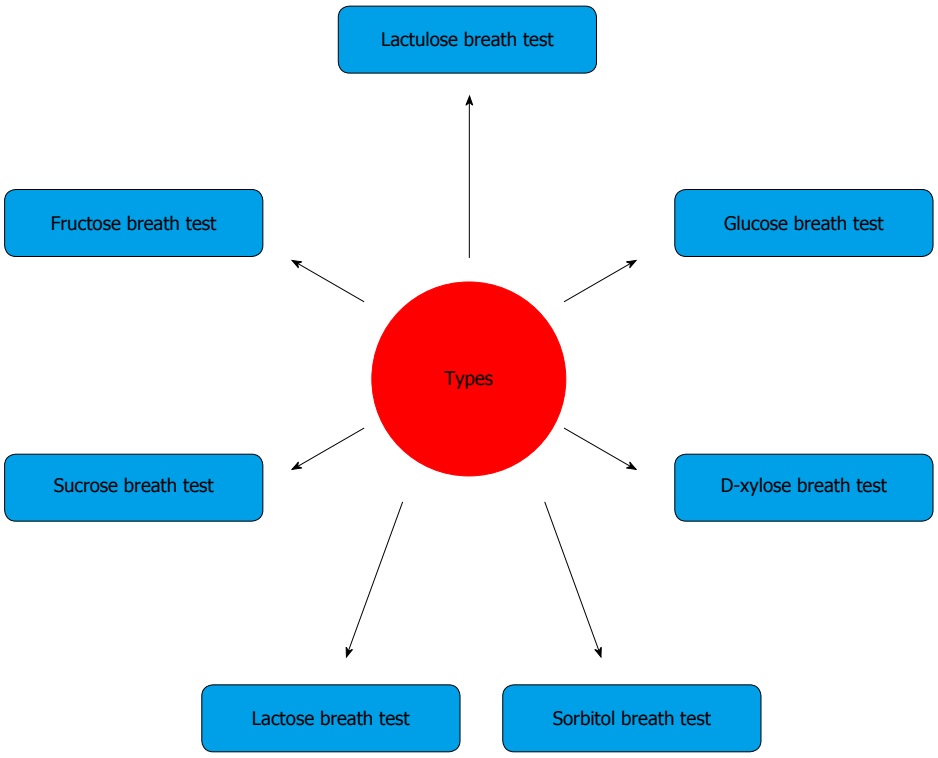

Figure 3 Types of breath tests.

geographical area. The GBT has been reported as a diagnostic test for SIBO[14,15]. It is the most extensively used test, as the substrate is inexpensive, and glucose is fermented by small intestinal bacteria into H_2/CH_4 and CO_2. Kerlin and Wong[16], have reported that GBT performed for 2-h had a sensitivity of 93% and a specificity of 78% in SIBO identification against the gold standard of a jejunal aspirate. The jejunal aspirate culture has been used as the gold standard to diagnose SIBO but limitations of this test include the challenges posed by attempting to culture all strains and species, possibility of contamination and the most important being its invasiveness[17,18]. Therefore, breath tests (lactulose or glucose breath tests) are most commonly used[19]. The different patterns observed in glucose and lactulose breath tests for detection of SIBO are shown in Figure 4.

Prevalence of SIBO in IBS patients was found to be 4% (based on the definition of $\geqslant 10^5$ CFU/mL of bacteria in jejunal aspirate) which is similar to that observed in healthy individuals[20]. However, Lupascu *et al*[21] observed that positive GBT was found in 31% (20/65) of IBS patients compared with 4% (4/102) in a control group. In comparison to this, a study was performed by Pimentel *et al*[22] in 111 IBS subjects using the lactulose breath test. He reported a prevalence of SIBO of 84% in IBS compared with 20% in healthy individuals. Additionally, the administration of neomycin significantly pacified IBS symptoms. The sensitivity and specificity of the GBT for SIBO were 62.5% and 82%, respectively, and of the lactulose breath test were 52% and 86%, respectively[23]. Another study also found a higher percentage of SIBO (76%) in IBS patients using the lactulose breath test[24]. The variation in lactulose and GBTs may be due to differences in the nature of the substrate and diagnostic method used. Another practice of breath sample analysis utilized substrates such as D-xylose or glycocholic acid labeled with ^{13}C and ^{14}C isotopes, followed by analysis by mass spectrography or scintillation counting of breath samples for isotopic CO_2[25-27]. ^{14}C-labeled substrate however are not applicable for testing children and pregnant women.

STUDIES RELATED TO SIBO IN IBS IN DIFFERENT POPULATIONS

Cuoco and Salvagnini[9] reported that 46% of 96 patients in North Italy with IBS had positive breath test after oral lactulose administration. European investigators reported increased gastrointestinal bacterial flora in 43% of IBS patients in comparison to 12% of controls[20]. United States-based clinicians have also reported positive test in around 80% of IBS patients[28-30]. In a study using a lactulose H_2 breast test and whole-gut scintigraphy in IBS patients, radio-labeled material almost always reached cecum before H_2 breath content rose by > 20 ppm[28,30]. This study provided convincing evidence that lactulose H_2 breath testing reflects variations in orocecal transit time rather than a diagnosis of SIBO. A meta-analysis in patients with IBS found that prevalence of positive lactulose or glucose H_2 breath test was 54% and 31%, respectively, with significant heterogeneity between studies[12]. Park *et al*[31] also observed that lactulose breath

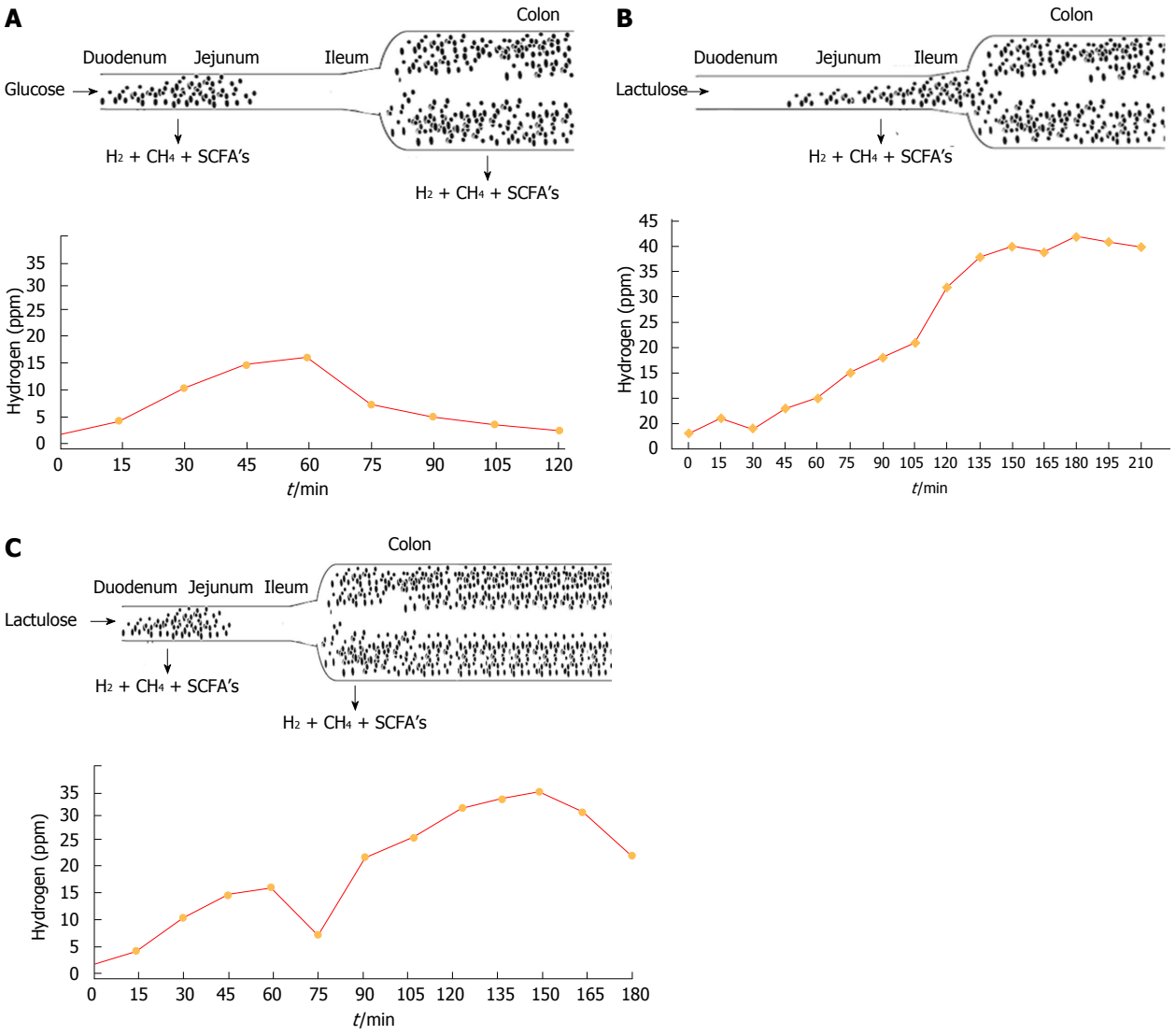

Figure 4 It shows pattern of breath test with bacterial overgrowth in duodenum and jejunum (A), more colonic type of bacteria in small Intestine (B) and more bacteria in duodenum, jejunum and colon showing 2 peaks with lactulose administration (C).

test was not useful for discriminating IBS patients from controls. A recent study by Meyrat *et al*[32] also observed a high percentage of positive lactulose breath tests among IBS patients (71%). IBS-associated symptoms improved following 2 wk of treatment with rifaximin. The authors concluded that rifaximin treatment pacifies symptoms in lactulose breath test-positive IBS patients. Similar results were observed by other authors in relation to SIBO and its treatment with rifaximin in IBS patients[33-37]. Law *et al*[38] observed that therapy with PPI did not affect production of H_2 on lactulose breath tests in IBS patients. Parodi *et al*[39] showed that GBT is useful to identify a subgroup of IBS-like patients, whose symptoms are a result of SIBO. Normalization of the GBT after antibiotic therapy was found to be associated with a significant improvement in symptoms. In a study from Pakistan, the lactose H_2 breath test was used to diagnose SIBO in IBS patients[40]. SIBO was observed by the lactose H_2 breath test in 14% (32/234) cases. It was positive in 19% (22/119) diarrheal type IBS (IBS-D) patients, while 9% (10/115) patients had chronic non-specific diarrhea. In another study, sucrose was used as a substrate to diagnose SIBO[41]. The authors observed that 32.9% (52/158) patients with IBS had abnormal breath tests compared with 17.9% (6/34) of controls while SIBO+ve and SIBO-ve patients did not differ in prevalence of IBS subtypes. Sachdeva *et al*[42] also showed that SIBO was more prevalent in IBS patients 23.7% (14/59) than healthy controls [2.7% (1/37)] using GBT. Patients with D-IBS suffered from SIBO more frequently as compared with non-D-IBS patients [37% (10/27) *vs* 12.5% (4/32)]. Constipation-type IBS (C-IBS) had the lowest number of patients with SIBO (9%, 1/11) among all IBS subgroups. The prevalence of SIBO in children affected by IBS was studied by Scarpellini *et al*[43]. They observed that an abnormal lactulose breath test was significantly higher in IBS patients (65%, 28/43) than in control subjects (7%, 4/56). The study conducted in our laboratory on SIBO in IBS patients showed that the prevalence of SIBO in IBS patients from North India was approximately 11.1%[44],

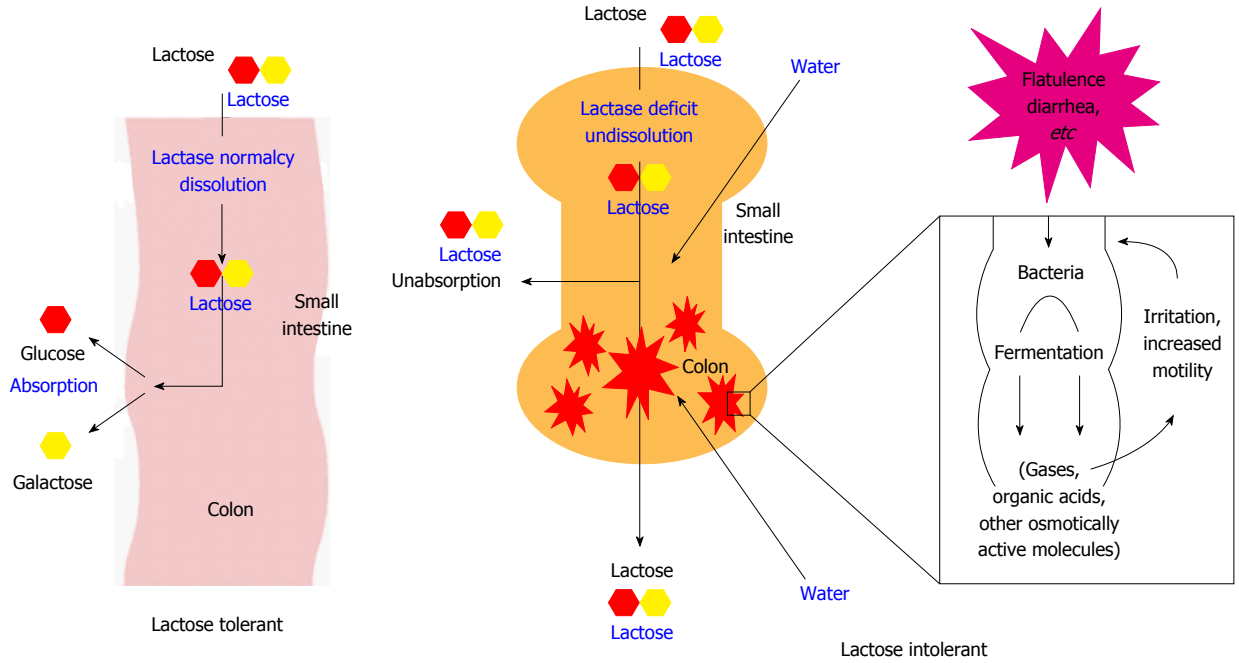

Figure 5 Mechanism of lactose intolerance.

Table 1 Comparison of glucose and lactulose breath tests for diagnosis of small intestinal bacterial overgrowth in patients with irritable bowel syndrome

Year	Ref.	Substrate	% age of SIBO + ve	Number of patients
2005	Lupascu *et al*[21]	Glucose	31	65
2007	Majewski *et al*[35]	Glucose	46	204
2008	Rana *et al*[45]	Glucose	11.1	225
2009	Parodi *et al*[39]	Glucose	16	130
2010	Reddymasu *et al*[15]	Glucose	36	98
2011	Sachdeva *et al*[42]	Glucose	23.7	59
2012	Rana *et al*[44]	Glucose	6.2	175
2008	Grover *et al*[41]	Sucrose	32.9	158
2011	Yakoob *et al*[40]	Lactose	14	234
2003	Pimentel *et al*[22]	Lactulose	84	111
2005	Nucera *et al*[79]	Lactulose	65	98
2007	Madrid *et al*[24]	Lactulose	76	367
2008	Bratten *et al*[47]	Lactulose	67	264
2009	Scarpellini *et al*[43]	Lactulose	65	43
2009	Peralta *et al*[33]	Lactulose	56	97
2010	Park *et al*[31]	Lactulose	56.3	555
2012	Meyrat *et al*[32]	Lactulose	71	150
2013	Scarpellini *et al*[36]	Lactulose	66	50

SIBO: Small intestinal bacterial overgrowth.

which is lower than the reported prevalence in Western countries[12]. GBT was found to be a more appropriate test for the SIBO detection than lactulose breath test as per thestudy performed in our laboratory. SIBO was positive in 34.3% (60/175) patients with lactulose and in 6.2% (11/175) patients using GBT. In controls, lactulose breath test was positive for SIBO in 30% (45/150) and in 0.66% (1/150) using GBT. It was also observed in this study that a positive lactulose breath test for SIBO was not significantly different in patients and controls; while using GBT, SIBO was significantly higher ($P < 0.01$) in patients than in controls. Thus, we concluded that the lactulose breath test was not a good test to discriminate SIBO in IBS patients from controls[45]. Various studies[46-48] have demonstrated the disadvantages of using lactulose in diagnosing SIBO, mainly because of the high rate of false positive results. Table 1 also clearly shows that the percentage of SIBO in IBS patients is high with the lactulose breath test compared with the GBT.

By analyzing the above literature, it can be concluded that the GBT is a better diagnostic test for SIBO in IBS patients compared with the lactulose breath test, and that occurrences of SIBO in IBS patients varies among different populations.

LACTOSE INTOLERANCE AND IBS

Lactose intolerance has been known for over a century. Figure 5 explains the mechanism of lactose intolerance. The lactose H_2 breath test[49] extensively used as test for lactose intolerance. Pattern of the breath test observed in lactose-tolerant and lactose-intolerant patients is shown in Figure 6A.

The lactose H_2 breath test is not sufficient for the diagnosis of lactose intolerance because lactose malabsorbers can also give negative H_2 breath test. It has been observed that individuals with methanogenic flora, measurement of breath CH_4 may improve accuracy of the lactose H_2 breath test in analysing lactose malabsorption[50].

STUDIES SHOWING INTERDEPENDENCE OF IBS AND LACTOSE INTOLERANCE

IBS and lactose intolerance have similar symptoms and

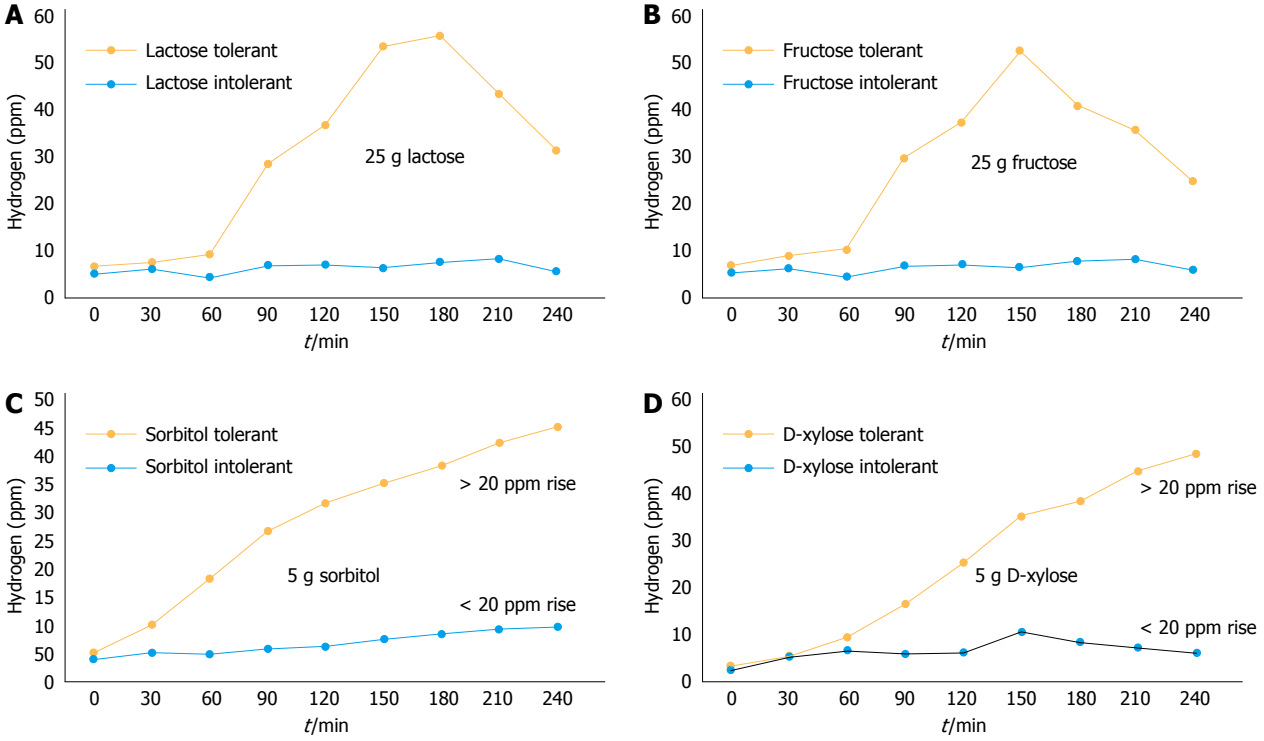

Figure 6 Pattern of lactose (A), fructose (B), sorbitol (C) and D-xylose (D) and tolerance and intolerance using lactose breath test.

both of them are common all over the world[51,52]. It is approximated that 4%-74% of healthy individuals in different geographic regions[53,54] and 4%-78% IBS patients[55,56] may have lactose intolerance. Symptoms of LI may be influenced by the type of diet taken by an individual like the type and amount of polysaccharides, caffeines, intake of fluid and the type of gut flora of that individual[57]. Lactose intolerance patients are at more risk of developing IBS[52] as they have higher visceral sensitivity to effect of lactose in the luminal as compared with lactose-tolerant subjects[58]. Studies have shown that lactose maldigestion affected 24%-27% of IBS patients by lactose breath test[59,60]. In study by Alpers, it was documented that 45% of IBS patients have lactose intolerance. However, only 30% were able to relate their symptoms with milk and other dairy products[61]. Strikingly, some IBS patients who did not suffer from lactose maldigestion complained about symptoms of lactose intolerance. Thus, this shows that lactose intolerance should be measured in IBS patients.

Studies have revealed the presence of lactose malabsorption patients suspected with IBS by H_2 breath testing[60-64]. One study observed that 23% (256/1122) patients with suspected IBS showed lactose malabsorption with 25 g of lactose[63]. In another study, 50 g of lactose was used to assess 186 patients with suspected IBS. They also observed that occurrence of LI in IBS was 25.8% (48/186)[64]. In a succeeding publication, authors showed that patients with lactose malabsorption had no significant relationship with their gastrointestinal (GI) symptoms compared with patients without lactose malabsorption[65]. Böhmer and Tuynman[56] also indicated similar lactose malabsorption *i.e.*, 24.3% by H_2 breath testing in IBS patients. In contrast to these findings, Tolliver *et al*[65] showed significant improvement in IBS symptom scores in 75% of IBS patients with lactose-intolerant after specific dietary intervention 5 years. In an North Indian study by Gupta *et al*[66], it was observed that persistence of lactose intolerance was similar IBS patients of IBS (72%, 89/124) and healthy controls (60%, 32/53). However, IBS patients more frequently complained about symptoms following lactose intake even though levels of breath H_2 were similar to healthy individuals[66]. Prevalance of lactose intolerance in IBS-D patients was commensurable to that in patients with other types of IBS. Their results further advocated that self-reported milk intolerance has 81% positive and 23% low negative predictive values for lactose intolerance diagnosis. Therefore, absence of such self-reported lactose intolerance should not be used to exclude lactose intolerance in IBS patients. These results are in similar lines with previous report from Italy[67]. In this study, LI was analyzed by self-reported symptoms with positive and negative predictive values observed to be 75% and 31%, respectively. In a recent study[68], however, production of H_2 and distention were similar among IBS patients and healthy controls using lactose breath test. However, lactose intolerance was more common in IBS (53.8%) than in controls (28.1%).

A study was also conducted in our laboratory to observe lactose intolerance in different types of IBS patients from north India[54]. 44% (11/25) patients were of D-IBS, 28% (7/25) patients of spastic and remaining seven (28%) patients had characteristics of both types of symptoms. Abnormal lactose H_2 breath test was

observed in 82% (9/11) D-IBS which was significantly higher than controls. Furthermore, patients with D-IBS had a higher incidence of lactose intolerance compared with patients with spastic type or features of both types. Furthermore, Yang *et al*[69] observed that malabsorption of 40 g lactose was observed in 93% of controls and 92% of patients with D-IBS. Fewer controls than D-IBS patients were intolerant to 10 g lactose (3% *vs* 18%), 20 g lactose (22% *vs* 47%), and 40 g lactose (68% *vs* 85%). Self-reported lactose intolerance was more frequently observed in D-IBS (63%) than controls (22%), and thus ateless dairy products.

In children, lactose intolerance was also found to be linked with IBS. Gremse *et al*[70] showed that lactose maldigestion may be an important contributory factor in IBS children.Lactose avoidance in these patients may reduce medication use to relieve symptoms.

The relationship of the lactose breath test with methanogenic flora has also been investigated in various studies. Vernia *et al*[71] showed that after an oral dose of lactose less H2 is excreted by patients with predominant fasting CH_4 low CH_4 producers (LMP). Lower prevalence of grave lactose intolerance and its symptoms during the test in predominant CH_4 producers (PMP) may be associated with lower and slower H_2 excretion. Thus, taking only H_2 excretion as effective means to quantify carbohydrate malabsorption is unrelaible in PMP. CH_4-producing patients are expected to have a increased false negative rate of lactose intolerance compared with LMP after lactose ingestion. As symptoms are related to the amount of gas produced in colon, lactose breath test recognizes patients with lactose intolerance irrespective of presence of lactose malabsorption and helps in predicting effect of a lactose-restricted diet. Similarly, we observed that lactose breath test was present in 50% (77/154) of IBS patients and in 49.6% (142/286) of controls. It was also observed that the lactose breath test was negative due to PMP in 6.49% (5/77) of IBS patients and in 20.14% (29/154) controls. The effect was more plausible in healthy subjects than in IBS patients[72]. However, in a recent study, Lee *et al*[73] observed that CH_4 and H_2 are not associated with specific symptoms in IBS patients.

Thus, it can be concluded that measurement of lactose intolerance using the lactose breath test is essential in IBS patients to modify their diet for improvement of symptoms. It also indicates the importance of CH_4 measurement along with H_2 gas to detect lactose intolerance.

CONTROVERSIAL STUDIES ON LACTOSE INTOLERANCE IN IBS PATIENTS

Farup *et al*[53] observed that IBS and lactose malabsorption were found to be unrelated disorders. A usual test for lactose malabsorption seems unnecessary in persons with IBS in an area with a low lactose malabsorptionprevalence. Milk-related symptoms and symptoms after lactose intake were inaccurate predictors for lactose malabsorption. In a study by Corlew-Roath *et al*[74], incidence of fructose and lactose malabsorption in populations with and without IBS was comparable. 33% of both groups had lactose malabsorption, fructose malabsorption or both. Both populations also had similar results with diets. IBS patients had 77% compliance and 72% in patients without IBS. However, patients without IBS showed improvement in symptoms with dietary changes than IBS patients. This advocates that IBS symptoms are not dependent on carbohydrate maldigestion, and dietary changes may not improve symptoms in patients with IBS.

COMBINATION OF SUBSTRATES AND IBS SYMPTOMS

Lactose[75], fructose[76] and sorbitol malabsorption[77,78] have also been blamed for symptoms present in IBS patients. In a study in IBS patients[79], SIBO was present in 65% (64/98) using the lactulose breath test. SIBO-positive patients further showed significantly higher prevalence of malabsorption by lactose breath test (83% *vs* 64%), fructose breath test (70% *vs* 36%) and sorbitol breath test (70% *vs* 36%) when compared with the SIBO negative IBS patients. SIBO eradication caused significant reduction in lactose, fructose and sorbitol positive breath tests.They concluded that SIBO positivity should always be assessed first, before analyzing for carbohydratemalabsorption and specific carbohydrateelimination diets in IBS patients. Fructose, sorbitol and lactose breath tests could become a useful diagnostic approach in SIBO-negative patients with refractory symptoms. Sugar malabsorption could be primary (congenital enzymatic/carrier deficiency) or acquired due to damage in intestine due to acute gastroenteritis, celiac disease, Crohn's disease or due to medications[80]. When carbohydrates malabsorption occurs, their passage in bowel causes production of short chain fatty acids and gas with initiation of syndrome characterized by abdominal pain, diarrhea and meteorism, thus mimicking IBS symptoms. In a study by Moukarzel *et al*[81] breath H_2 tolerance tests with lactose, sucrose and apple juice in the amount patients normally consumed were positive in 32%, 0%, and 50%, respectively. They concluded that some individuals with IBS have symptoms depending upon malabsorption of carbohydrates present in apple juice, pear nectar and may improve with correct choices of fruit juice. Moreover, in a recent study by Wilder-Smith *et al*[82], it was observed that intolerance due to fructose intolerance was more frequent than lactose intolerance in all subgroups of functional gastrointestinal disorders. However, in an IBS-constipation subgroup, lactose intolerance was found to be more common. Table 2 summarizes the incidence of lactose intolerance reported in IBS patients by various authors.

FRUCTOSE INTOLERANCE AND IBS

It has been advocated that fructose malabsorption was present in 36% of European population[83]. The symptoms include both intestinal complaints as well as extrain-

Table 2 Lactose Intolerance in irritable bowel syndrome patients using lactose breath test

Year	Ref.	% age of lactose intolerance	Number of patients
1994	Corazza *et al*[50]	34.4	32
1994	Tolliver *et al*[64]	25.8	186
1998	Vesa *et al*[63]	23.0	1122
2001	Böhmer *et al*[56]	24.3	70
2001	Rana *et al*[54]	82.0	11
2002	Moukarzel *et al*[81]	32.0	28
2004	Vernia *et al*[55]	75.6	475
2006	Alpers *et al*[61]	45.0	150
2007	Gupta *et al*[66]	72.0	124
2009	Rana *et al*[72]	50.0	154
2009	Corlew-Roath *et al*[74]	33.0	66
2012	Knudsen *et al*[67]	64.7	406
2013	Zhu *et al*[68]	53.8	277
2013	Yang *et al*[69]	47.0	60
2013	de Roest *et al*[105]	37.8	90

testinal symptoms such as depression[84]. In studies with an uncontrolled diet, occurrence of malabsorption due to fructose was higher in IBS patients (30%-70%[85,86]) than in healthy subjects (0%-50%[87,88]). However, no difference was observed in a diet controlled study[89]. Goldstein *et al*[78] reported that, among patients with IBS or functional abdominal complaints, 44% suffered from fructose malabsorption based on consumption of 50 g fructose, and 56%-60% improved on a low-fructose diet. Improvement with a fructose-reduced diet has also been observed in other uncontrolled studies[90,91]. The association between IBS and fructose malabsorption is thus far from settled. Most likely, the diverging data can be explained by the fact that there is no general agreement on the criteria for diagnosis of fructose malabsorption. Finally, from a pathophysiological viewpoint, it would be matter of concern to further determine response to a fructose-restricted diet in IBS patients and the correlations with both the daily intake of fructose and the fructose absorption capacity of IBS patients. However, further studies are needed for validation. All data taken together indicate that fructose malabsorption should be kept in mind while managing IBS patients. A study by Reyes-Huerta *et al*[92] observed that 52% (13/25) IBS patients had fructose intolerance compared with 16% (4/25) control subjects (P = 0.01). They concluded that intolerance in fructose may be responsible for gastrointestinal symptoms in at least half of IBS patients, especially in the group of IBS-D patients. The pattern observed for fructose tolerance and intolerance using the fructose breath test is shown in Figure 6B.

80% of functional bowel disease patients suffered from fructose malabsorption. However, few randomized controlled studies advocated that there is lower prevalence of fructose malabsorption among IBS patients compared with healthy individuals[89,93]. The number of patients in these studies was small, but there was general agreement that IBS patients reported more frequently. This again highlights the problem with identifying specific diagnostic

Table 3 Fructose Intolerance in irritable bowel syndrome patients using fructose breath test

Year	Ref.	% age of fructose intolerance	Number of patients
1986	Rumessen *et al*[87]	40.0	10
2000	Goldstein *et al*[78]	44.0	94
2003	Choi *et al*[85]	73.0	183
2010	Reyes-Huerta *et al*[92]	52.0	25
2013	de Roest *et al*[105]	75.6	90

criteria with both positive breath test and symptoms for practical working definition. Effect of dietary treatment for fructose malabsorption in IBS patients is also very significant. Fernández-Bañares *et al*[94] reported that after fructose-free diet, symptom improvement was present at 1 mo and 12 mo in 81% and 76% of patients with Rome Ⅱ criteria of functional abdominal bloating and gas-related symptoms. Shepherd and Gibson[95] advocated that 77% patients improved with restriction in diet. Better response was seen in in those that were adherent (85%) to diet restriction than non-adherent (36%). Another study on dietary restriction by Choi *et al*[91] observed significant improvement in belching, pain, fullness, bloating, diarrhea and indigestion with diet. However, Berg *et al*[96] observed that the fructose breath test did not discriminate between patients with and without a response to a diet restricted with fructose. Even in the group with a negative fructose breath test, a significant improvement in symptom scores was observed. A summary of fructose intolerance in IBS patients is presented in Table 3.

SORBITOL INTOLERANCE AND IBS

Sorbitol is not completely absorbed and lead to osmotic diarrhea if large amounts (20-50 g) are ingested. A positive breath test can be seen observed with a dose as small as 5 g in healthy subjects. Most participants experienced mild gastrointestinal symptoms after 10 g of sorbitol but after 20 g severe gastrointestinal symptoms[97]. In this method, H_2 or CH_4 are measured in end-expiratory breath samples every 30 min for 4 h. An increase $\geqslant$ 20 ppm in 2 consecutive readings is considered a positive test.

FRUCTOSE AND SORBITOL AS SUBSTRATE FOR IBS SYMPTOMS

Small bowel transit is accelerated due to mixture of fructose (25 g) and sorbitol (5 g)[98]. Precise mechanism of this phenomenon is not known but there is some evidence that bacterial fermentation products may lead to activation of feedback pathways that play a role in regulation of gut motility[99]. Limited data have suggested that SIBO and fructose malabsorption might have a bi-directional cause and effect relationship. On one hand, fructose may cause survival of intestinal bacteria in distal

small intestine as easily available metabolic substrate for the synthesis of fructans as adherence factors. There is no direct evidence supporting or rejecting that these events occur in distal small intestine. By eliminating all potential metabolic substrates for bacteria by feeding patients with an elemental diet resulted in loss of features of SIBO along with improvement in symptoms of IBS[100]. On the other hand, patients with presumed SIBO abolished fructose malabsorption when treated with antibiotics along with reduction in associated symptoms[79].

Recently, Yao *et al*[101] observed that sorbitol was completely absorbed by similar proportion of IBS patients (40%) and healthy subjects (33%). Although IBS patients absorbed more mannitol (80% *vs* 43%). Production of breath H_2 was similar in both groups after lactulose but it reduced in IBS patients after ingestion of both polyols. Overall GI symptoms significantly increased after consumption of both polyols in IBS patients only. However, symptoms were independent of malabsorption of both polyols.

Thus,data in literature shows possible association between fructose, sorbitol and lactose malabsorption with IBS, suggesting that an exclusion of appropriate carbohydrate from diet may improve symptoms in IBS patients who have positive breath test with respect to that specific carbohydrate.However, need for breath testing to recognize individuals with specific carbohydrate malabsorption prior to dietary changes has been debated.

D-XYLOSE INTOLERANCE AND IBS

When D-xylose is absorbed incompletely, enteric bacteria metabolize the non-absorbed D-xylose in the colon, or in the small bowel with bacterial overgrowth, yielding H_2, which can be measured in the breath. The direct measurement of breath H_2after oral intake of D-xylose avoids necessity of using radioactive tracers[14]. Most breath H_2 is formed in colon due to carbohydrate fermentation by the indigenous flora, which allows measurement of intestinal transit[102]. Increased rates of H_2 production occur in small intestine when bacterial overgrowth is present. Study by Lembcke *et al*[103], showed that H_2 breath test with 25 g D-xylose was of no clinical relevance for diagnosis of celiac sprue. D-xylose tests were indicative of the IBS in 5 out of 10 (50%) patients. However, the diagnostic impact of this needs further investigation.

FERMENTABLE OLIGOSACCHARIDES, DISACCHARIDES, MONOSACCHARIDES AND POLYOLS IN IBS

It is apparent from the available literature that the consumption of fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs) may result in symptoms in some IBS patients. In a study by Ong *et al*[104], breath test was performed after intake of a FODMAP diet. They observed that over the entire day with high FODMAP diet in volunteers and IBS patients, increased levels of H_2 breath was produced. However, breath CH_4 were reduced in 10 healthy subjects but not in patients of IBS. Thus, they concluded that FODMAPs in diet induce increased H_2 production in intestine, influence CH_4 production and thus, induce gastrointestinal symptoms in IBS patients. Similar observations were seen in a recent study by de Roest *et al*[105]. Fructose malabsorption (75.6%), lactose malabsorption (37.8%) and SIBO (13.3%) was present in patients in this study. 75.6% patients who were adherent to diet, showed improvement in IBS symptoms. They further concluded that diet with less FODMAP is better for IBS patients. Thus, the current techniques of testing breath and dietary advice forms a good basis to manage IBS patients.

The patterns observed for sorbitol and D-xylose intolerance during respective breath tests are shown in Figure 6C and D, respectively.

CH_4 IN IBS PATIENTS

In humans, CH_4 is mostly produced by *Methanobrevibacter-smithii* (*M. smithii*) as a result of the conversion of 4 mol H_2 and 1 mol CO_2 to 1 mol CH_4, competing for H_2 with sulfate reducing bacteria. This process occurs mainly in the left colon[106,107]. It is an important reason for measuring both gases by breath tests (Figure 7). There is proof of slow transit time in CH_4 producers[108]. In one study, it has been reported that mean of transit time in CH_4 producers was 84.6 h and in non-producers was 48.6 h. Thus, indicating that some association may exist between delayed gut motility and CH_4.

CONSTIPATION-DIARRHEA-CH_4: ANY RELATIONSHIP?

Studies have advocated that production of CH_4 and constipation are strongly related. A study[109] showed that when patients with constipation and increased CH4 production at fasting state and after intake was glucose were treated with rifaximin, their breath CH_4 levels were reduced and constipation symptoms were also improved. CH_4 excretion mean was found to increase along with reduction in bowel movements in C-IBS patients using lactulose H_2 breath test[110,111]. However, apprehension remains as to whether CH_4 causes constipation or rather is result intestinal hypomotility. In contrast, patients suffering from diarrhea generally have higher excretion of breath H_2, during fasting state and after glucose intake[13]. CH_4 was observed to be associated with presence and degree of constipation in a study on 87 patients of IBS. 24% (20/87) produced CH_4 in lactulose H_2 breath test[112]. In a study by Kajs *et al*[113] it was found that low CH_4 producers had a significantly higher breath H_2 than high CH_4 producers on consumption of basal diet and after ingestion of sorbitol (27.1 ± 2.7 ppm *vs* 15.8 ± 3.6 ppm) or oat fiber (13.1 ± 0.08 ppm *vs* 9.6 ± 1.2 ppm). Low producers of CH_4 showed extremely increased

$$4H_2 + CO_2 \xrightarrow{\text{Methanogenic flora}} CH_4 + 2H_2O$$

Figure 7 Production of methane by methanogenic flora.

cramping and bloating after ingestion of sorbitol and increased bloating after fiber ingestion. However, high CH_4 producers showed no such symptoms. Thus, they concluded that methanogenic flora is linked with decreased symptomatic response to ingestion of non-absorbable, carbohydrates in healthy individuals. This indicates that normal flora manipulation could be of therapeutic value in non-methanogenic IBS patients.

Experiments in animals[114] have also suggested an active role for CH_4 in affecting intestinal motility, while other human investigations have shown that slow transit may facilitate growth of methanogenic bacteria[115,116]. However, it cannot be excluded that methanogenic organisms lead to constipation indirectly through the modification of the luminal environment, by producing active substrates or by competing with other bacterial species[117-119]. Recent study has advocated that degree of CH_4 production in breath testing may be related to constipation in IBS patients. Therefore, CH_4 testing may be useful for identification of candidates with constipation for antibiotic treatment to pacify IBS symptoms[120]. Moreover in a Spanish study[121], it was observed that patients of IBS who had low production of H_2 were 6 times more frequently constipated in lactulose breath test.In another study on subjects of IBS by Pimentel *et al*[114], fasting motility index in CH_4-producing subjects was significantly increased compared with H_2-producing subjects. Testing of H_2 alone overlooks the importance of CH_4 as a fermentation product[119]. 30%-50% of human population are producers of CH_4. Synthesis of CH_4 mostly consumes large amounts of H_2, this may waiver diagnostic accuracy of breath testing when alone H_2 is considered[122]. In a similar study by Lasa *et al*[123], it was observed that patients having low level of breath H_2 excretion after lactulose ingestion had significantly greater abdominal bloating than those with increased level of breath H_2 excretion. Kim *et al*[124] further observed in C-IBS patients with CH_4 on breath testing, *M. smithii* is predominant methanogen. They reported that number and proportion of *M. smithii* in stool is well correlated with breath CH_4 in their study.

It is apparent from the above-mentioned literature that CH_4 should also be measured during breath testing in IBS patients so that manipulation of gut flora can be performed in these patients.

RECOMMENDATIONS FOR USE OF BREATH TESTS FOR IBS PATIENTS

On the basis of this review, it is apparent that breath tests are useful for the management of IBS patients: (1) breath tests can be useful in evaluating diarrhea, constipation, functional bloating and suspected malabsorption in IBS patients; (2) Breath test analyzing both H_2 and CH_4 has been shown to be of more importance than breath test using only H_2 measurement for carbohydrate malabsorption and SIBO diagnosis; (3) GBT is a better diagnostic test for SIBO than the lactulose breath test, which gives false positive results; (4) breath tests are non-invasive, simple and safe alternatives to more invasive procedures such as obtaining aspirates for culturing and/or biopsies; (5) some errors may exist. In carbohydrate malabsorption false positive tests for SIBO may occur due to colonic fermentation and production of gas. In gastrointestinal motor disorders, delayed gastric emptying may cause false negative tests, and rapid transit through small bowel may result in false positive breath tests; (6) false positive results may also occur if the subject does not adhere to a low fiber diet the day before the test. Thus, patient is advised to reduce fiber intake prior to test, as this will effect a significant reduction in H_2 production in the intestine, thus creating better testing environment; (7) accurate results are also not obtained if the patient has taken antibiotics, which change intestinal flora and are thus avoided within 4 wk prior to testing; and (8) laxatives and enemas also result in decreased transit time through the intestine, leading to reduced time for bacterial fermentation or loss of bacteria producing H_2 or CH_4.

CONCLUSION

This review summarizes the use of breath tests, not only to direct about dietary interventions but they also to provide prognostic information. These breath tests can help in the diagnosis of SIBO and carbohydrate malabsorption in IBS patients. Further studies analyzing H_2 and CH_4 concentrations in breath samples may improve diagnostic criteria for carbohydrate malabsorption in IBS patients. Moreover, area-under-the-curve analysis of the change in H_2/CH_4 concentration in breath samples over time after administering lactulose as a substrate may in future help to analyze the bacterial level in the bowel. Breath testing is also a useful to the low-FODMAP diet in IBS patients. In most cases of food intolerance, diagnosis is difficult. Thus, breath testing provides accurate, reliable and a non-invasive measure of absorption of a test sugar by assessment of breath H_2/CH_4 levels. Breath tests are performed to determine whether fructose and/or lactose and/or sorbitol are FODMAPs for an individual who has IBS symptoms. Thus, it can be shown whether an individual can or cannot completely digest fructose, lactose and sorbitol. This can be helpful to patients as well as physicians to formulate a particular diet which may help to reduce gastrointestinal symptoms present in IBS patients.

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Irritable bowel syndrome: A concise review of current treatment concepts

Geoffrey C Wall, Ginelle A Bryant, Michelle M Bottenberg, Erik D Maki, Andrew R Miesner

Geoffrey C Wall, Ginelle A Bryant, Michelle M Bottenberg, Erik D Maki, Andrew R Miesner, Department of Pharmacy Practice, Drake University College of Pharmacy, Des Moines, IA 50311, United States
Author contributions: All authors participated in literature review, retrieval, synthesis as well as writing this paper
Correspondence to: Geoffrey C Wall, Pharm.D., FCCP, BCPS, CGP, Department of Pharmacy Practice, Drake University College of Pharmacy, 2507 University Ave., Des Moines, IA 50311, United States. geoff.wall@drake.edu
Telephone: +1-515-2414297 Fax: +1-515-2415085
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Abstract

Irritable bowel syndrome (IBS) is one of the most common gastrointestinal disorders causing patients to seek medical treatment. It is relatively resource intensive and the source of significant morbidity. Recent insights into the pathophysiology and treatment of IBS has given clinicians more options than ever to contend with this disorder. The purpose of our paper is to review older, "classic" treatments for IBS as well as newer agents and "alternative" therapies. We discuss the evidence base of these drugs and provide context to help develop appropriate treatment plans for IBS patients.

Key words: Irritable bowel syndrome; Probiotics; Rifaximin; Lubiprostone; Linaclotide; Peppermint oil

Core tip: Gastroenterology practitioners have more agents than ever before to treat the symptoms associated with irritable bowel syndrome. Unfortunately, despite advances in our understanding of the pathophysiology of this disorder, targeted treatments do not yet exist. This review summarizes the recent evidence-based treatment of this disorder, including, older and newer agents.

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INTRODUCTION

Irritable bowel syndrome (IBS) is one of the most common gastrointestinal disorders causing patients to seek medical treatment. It exerts significant economic burden and is responsible for considerable morbidity in Western countries[1]. Despite these costs and numerous investigations into the pathophysiology and treatment of this disorder, our understanding of IBS is still incomplete. Over the last ten years, increasing insight into the enteric nervous system and how its dysfunction may play a role in IBS pathology has emerged[2]. Additionally our increasing understanding of the gut microbiome and how its potential disruption may lead to IBS symptoms has also been highlighted[3]. However, with few exceptions, these insights have yet to lead to targeted treatment strategies for IBS. Currently, many clinicians use a treatment approach based on the predominant symptoms of the patient: constipation (IBS-C), diarrhea (IBS-D), or mixed symptoms (IBS-M) (Table 1)[4]. Several new drugs have recently been examined for IBS using this symptom-based approach. Two agents for IBS-C, lubiprostone and linaclotide have been approved by the United States Food and Drug Administration (FDA) for that specific indication[5]. To improve the evidence by which drugs for IBS are approved, the FDA has recently proposed standardized outcomes for approval studies as is discussed later in this paper. The purpose of this paper is to provide the clinician with a concise review

Table 1 Irritable bowel syndrome subtypes

Subtype	Definition (symptoms classified using Bristol stool form scale)
IBS with constipation (IBS-C)	> 25% of stools are hard or lumpy and < 25% of stools are loose/mushy or watery
IBS with diarrhea (IBS-D)	> 25% of stools are loose/mushy or watery stools and < 25% are hard or lumpy
Mixed IBS (IBS-M)	> 25% of stools are loose/mushy or watery stools and > 25% and hard or lumpy
Unsubtyped IBS	insufficient abnormality of stool consistency to meet criteria for IBS-C, D, or M (in the absence of antidiarrheals or laxatives)

IBS: Irritable bowel syndrome.

of pharmacotherapy strategies for IBS. Consequently, it is divided into three sections: "classic" treatment options, "newer drugs," such as lubiprostone and linaclotide, and "alternative" treatments such as probiotics and peppermint oil. In the last section we will also discuss emerging information on the so-called "pre-cebo" effect in IBS.

CLASSIC TREATMENTS FOR IBS

Antidiarrheals

Loperamide is a synthetic opioid, which acts on intestinal muscles to prolong transit time and inhibit peristalsis. While loperamide has been studied in different subtypes of IBS, it may be particularly effective in IBS-D because of its ability to decrease fecal volume and transit time. A meta-analysis in 2000 found loperamide to be an effective agent in decreasing stool frequency and improving stool consistency, as well as demonstrating a modest improvement in global well being[6]. However, it does not appear that loperamide is effective in reducing abdominal pain in comparison to placebo. In fact, some studies show an increase in abdominal pain particularly when loperamide is used in IBS-C[7]. Other common antidiarrheal agents, such as diphenoxylate with atropine, have not been well studied in IBS and are likely to be less tolerated due to anticholinergic effects, such as sedation, dry mouth, constipation, and urinary retention.

Antidepressants

Antidepressants, such as the tricyclic antidepressants (TCA), selective serotonin reuptake inhibitors (SSRI), and serotonin norepinephrine reuptake inhibitors, have been utilized in the treatment of various functional gastrointestinal disorders. Current treatment guidelines endorse the use of either TCAs or SSRIs for patients with IBS, although duloxetine has also been studied in small trials for this population[8,9]. These agents are believed to act *via* centrally-mediated antinociceptive pathways decreasing abdominal pain associated with IBS. In addition, these agents may affect the gastrointestinal tract by peripheral means particularly in gut transit times[8]. A recent Cochrane review pooled 15 TCA and SSRI trials[10]. The antidepressant class as a whole significantly decreased pain, IBS symptom scores, and overall global assessment. A subgroup analysis revealed only TCAs remained statistically significant for abdominal pain and improvement in symptom scores. However, this may be due to a smaller number of patients and trials studying the use of SSRIs to treat IBS. In addition, an earlier meta-analysis demonstrated a reduction in pain, bloating, and other symptoms, although it contained mostly TCA trials[11].

Despite the large differences in the amount of supporting data, many clinicians are reticent to prescribe TCAs instead of SSRIs given the poor tolerability of these agents. In fact, one trial utilizing desipramine found nearly one in five subjects of that treatment arm dropped out due to adverse reactions[12]. Secondary amine tricyclic antidepressants (*e.g.*, nortriptyline) are typically better tolerated than tertiary amines (*e.g.*, amitriptyline) because of decreased anticholinergic adverse effects. In addition, lower doses of TCAs as compared to doses used to treat depression seem adequate to provide IBS symptom relief. Despite a more favorable side effect profile, SSRI use is more controversial in IBS patients as the supporting evidence is not nearly as robust. Clinical guidelines do suggest hypothetically that SSRIs may be of more utility in IBS-C and TCAs may be of more benefit in IBS-D due to their respective effects on whole gut transit times[8]. Clinicians await head-to-head trials with these agents.

Antispasmodics

Medications that relax smooth muscle *via* anticholinergic mechanisms or calcium channel antagonism have been commonly utilized for the treatment of IBS. Among these are alverine, dicyclomine (with or without cimetropium), hyoscyamine, otilonium, pinaverium, scopolamine, and trimebutine. The availability of many of these medications varies from country to country. Generally, antispasmodics have been utilized for their effects on gastrointestinal motility in attempts to reduce abdominal pain associated with IBS. They have also been evaluated in combination with agents such as acetaminophen, simethacone, and benzodiazepines in attempts to improve gastrointestinal discomfort[13-15].

Unfortunately many of the studies evaluating antispasmodics are small, suffer from methodological issues, and often fail to evaluate individual symptoms or effect on IBS subtypes. Only a small number of trials include active comparators. A recent Cochrane review of 29 antispasmodic trials for IBS suggested that some, but not all antispasmodics may decrease abdominal pain[10]. Similarly some, but not all, antispasmodics improved IBS symptom scores and global assessment. A subgroup analysis showed benefit of the use of trimebutine, pinaverium, and combined dicyclomine/cimetropium in the treatment of IBS. Anticholinergic side effects of these agents often include dose-related vision disturbances, dry mouth, and dizziness. Moreover, antispasmodics can also cause constipation, thus they should be used cautiously in patients with IBS-C. Prescribers should consider the limitations of these medications when using them for IBS.

Table 2 Response rates for lubiprostone 12 wk phase Ⅲ irritable bowel syndrome with constipation studies

	Lubiprostone	Placebo	*P* value
Overall responder	17.90%	10.10%	0.001
Month 1	10.80%	7.50%	0.078
Month 2	18.20%	11.40%	0.003
Month 3	22.00%	14.50%	0.003

Bulking agents

Several bulking agents have been examined in the treatment of IBS. These include psyllium, calcium polycarbophil, bran, and ispaghula husk. These synthetic and naturally occurring fiber supplements are often used for their ability to increase stool frequency, quality, and transit time. Consequently, they are often attractive options in all subtypes of IBS, particularly IBS-C. Most of the trials involving these agents have been small and as a result, multiple meta-analyses have been undertaken. An early systematic review found that there may be a significant improvement in global IBS symptoms with soluble fibers (psyllium, calcium polycarbophil, ispaghula), but worsening symptoms with insoluble fiber (bran)[16]. However, this review suffered from significant heterogeneity. Furthermore, a recent Cochrane review of 12 randomized control trials showed that fiber supplements do not improve abdominal pain, IBS symptom scores, or global assessment. Other meta-analyses have had similar results[17].

Osmotic laxatives

Osmotic laxatives are often used in the treatment of IBS-C due their efficacy in chronic idiopathic constipation. These agents, including polyethylene glycol (PEG) 3350 and lactulose, work by increasing water in the intestinal lumen to decrease intestinal transit time. PEG 3350 (with or without electrolytes) has been utilized in only a few randomized control trials for the treatment of IBS[18,19]. It has been shown to be effective for relieving constipation associated with IBS, but no more effective than placebo for reducing abdominal pain, bloating, or other symptoms associated with IBS[18]. Lactulose has not been rigorously studied in IBS. In addition, lactulose may cause bloating resulting from fermentation in the intestinal lumen. Thus it should not be recommended for patients with IBS.

NEWER TREATMENTS FOR IBS

Lubiprostone

Lubiprostone is a gastrointestinal chloride-channel activator (specifically at the chloride channel 2 receptor) that enhances intestinal fluid secretion which leads to increased intestinal motility and facilitation of stool passage[20]. It was FDA approved in 2006 for the treatment of chronic idiopathic constipation (CIC) at a dose of 24 μg taken twice per day. Subsequently in 2008, its use was approved for IBS-C in women older than 18 years of age at a dose of 8 μg taken twice per day. This approval was based on the results of two 12 wk randomized phase Ⅲ trials that were published in one manuscript in 2009[21]. The primary endpoint in this study was monthly responder status at three months. The definition of responder was developed between the study investigators and the FDA and thought to be more rigorous than previous trials of IBS treatments. In this study, a "monthly responder" was defined as subjects who reported moderate relief of IBS symptoms for four of four weeks or significant relief for more than two of 4 wk (Table 2). To be considered an "overall responder" (the primary efficacy endpoint), patients had to be a monthly responder for two of three months of the trial. Symptoms were recorded in a weekly electronic diary in which patients were asked "How would you rate your relief of IBS symptoms over the past week compared to how you felt before you entered the study?" Subjects' responses were recorded on a seven-point scale that ranged from "significantly worse" to "significantly relieved". Results of this study showed a statistically significant improvement in the primary efficacy endpoint (17.9% lubiprostone *vs* 10.1% placebo, $P = 0.001$; NNT = 13), as well as monthly response at months two and three (Table 2). Adverse events were frequent but similar between the lubiprostone and placebo groups, with gastrointestinal events occurring most frequently. There was no difference in serious adverse drug reactions (ADRs) or patients who discontinued treatment due to an adverse event (Table 3). ADRs to lubiprostone were reported at a lower rate in the IBS-C trials when compared to trials examining its other indications of opioid-induced constipation and CIC. This is likely due to lower systemic exposure (16 μg/d *vs* 48 μg/d) and the differences between disease states since placebo rates were higher in those trials.

Table 3 Adverse events for lubiprostone phase Ⅲ irritable bowel syndrome with constipation studies

	Lubiprostone 12 wk	Placebo 12 wk	Lubiprostone 36 wk
Serious	1%	1%	1.90%
Treatment related	22%	21%	25.40%
Nausea	8%	4%	11%
Diarrhea	6%	4%	11%
Abdominal distension	2%	2%	3.70%
Discontinuation due to ADR	5%	7%	4%

ADR: Adverse drug reaction.

Patients who completed the 12 wk study were eligible for an additional open-label 36 wk extension study if they had been at least 70% compliant with the study medication[22]. The primary objective of this study was to assess long-term safety and tolerability. Treatment related ADRs were more frequent but similar to the 12 wk study with nausea and diarrhea reported most commonly (Table 3). The drug was tolerated well with only 4% of patients withdrawing due to adverse events. This rate was lower than the 12 wk study, however this likely reflects some selection bias since patients were not treatment naive (except

those previously in the placebo arm).

Too few men with IBS-C were enrolled in the clinical trials with lubiprostone to draw any conclusions about its effectiveness in this population. Because the drug is associated with teratogenic effects in animals, the manufacturer recommends that women who could become pregnant have a negative pregnancy test before beginning therapy, as well as be able to comply with effective contraceptive measures during therapy. The drug is significantly more expensive than traditional laxatives, and should generally be reserved for patients who have failed other therapy for IBS-C.

Linaclotide

An agonist of guanylate cyclase, linaclotide is a unique agent which was recently approved by both American and European regulatory agencies for the treatment of IBS-C[23,24]. Stimulation of guanylate cyclase receptors leads to increased secretion of both guanylin and uroguanylin into the intestinal lumen where they act as a second messenger for both fluid and electrolyte release into the large bowel[25]. Linaclotide is minimally absorbed and has a strong affinity for the guanylate cyclase receptor. Preliminary clinical studies were conducted in the mid-2000s and found the drug to have significant effects on ascending colonic transit time and clinical symptoms related to stooling[26,27]. This led to phase Ⅲ studies that were submitted for regulatory approval. One such study was performed by Rao and colleagues in a randomized, double-blinded fashion on 800 patients with IBS-C[28]. These patients were randomized in this 12-wk trial to linaclotide 266 mcg (n = 405) *vs* placebo (n = 395). As with most IBS studies, the majority of patients were white females who had met Rome Ⅱ criteria for IBS-C. Exclusion criteria included cathartic colon, laxative or enema abuse, ischemic colitis, pelvic floor dysfunction, recent abdominal or pelvic surgery, or other conditions that would explain symptoms, such as inflammatory bowel disease. Of interest, this study was one of the first to use the United States FDA recommendations for trial design and outcomes in IBS studies[29]. Thus, one of the four primary outcomes in the trial was the combination of (1) an improvement of ≥ 30% from baseline in the average of the daily worst abdominal pain scores on standardized scales; and (2) an increase of ≥ 1 spontaneous bowel movements from baseline. Numerous secondary endpoints including patient assessed symptoms, such as abdominal discomfort, abdominal bloating, stool frequency and stool consistency were evaluated. In this study, the primary FDA endpoint was reached by 33.6% receiving linaclotide compared with 21.0% receiving placebo (OR = 1.9, 95%CI: 1.4-2.7, P < 0.0001; NNT = 8). All other primary and secondary efficacy endpoints were similar. Of interest was the group of patients who had improvement in abdominal pain of ≥ 30% (34.3% of linaclotide *vs* 27.1% placebo, OR = 1.4, 95%CI: 1.0-1.9, P = 0.0262; NNT = 14). This suggests that in addition to acting as a laxative, linaclotide has gut anti-nociceptive properties. The safety profile of linaclotide was favorable with diarrhea being the most common adverse effect reported (5.7% *vs* 0.3% in placebo-treated patients). Additionally, no serious or life-threatening adverse effects were reported in this study.

A similarly designed study was performed by Chey *et al*[30] to examine the long-term safety and efficacy of linaclotide in IBS-C. Subjects included 804 patients classified as having IBS-C by Rome Ⅱ criteria and were randomized to either linaclotide 290 mcg or placebo once daily for 26 wk. Exclusion criteria and outcomes were virtually identical to the study discussed above. In this 26 wk study, linaclotide achieved the FDA outcome more frequently than placebo (33.7% *vs* 13.9% respectively, P < 0.0001; NNT = 6). As with the 12 wk study, all other primary and secondary efficacy endpoints showed similar benefits with linaclotide. Diarrhea was again the most common adverse effect reported, with 5.7% of patients dropping out of the study due to this effect. This study not only helped confirm linaclotide's role in treating IBS-C, but it also showed durability of response, a notorious problem when addressing the evidence base of older treatments for this disorder. As mentioned above, these two studies were among the first to utilize the FDA recommended outcomes for IBS trials. It should be noted that other investigators have examined these outcomes and have suggested they may be conservative. Consequently, the true effect size of linaclotide in IBS-C may be greater than these studies suggest[31].

Most recently, a meta-analysis assessed all current randomized controlled trials of linaclotide for both chronic constipation as well as IBS-C[32]. For IBS-C, the investigators utilized the two studies listed above as well as a third trial for which the FDA primary outcome was compiled. When analyzing the data from these studies together, linaclotide was associated with a significant improvement in the FDA outcome [RR = 1.95 (95%CI: 1.3-2.9); NNT = 7 (95%CI: 5-11)]. The authors concluded that linaclotide was effective and had a robust effect size in treating IBS-C. Despite the growing evidence, the role of linaclotide for treating IBS-C in the United States is still uncertain. Given the published data, some experts have called for its placement as a first-line option for this disorder[33]. However, given its cost in the United States (roughly United States $900 monthly), and the reluctance of many third-party payers to cover it, its use will likely be reserved for those patients with IBS-C who have failed other treatments.

Rifaximin

As previously mentioned, a number of avenues concerning the pathogenesis of IBS have received considerable investigation in recent years. Among these lines of research is the relationship between host-gut microbiome. Disruption of this complex relationship, perhaps caused by small intestinal bacterial overgrowth (SIBO), may lead to symptoms attributed to IBS: constipation, abdominal pain and bloating, and change in bowel habit[34]. This

may explain the subset of IBS patients who develop symptoms after a gut infection (so-called "post-infectious IBS"). After disruption of the normal gut microbiome and overgrowth of the small bowel by bacteria, the resulting inflammation may lead to chronic IBS-like symptoms[35]. For the practicing clinician, this does raise interesting questions, such as is SIBO a cause of IBS, particularly the diarrhea-predominant version of the disorder[36]? Or conversely, are some patients labeled as having IBS in reality suffering from SIBO? In either event, a therapeutic strategy aimed at treating SIBO in select patients with IBS-D may be rational.

Since traditional bacterial culture of the entire small bowel is impractical, experts have recommended using breath tests, such as the hydrogen or lactulose test to assess the possibility of SIBO[37]. Selective utilization of these tests, combined with assessment of patient symptoms may help to delineate IBS patients with a SIBO component to their disorder. A recent review of this subject provides an excellent overview for the clinician[37]. Once the determination that SIBO may be playing a factor in a patient's IBS symptoms, should antimicrobials be used for treatment? And, if so, which agent would be preferred? The ideal agent would have little to no systemic absorption, would be active against common gut flora, and would have few adverse effects. Older agents traditionally used for bowel decontamination such as neomycin or metronidazole largely do not meet these criteria. Rifaximin is a drug chemically related to rifampin that has little to no systemic absorption and is well tolerated[38]. This agent has been used in patients with SIBO and has been examined in patients with IBS who do not have constipation. Currently, rifaximin is not FDA approved in the United States for IBS, however, several trials support its use for this indication.

An initial small randomized, controlled trial by Pimentel and co-workers in 87 patients with IBS suggested that a 10-d course of rifaximin 400 mg three times daily improved patient global scores of symptoms compared to placebo[39]. This improvement seemed to persist for the duration of the trial (10 wk) and led these investigators to confirm rifaximin's utility in two larger studies named TARGET-1 and TARGET-2. The results of these trials were combined and published in 2011[40]. Both studies were identically designed and enrolled patients with IBS as assessed by the Rome Ⅱ criteria. Key exclusion criteria included patients with a recent exposure to antibiotics, inflammatory bowel disease, diabetes, or use of other medications exclusively for IBS symptoms. Patients were randomized to rifaximin 550 mg twice daily for two wk or placebo and were followed for up to 10 wk after medication completion. The primary outcome was patients who reported qualitative relief of their global IBS symptoms. A key secondary endpoint was patient assessment of relief from abdominal bloating. A total of 1260 patients were enrolled in the two trials, making these studies among the largest in the IBS literature. In looking at the combined primary endpoint, 40.7% of rifaximin patients reported global improvement in symptoms compared to 31.7% of placebo patients ($P < 0.001$; NNT = 12) in the two studies combined. Numerous secondary endpoints, including abdominal bloating, were statistically better in the active treatment arm compared to placebo. This benefit was largely maintained throughout the study period, up to 10 wk after treatment ended. No significant adverse effects were reported in the rifaximin arm, and no cases of *Clostridium difficile*-associated diarrhea or ischemic colitis were seen. The authors concluded that a two-week course of rifaximin may provide lasting improvement of symptoms in patients with IBS without constipation.

One concern with the aforementioned study was the need to know durability of response to see if or when patients would need retreatment. The TARGET lead investigators performed a retrospective review of patients in their health-system who had received rifaximin for IBS[41]. Of the 71 patients evaluated, the majority did require retreatment for relapsing symptoms. However, patients who responded to one treatment generally also responded to subsequent ones. This is in accordance with a study in only SIBO patients that found a recurrence of symptoms in approximately half of patients nine months after rifaximin treatment[42]. Such patients may be required to receive multiple doses of an expensive antibiotic (roughly United States $700 per treatment course), raising the possibility of developing resistance[43].

Most recently, a meta-analysis was published examining the treatment effect of rifaximin in IBS patients[44]. The authors performed a systematic review that culminated in five articles subject to meta-analysis. The results of this analysis are consistent with individual trial data. Rifaximin was found to improve global IBS symptoms compared to placebo (OR = 1.57, 95%CI: 1.22-2.01; NNT = 11). Bloating symptoms also improved compared to placebo (OR = 1.55, 95%CI: 1.23-1.96; NNT = 11).

Given the price of rifaximin in the US, many patients or payers will be unwilling to assume the cost of the drug. Yet another cost consideration is whether all patients should undergo hydrogen or lactulose breath testing before rifaximin therapy. A recent study from Switzerland suggests that a high percentage of patients diagnosed with IBS will have positive breath testing, and when treated with rifaximin, will have a sustained response[45]. This suggests that, if available to the clinician, such testing should be performed to help guide therapy with rifaximin.

Other treatments, including prucalopride (a selective serotonin receptor agonist with prokinetic activity) may become viable options for IBS, but data to date are limited[46].

ALTERNATIVE TREATMENTS

Peppermint oil

Peppermint oil is an antispasmodic available over the counter in the United States that blocks calcium channels resulting in gastrointestinal smooth muscle relaxation. [8]According to the American College of Gastroen-

Table 4 Probiotic strains

Clinical condition	Effectiveness	Specific strain
IBS	B	*Bifidobacterium infantis* B5624
IBS	B	VSL33 (composite containing multiple strains): 3 strains of *Bifidobacterium*: *Bifidobacterium longum* *Bifidobacterium finfantis* *Bifidobacterium breve* 4 strains of *Lactobacillus*: *Lactobacillus acidophilus* *Lactobacillus casei* *Lactobacillus bulgaricus* *Lactobacillus plantarum* 1 strain of *Streptococcus salivarius*, subspecies *Thermophilus*
IBS	C	*Bifidobacterium animalis*
IBS	C	*Lactobacillus plantarum* 299V

IBS: Irritable bowel syndrome.

terology, peppermint oil may provide short-term relief of discomfort and abdominal pain in IBS and appears to be superior to placebo[17]. However, this conclusion is based on a small number of studies (Grade 2B), and there are no long-term studies to support chronic use. Additionally, there is large variation in the doses of oral peppermint oil (450-900 mg/d in 2-3 divided doses) and duration of therapy used in clinical trials (1-3 mo)[47-51]. The most common adverse effect reported with oral peppermint oil is gastroesophageal reflux. This is thought to be due in part to relaxation of the lower esophageal sphincter, and has led to the popularity of enteric-coated preparations that can bypass the upper gastrointestinal tract[52].

A 2008 meta-analysis including 4 trials (n = 392) provides support for the use of peppermint oil in IBS[17]. In this study, peppermint oil (n = 197) resulted in fewer patients reporting persistent symptoms compared to treatment with placebo (n = 195) for a duration of one to three months (26% *vs* 65% respectively, RR = 0.43, 95%CI: 0.32-0.59; NNT = 2.5). However, statistically significant heterogeneity was detected between studies (I^2 = 31.1%, P = 0.23). Only one of the trials (n = 57) reported the type of IBS according to stool pattern, as two of the four trials included predate the use of these subgroups which were developed with the publication of the Rome Ⅱ criteria in 1999. In this study, 25% of patients had predominant IBS-C and 75% had IBS-D[49]. Additionally, the treatment effect of peppermint oil was found to last for 4 wk after stopping therapy in over 50% of patients in this trial. Although other alternative therapies have been advocated to treat IBS, data on many of these treatments are limited. Oral capsaicin was examined in one small trial found a small improved in abdominal pain and bloating scores, but discontinuations due to initial intolerance was high[53].

Probiotics

Probiotics are dietary supplements that contain live or attenuated bacteria, or bacterial products, which when ingested, may have beneficial effects to a patient's health by altering the gastrointestinal flora[54]. The precise mechanism of action of probiotics is not known. It is hypothesized that inflammation or disproportion of the gastrointestinal bacterial flora may play a part in the pathogenesis of IBS. The probiotic theory suggests that supplementation of the gastrointestinal flora with the right types and numbers of live microorganisms can improve the gut flora and promote health[55]. Additionally, there is evidence to suggest that certain strains of probiotics may stimulate an anti-inflammatory response or improve visceral hypersensitivity, which could theoretically lead to an improvement in symptoms of IBS[56]. Probiotics may comprise a formulation containing a single or mixed-culture of live microbes and are obtainable in diverse preparations, including fermented milk drinks, food products (snacks, chocolates, *etc*.), capsules, pills, and powders[57]. Side effects are generally minimal, although there are risks for patients who are immune compromised[58].

Several strains of probiotics have been studied, but the most commonly used organisms are the lactobacillae and bifidobacteria (Table 4). Several clinical trials have evaluated the effectiveness of a variety of probiotics in patients with IBS, and in general, probiotics can be used for patients with all types of IBS (IBS-D, IBS-C, and IBS-M). Nonetheless, the supportive evidence for treating IBS with probiotics is weak due to the heterogeneity of the studies and the varying probiotics evaluated[59]. Relating and summarizing these trials is difficult due to differences in study design, patient populations, dosing regimens, probiotic species utilized, and reported clinical end points. Regardless of these limitations, some recent systematic reviews and meta-analyses concluded that probiotics seem to be effective in patients with IBS[60-63]. A systematic review of data pooled from 10 randomized controlled trials (RCTs) involving 918 patients with IBS showed a significant benefit for probiotics *vs* placebo in reducing IBS symptoms and decreasing pain and flatulence [RR = 0.71, 95%CI: 0.57-0.88, I^2 = 68%; NNT = 4 (95%CI: 3-12.5)][61]. An additional systematic review of 14 RCTs showed a moderate improvement in overall symptoms, abdominal pain, and flatulence in patients taking probiotics *vs* placebo (OR = 1.6; 95%CI: 1.2-2.2 for dichotomous data from seven trials and standardized mean difference = 0.23; 95%CI: 0.07-0.38 for continuous data from six trials)[60]. Several of the studies found improvement in primary end points compared with baseline, but only some were able to show significant improvement over placebo.

Two types of probiotics were granted the highest rating for efficacy in the treatment of IBS (level "B": based on positive, controlled studies and in spite of the presence of some negative studies) in the Recommendations for Probiotic Use from a Yale University Workshop[64]. The recommendation for *Bifidobacterium infantis* 35624 was concluded from two well-designed clinical trials[65,66] and has been labeled with the "B" rating since the 2008 update[67]. One particular mixture of probiotics, VSL#3

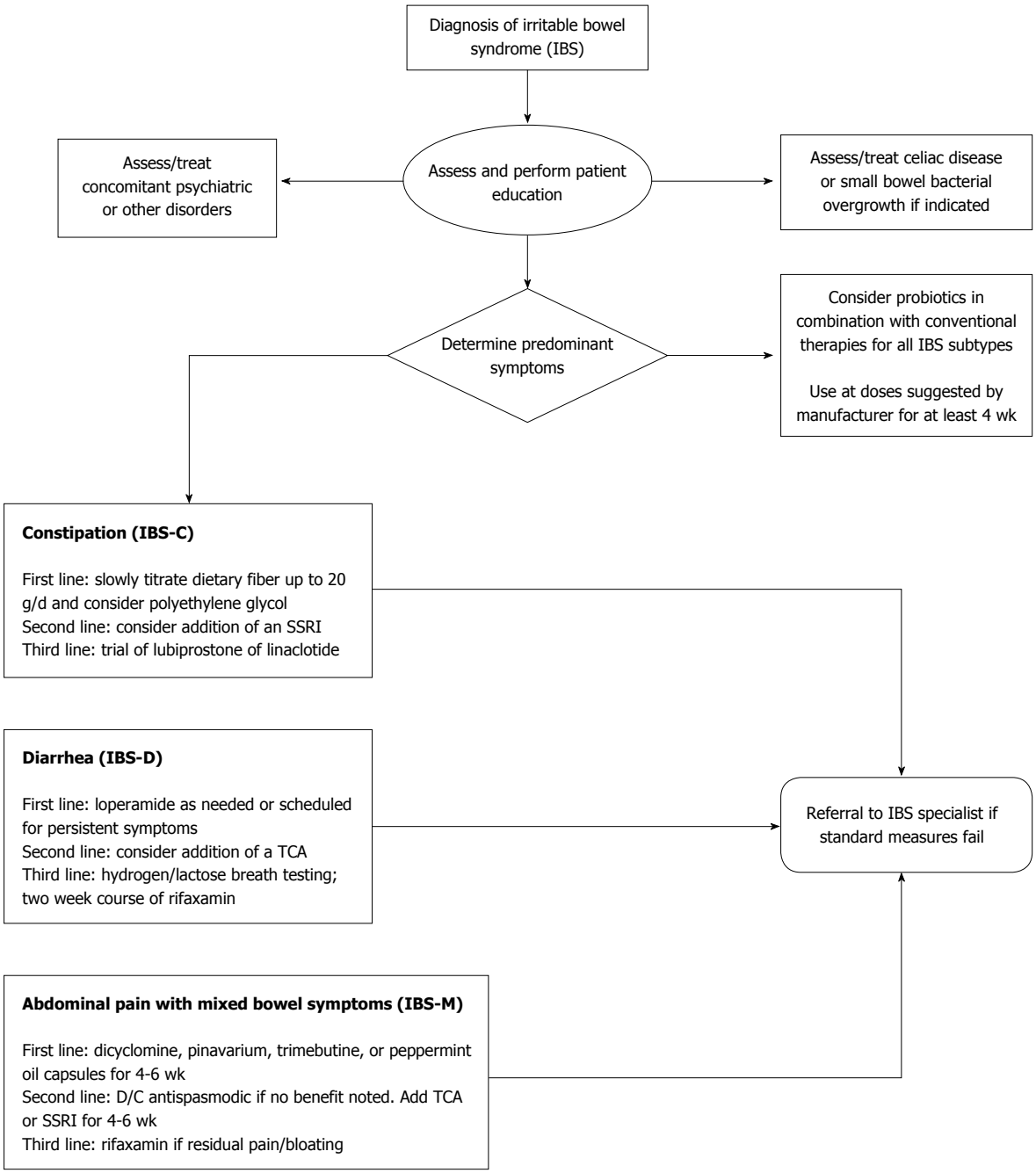

Figure 1 Treatment algorithm for irritable bowel syndrome. IBS: Irritable bowel syndrome; TCA: Tricyclic antidepressant; SSRI: Selective serotonin reuptake inhibitor; D/C: Discontinue.

moved up from a "C" rating to a "B" rating based on the results from two trials[68,69]. A more recent study evaluated patients randomized to receive either placebo or *Bifidobacterium bifidum* MIMBb75[70]. This particular probiotic type reduced the global assessment of IBS symptoms (on a 7-point Likert scale) by 0.88 points *vs* 0.16 points ($P < 0.0001$) and had adequate symptom relief in 47% *vs* 11% ($P < 0.0001$, NNT = 3).

It is important to note that in the United States, regulatory authorities consider probiotics as dietary supplements that are not intended to diagnose, treat, cure, or mitigate the effects of diseases. It is advised that consumers should consult with a health care professional before consuming these products. Many of the available products have not been sufficiently tested for their effectiveness in IBS in satisfactorily designed clinical trials. Another critical factor is the issue of the type products being sold to the public and if their content have enough viable amounts of organisms to make a clinical difference[71]. Furthermore, a study by Mercer *et al*[72] evaluated how patients with IBS viewed probiotics. In this study, patients conveyed frustration that their more traditional IBS medications had worked at first, but became less effective over time. Patients in this study considered probiotics as an appealing potential therapeutic approach for those running out of pharmaceutical options.

Further research is needed to help identify the most effective probiotic species and strains, and the ideal regimen. However, with limited available treatments for IBS, the overall safety of probiotics lowers the bar for trying probiotic products in patients with IBS. Clinicians should not recommend probiotics as monotherapy in symptomatic patients with IBS, but rather in combination with current conventional treatments[57]. Based on the limited evidence for the use of probiotics in patients with IBS, the following organizations have developed guidelines to aid clinicians in their recommendations of products to patients. The National Institute for Health and Clinical Excellence in the United Kingdom has the following recommendation about the use of probiotics in IBS: "Probiotics do not appear to be harmful (unless they come from an unreliable source) and they might benefit people with IBS; they should be advised to take the product at the dose recommended by the manufacturer for at least four wk while monitoring the effect[73]." Additionally, recommendations from the American College of Gastroenterology Task Force on IBS resolved that Bifidobacteria and certain combinations of probiotics demonstrate some efficacy, and that in single-organism studies, lactobacilli do not appear effective for patients with IBS[8].

"Pre-cebo" effect

The placebo effect in clinical trials has long been known, and because of the vague nature of IBS symptoms and the use of primary outcomes that are often subjective in nature, high placebo response rates have been noted in IBS trials. However, Kim *et al*[74] have also described the potential for a "pre-cebo" effect in IBS, which impacts the treatment outcome even before the study begins. The pre-cebo effect describes the impact of consent language used in clinical trials on expectations of benefit from the study medication. This was studied in 59 patients with IBS-D who were randomized to one of 3 medication questionnaires (desipramine, alosetron, or rifaximin). Subjects were asked to rate the percent (0%-100%) improvement in symptoms that would be sufficient for the subject to feel adequate relief. Patients anticipating therapy for any of the three drugs had very high expectations of benefit (> 70%), and patients anticipating rifaxamin treatment had the highest expectation of improvement needed for satisfactory symptom relief (87.3%) compared to desipramine (73.4%, $P < 0.001$) and alosetron (76.8%, $P = 0.049$). This was thought to be due both to the wording used in the consent process, as well as any preconceived ideas about the study medication. The authors note that the high overall expectations may be a challenge to positive outcomes of therapy in any study, and this is particularly noteworthy in IBS because many trials depend on subjective measures of improvement that are patient driven.

CONCLUSION

Gastroenterology practitioners have more agents than ever before to treat the symptoms associated with IBS. Unfortunately, despite advances in our understanding of the pathophysiology of this disorder, targeted treatments do not yet exist. Based on the literature reviewed in this paper, the authors have constructed an algorithm to guide practicing clinicians who encounter this disorder (Figure 1). This algorithm is stratified to symptoms, economic costs, and level of evidence. Using this, or another systematic approach will enable practitioners who treat IBS to do so more efficiently, yet provide relief to a significant number of their patients with this disorder.

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Irritable bowel syndrome: A disease still searching for pathogenesis, diagnosis and therapy

Massimo Bellini, Dario Gambaccini, Cristina Stasi, Maria Teresa Urbano, Santino Marchi, Paolo Usai-Satta

Massimo Bellini, Dario Gambaccini, Maria Teresa Urbano, Santino Marchi, Gastrointestinal Unit, Department of Gastroenterology, University of Pisa, 56124 Pisa, Italy
Cristina Stasi, Department of Experimental and Clinical Medicine University of Florence, 50134 Florence, Italy
Cristina Stasi, Epidemiological Observatory, Health Agency of Tuscany, 50141 Florence, Italy
Paolo Usai-Satta, Gastrointestinal Unit, P. Brotzu Hospital, 09124 Cagliari, Italy
Author contributions: Bellini M contributed to the conception and design of the study and wrote the manuscript; Gambaccini D and Urbano MT contributed to the conception of the study and wrote the manuscript; Stasi C contributed to the conception of the study and revised the manuscript; Marchi S revised the manuscript; Usai-Satta P contributed to the study design and wrote the manuscript.
Correspondence to: Massimo Bellini, MD, PhD, Gastrointestinal Unit, Department of Gastroenterology, University of Pisa, via Paradisa n 2, 56124 Pisa, Italy. mbellini@med.unipi.it
Telephone: +39-50-997448 Fax: +39-50-997368
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Abstract

Irritable bowel syndrome (IBS) is the most frequently diagnosed functional gastrointestinal disorder in primary and secondary care. It is characterised by abdominal discomfort, pain and changes in bowel habits that can have a serious impact on the patient's quality of life. The pathophysiology of IBS is not yet completely clear. Genetic, immune, environmental, inflammatory, neurological and psychological factors, in addition to visceral hypersensitivity, can all play an important role, one that most likely involves the complex interactions between the gut and the brain (gut-brain axis). The diagnosis of IBS can only be made on the basis of the symptoms of the Rome Ⅲ criteria. Because the probability of organic disease in patients fulfilling the IBS criteria is very low, a careful medical history is critical and should pay particular attention to the possible comorbidities. Nevertheless, the severity of the patient's symptoms or concerns sometimes compels the physician to perform useless and/or expensive diagnostic tests, transforming IBS into a diagnosis of exclusion. The presence of alarming symptoms (fever, weight loss, rectal bleeding, significant changes in blood chemistry), the presence of palpable abdominal masses, any recent onset of symptoms in patient aged over 50 years, the presence of symptoms at night, and a familial history of celiac disease, colorectal cancer and/or inflammatory bowel diseases all warrant investigation. Treatment strategies are based on the nature and severity of the symptoms, the degree of functional impairment of the bowel habits, and the presence of psychosocial disorders. This review examines and discusses the pathophysiological aspects and the diagnostic and therapeutic approaches available for patients with symptoms possibly related to IBS, pointing out controversial issues and the strengths and weaknesses of the current knowledge.

Key words: Irritable bowel syndrome; Pathogenesis; Diagnosis; Therapy

Core tip: The pathophysiology of irritable bowel syndrome (IBS) is not definitely known and many fundamental questions remain unanswered about its pathophysiology, diagnosis and therapy. Conflicting results reflect the largely overlapping data of healthy controls and the wide heterogeneity of the IBS patients. This review summarises the main pathophysiological aspects, practical diagnostic approaches and therapeutic management strategies for patients with symptoms possibly related to IBS, in addition to pointing out some controversial issues and pointing out the strengths and the weaknesses of our current knowledge.

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INTRODUCTION

Irritable bowel syndrome (IBS) is quite prevalent in the general population (from 5% to 20%) and represents the functional gastrointestinal (GI) disorder most frequently encountered in primary and secondary care[1,2]. IBS is characterised by abdominal discomfort, pain and changes in bowel habits (constipation and/or diarrhoea)[3] that wax and wane over time. Moreover, it is often associated with other functional digestive and non-digestive disorders[4-8].

The pathophysiology of IBS is not definitely known but most likely involves central and peripheral mechanisms. A disruption of the so called "brain-gut axis" that determines changes in digestive motility and secretion, causes visceral hypersensitivity and leads to cellular and molecular abnormalities in the enteroendocrine and immune systems has been suggested. In addition, genetic factors, infections and alterations of the intestinal microbiota, inflammation and food intolerance and/or hypersensitivity could play a role by altering the integrity of the intestinal barrier and increasing intestinal permeability[9,10]. Up to now, unfortunately, conflicting results have been achieved, most likely reflecting the largely overlapping data of healthy controls and the wide heterogeneity of the IBS population.

The direct and indirect costs of the syndrome are significant, as IBS can have a serious impact on patient quality of life. Because there are not yet any available biological markers or resolving therapies, the patient may undergo expensive tests and treatments[11-13].

The therapeutic approach depends on the intensity of symptoms and the degree of psychosocial comorbidities. Initial treatment is directed towards education, reassurance and lifestyle modification. In a second phase, an appropriate pharmacotherapy can be proposed on the basis of individual or global intestinal symptoms and/or psychological disturbances.

Many different drugs have been suggested for IBS treatment, but their real benefits are very debatable. Based on the multifaceted pathophysiology of the disease, it is unlikely that drugs acting on a single receptor and/or a unique pathophysiologic mechanism would be able to provide any substantial therapeutic gain over a placebo in this disease, for which the placebo response rate is approximately 40%[14].

Essentially, we are still far from having discovered the magic bullet capable of treating all IBS symptoms. Although many papers have been published on this syndrome in recent years, up to now, many fundamental questions remain unanswered about its pathophysiology, diagnosis and therapy.

This review summarises the main pathophysiological aspects, practical diagnostic approaches and therapeutic management strategies for patients with symptoms possibly related to IBS, in addition to pointing out some controversial issues and pointing out the strengths and the weaknesses of our current knowledge.

A search of the literature was carried out using the online databases of PubMed, Medline and Cochrane to identify articles published in English concerning pathophysiology, diagnosis and treatment of IBS.

PATHOPHYSIOLOGICAL ASPECTS

The pathophysiology of IBS, as in all functional digestive disorders, is complicated because there is no clearly identified pathophysiological basis for the disease. In fact, IBS is identified by a combination of chronic or recurrent GI symptoms in the absence of structural abnormalities (radiological/endoscopic) or biomarkers capable of positively identifying this condition. Aside from these drawbacks, the clinical manifestations of IBS are themselves extremely heterogeneous, a sort of "semantic umbrella" under which different clinical situations related to phenotypic aspect (traditionally subtyped as diarrhoea predominant, constipation predominant and mixed type) and the modality of clinical onset (post-infectious, food-related, stress-linked, *etc.*) fall[15].

The aetiology of IBS is multifactorial. Many pathogenetic factors, in various combinations and not all necessarily present in each patient, can play an important role (Table 1). Genetics, immune factors, environmental influences, inflammatory and infective agents, neurological and psychological factors, hypersensitivity to food and to bile salts and altered intestinal microbiota and permeability can all influence the brain-gut axis, leading to abnormal GI function and motility. It is unclear which among these factors is the trigger or how these conditions converge to initiate the IBS; previous studies aiming to identify a factor as more of a trigger over the others all failed to distinguish any one trigger.

The genetic factors have been extensively studied. Up to 33% of IBS patients have a family history of IBS, compared to 2% of controls[16]. There is a higher prevalence of the disease in families of patients with IBS compared to the families of the spouses without IBS[17]. Moreover, some studies have reported a higher prevalence in monozygotic twins compared with heterozygotes, indicating a hypothetical genetic component[18]. However, other studies[19] demonstrated that having a parent with IBS was a better predictive factor than having a twin affected with IBS, suggesting that the environmental factor is more important.

The genetic factors involved in the pathogenesis of IBS has also been evaluated by a number of studies investigating the possible role of gene polymorphisms coding for serotonin (SERT), cholecystokinin (CCK) receptors 1, anti-inflammatory and pro-inflammatory interleukins and alpha 2 adrenergic receptors[20-22]. As sero-

Table 1 Factors potentially involved in the pathogenesis of irritable bowel syndrome

Altered intestinal motility
Food intolerance/allergy
Enteric infection/inflammation
Altered intestinal immunity
Altered gut microbiota
Genetics
Psychological distress and disorders; sexual abuse

tonin was involved in the regulation of digestive motility, secretion and visceral sensitivity, particular investigative emphasis has been placed on polymorphisms of the gene regulating the reuptake of serotonin (SERT), which can induce a variation of its synaptic concentration[23]. SERT polymorphisms are not related to the development or onset of IBS, but rather to a different clinical expression, a greater perception of abdominal pain and an increased dissatisfaction regarding bowel habits[24].

Recently, a "biopsychosocial" model[25,26] has been introduced, in an attempt to integrate and harmonise the different factors (genetic, environmental and psychological) acting in a synergistic way to produce these symptoms.

These deficiencies in understanding the pathophysiological mechanisms of IBS have a heavy negative effect on clinical practice and may explain the disappointing results of previous therapeutic attempts, as well as the high costs of management. Currently, there is no single drug that is able to treat all of the symptoms related to IBS; rather, a "drug cocktail" is administered, having different effects on different symptoms.

Previous studies[27] have considered this syndrome a result of alterations in the normal digestive motility pattern, the so-called "spastic colon". Subsequently, much interest was directed toward visceral hypersensitivity, under the hypothesis that IBS patients experienced visceral stimuli more strongly than healthy subjects. Later, IBS came to be considered a two-way interaction between the gut and the brain, with much interest directed not only toward the activation/deactivation of afferent and efferent nervous stimuli but also toward the effects of neuromodulators.

The possibility that IBS could be initiated after an enteric infection and the evidence that, in inflammatory bowel disease limited to the mucosa, patients suffer from enhanced sensory perception and motor dysfunction have driven researchers to study these as further potential causes of IBS.

Some previous studies[28,29] attempted to assess whether an abnormal motility pattern is typical in cases of IBS; however, despite identifying cluster contractions in phase Ⅱ of the migrating motor complex in the jejunum, propagated ileal contractions related to pain and an increased postprandial motor activity of the colon, up to now, all attempts made have failed to reach a single typical pattern.

An altered colonic transit rate [accelerated in IBS and diarrhoea (IBS-D) and slowed in IBS with constipation (IBS-C)] was described in some studies[30,31] but these results have not been confirmed by more recent studies[32,33]. Salvioli *et al*[34] reported a decreased capacity of the motor activity in the small intestine to eliminate intestinal gas, resulting in abdominal distension and typical symptoms of IBS. IBS patients likely experience psychological stress, foods, neurotransmitters and/or rectal or bowel distension, which can lead to an altered motor response that leads to the same motor events being perceived more strongly and painfully[35].

Visceral hypersensitivity in IBS patients is supported by several studies[36-38]. Verne *et al*[39] used functional nuclear magnetic resonance (RMN) to show that a mechanical stimulus (rectal distension) active different regions of the brain in healthy volunteers, compared to patients with IBS. Unfortunately, this technique is expensive and not widely available. Moreover, comorbidities, such as fibromyalgia and psychological disturbances, can significantly affect its outcome.

Psychological disorders, including sexual and physical abuse, result in a high percentage of patients with functional disorders. Even if the disorders are not directly responsible for the onset or progression of the IBS symptoms, they certainly determine a different perception of the symptoms and result in more frequent requests for medical aid. In fact, these disorders are more common in IBS patients who seek medical care than in patients who do not ask for medical help or healthy volunteers[15,40].

Psychological distress and disorders can affect the brain-gut axis, promoting the release of corticotropin-releasing hormone, which is able to influence mood, digestive motility, permeability, visceral sensitivity and inflammatory pathways *via* neuroendocrine and autonomic outflows[41-44]. Dinan *et al*[44] showed that physical and mental stress in IBS patients increased the levels of pro-inflammatory interleukins, activating both the hypothalamic-autonomic nervous system and the hypothalamic-pituitary-adrenal (HPA) axes and consequently increasing the serological adreno-cortico-tropic-hormone and cortisol levels. Recent studies[44,45] introduced the hypothesis that IBS could be an inflammatory disorder that is supported by a dysregulation of the HPA.

On the other hand, it has been shown that physical and psychological stress activates different regions of the brain among patients with IBS than among healthy volunteers. In particular, IBS patients have a greater activation of the mid-anterior cingulate cortex, an area linked to anxiety, fear and hypervigilance[46]. This area is the target of many antidepressant drugs and psychotherapy. In healthy controls, stress instead activates the perigenual area, from which originate the descending inhibitory pathways that control visceral afferents to the posterior horn of the spinal cord[47].

A continuous and mutual interaction between the gut and the brain is made possible through the autonomic nervous system and the enteric nervous system *via* neuroendocrine mediators (VIP, 5HT, Ach, NO, NO, CCK, *etc.*); this system comprises the so-called "gut - brain axis".

Signals received from the GI tract affect the brain that, in turn, can affect the motility, secretion and immune functions of the digestive tract. Thus, alterations to this system may cause many digestive disorders, and particularly IBS, compared to normal, unaltered subjects[41,48,49].

The neuroendocrine system is potentially involved in the pathogenesis of IBS. This system is very complex and consists essentially of two components.

The endocrine cells (at least 14 endocrine or paracrine cell populations), which are distributed between the epithelial cells of the digestive mucosa and directly in contact with the intestinal lumen and its contents; and the nerve fibres (peptidergic, serotonergic, nitrergic, *etc.*) of the enteric nervous system[15].

Motility, secretion, absorption and intestinal microcirculation are all influenced by this system by the means of several mediators that have endocrine (released directly into the blood stream), autocrine/paracrine (local effects) or neuroendocrine (released from synapses into the bloodstream) functions[41].

An alteration to this system has been hypothesised, in which a decreased density of cells producing gastric inhibitory polypeptide (GIP) and somatostatin (in D-IBS and C-IBS) and in those producing secretin and CCK (in D-IBS) was reported in the small intestine, whereas a lower expression of cells producing 5-hydroxytryptamine and PYY was detected in the colons of patients with D-IBS and C-IBS[15,41]. An abnormal inflammatory response to different events (stress, infections, food, *etc.*) could be responsible for the abnormal cellularity in the colonic mucosa and the increased concentration of pro-inflammatory interleukins detected in the colons of some IBS patients[50]. These studies suggest that the activation of mast cells, macrophages or leukocytes producing inflammatory mediators is able to affect the motility, secretion, sensitive nerve endings and ultimate perception of pain.

Biopsies from the colons of IBS patients showed an increased activation of lymphocytes and mast cells in close proximity to the enteric neurons, with increased production of cytokines and other proinflammatory and vasoactive peptides[51,52]. Degranulation of these cells (especially mast cells) has been associated with the onset of the typical abdominal pain endured by IBS patients[53]. Moreover, the density of immunocompetent cells gradually increases on a spectrum from controls to patients with IBS, then to patients with microscopic colitis and, finally, to those with ulcerative colitis[54].

Inflammation can also result from a previous enteric infection. The onset of IBS follows an infection in approximately 10% of patients. In these patients, there are increases in the levels of CD3 serum lymphocytes, CD8 intraepithelial lymphocytes, and macrophage calprotectin-positive cells. Moreover, cells producing serotonin and CCK were found to be increased in the small bowel, while those producing serotonin and PYY were decreased in the colon. These alterations were usually transient but tended to persist in patients who developed IBS[55].

In post-infectious IBS and D-IBS, intestinal permeability has also been studied. The findings included a decreased expression and remodelling of the structural proteins constituting the epithelial "tight junctions" in the cells of the small intestine and colon. These changes increased the intestinal permeability, resulting in an easier passage of antigenic material through the epithelium and a stimulation of the intestinal immune system (especially mast cells) with the production of the proteases, histamine and prostanoids able to maintain the permeability and to produce abnormal neuronal responses, inducing the motor and sensory results typical in IBS[42].

Based on these results, it is evident that preserving, maintaining or restoring the normal composition of the intestinal microbiota is essential for good bowel function[42]. The intestinal microbiota is a major target of many therapeutic options for relieving IBS symptoms. The colon of each individual contains from 300 to 500 different species of bacteria. Thus, each of our microbiota is individual and unique. The microbiota is influenced by the environment, diet, previous infections, genetics, age, and antibiotic therapy. In normal conditions, the lactobacilli and bifidobacteria bind to epithelial cells, inhibiting the binding of pathogens and reinforcing the defences of the mucosal barrier. In addition, lactobacilli and bifidobacteria do not produce gas by fermenting carbohydrates and inhibiting the growth of the Clostridia species, which do produce this effect. Lactobacilli and bifidobacteria were found to be decreased in IBS patients, and their activities were found to be heavily compromised[56]. Moreover, some evidence indicates that probiotics affect intestinal fermentation and stabilise the intestinal microbiota, normalising the relationship between pro-inflammatory and anti-inflammatory cytokines with beneficial effects on intestinal inflammation, permeability and visceral sensitivity[57,58].

Unfortunately, at present, there are intrinsic difficulties in clearly establishing the role of the gut microbiota in the pathophysiology of IBS, both due to the great heterogeneity in the clinical presentation of IBS and to the limitations of the available studies (study design, length of observation, small sample, *etc.*).

Finally, the role of food in IBS merits specific mention. Patients with IBS tend to declare that their symptoms are often exacerbated by meals or by certain foods (sweeteners, fats, *etc.*). The classical IgE-mediated food allergy does not seem to play an important role in IBS. In the recent past, high levels of the specific IgG4 for wheat, beef, pork and lamb were found in IBS patients, compared to healthy subjects, and based on this, an exclusion diet was proposed[59]. On the other hand, this subgroup of Ig seems to be only an epiphenomenon of mucosal production, according to recent evidence[60].

In any case, up to 60% of patients with IBS reported a worsening of symptoms after food intake, in particular after specific foods like milk and dairy products, wheat, onions, beans, spices, cabbage, red meat, fried, smoked products, and caffeine. These foods represent the so-

Table 2 Most frequently reported comorbidities in irritable bowel syndrome patients

Functional dyspepsia and functional heartburn
Fibromyalgia
Chronic fatigue syndrome
Back pain
Multiple chemical sensitivity syndrome
Post-traumatic stress disorder
Psychological/psychiatric disorders
Sleep disturbances
Migraine and tension headaches

called fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs). However, studies supporting this are limited and demonstrate only a partial improvement in patients after the restriction of these foods. More frequently, IBS patients seem to have an exaggerated gastric-colic reflex after eating any item of food.

In recent years, it has been observed that the ingestion of gluten causes abdominal discomfort and IBS-like symptoms in subjects without a diagnosis of celiac disease (the so-called gluten sensitivity).

At the moment, the mechanisms responsible for these symptoms are not clear. Most likely, the gluten, as other well-known factors, alters the intestinal permeability, activating the enteric and autonomous nervous systems and producing the typical symptoms of IBS. Recently, authors have disagreed on the topic of gluten sensitivity, instead attempting to explain the problem with a simpler hypothesis: gluten-rich foods may cause symptoms with the same mechanisms of the FODMAPs[61,62]. The positive effect of the gluten-free diet on abdominal disorders could be due to the drastic reduction of FODMAPs that is inevitable in a diet of this type.

Up to now, the available results in the literature conflict; thus, further studies are needed to clarify this intriguing matter.

DIAGNOSTIC APPROACHES

A careful medical history is critical for the evaluation of a patient with a possible diagnosis of IBS. Particular attention has to be devoted to many different issues, such as dietary habits, therapies (especially the intake of drugs capable of altering the bowel frequency and/or causing abdominal pain), the degree of physical activity, comorbidities, previous surgical interventions, presence of symptoms suggesting anxiety or depression, and recent trips to exotic locations[3,63].

In the absence of accepted and shared biological markers, symptoms remain the cornerstone for the diagnosis of IBS.

Regarding the symptom "pain", it is useful to assess its type (cramping, tensive, stabbing, burning), localisation, frequency, duration, mode of occurrence and possible changes in relation to defecation, to food intake (or to intake of particular foods), to stressful events and to the menstrual cycle[63,64].

As for abdominal distension or tension, it is mandatory to ask the patient if it is visible from others or if it is otherwise measurable (changes in size, inability to tie the skirt or pants, *etc.*). Additionally, patients should be asked whether their pain gets worse at certain times or improves with evacuation or emission of the flatus.

It is also necessary to investigate the characteristics of the defecation: difficult or prolonged, painful or simply incomplete, the presence of a sensation of anorectal blocking, the need for manual help, the presence of ineffective attempts or, on the contrary, of an urgency at defecation and real episodes of faecal incontinence[64].

Moreover, it is important to check for the presence of blood, mucus or pus in the faeces and to assess the usual shape of the stool using the Bristol Scale that, by relating the rate of intestinal transit with faecal consistency, provides a visual aid to help the patient better classify a topic otherwise difficult to objectify[65].

Additionally, it is mandatory to look for the possible co-morbidities that can occur in a patient with IBS, because they can increase the perception of the disease severity[8,13,66,67].

In Table 2, the most frequent co-morbidities are represented. These share common characteristics, such as the following: (1) a higher prevalence in females; (2) pathophysiology linked to low-grade inflammation, stress, somatisation, hypersensitivity, changes in the central processing of peripheral afferents and/or alterations of substances acting as neuromodulators; (3) a diagnosis mainly based on symptoms; (4) possible responsiveness to antidepressant medications and cognitive-behavioural therapies; (5) frequent multidisciplinary management; and (6) a considerable reduction of the quality of life and high, direct and indirect, costs.

The presence of alarm symptoms, the so-called "red flags" like fever, weight loss, rectal bleeding, and significant changes in blood chemistry, should be investigated, as well as the presence of palpable abdominal masses, any recent onset of symptoms in patients aged over 50 years, the presence of symptoms at night, and a familiar history positive for celiac disease, colorectal cancer and/or inflammatory bowel disease[64,68].

Still, some authors[69] believe that the accuracy of the "alarm symptoms" is disappointing. In particular, rectal bleeding and nocturnal pain would be of little value in discriminating patients with IBS from patients with organic disease, while anaemia and weight loss would have low sensitivity, but high specificity, to identify an organic disease.

A physical examination would not be very rich in information, as it could only detect abdominal tenderness (localised or diffuse) and abdominal hypertympanism or bowel sounds at auscultation, but this practice reassures the patient and can provide a first, coarse exclusion of organic diseases (abdominal masses, *etc.*). The examination should include the inspection of the anorectal region and a digital rectal examination, preferably in the left-lateral decubitus, which would provide useful information

Table 3 Diseases and conditions considered in the differential diagnosis

Celiac disease and malabsorption
Lactose intolerance, fructose intolerance
Inflammatory bowel disease
Lymphocytic and collagenous colitis
Whipple's disease
Colonic cancer
Enteric infections
Metabolism disorders (*e.g.*, thyroid, diabetes, *etc.*)
Food allergy and intolerance
Endometriosis
SIBO
Neuroendocrine tumors
Drugs

SIBO: Small intestinal bacterial overgrowth.

about the dynamics of the pelvic floor, especially if any functional alteration is suspected. Thus, the presence of comorbidities and organic diseases can be detected[63,70-72].

The use of specifically dedicated scores to measure the impairment of the quality of life and symptom severity has been debated in clinical practice, both at the initial stages and later, in order to verify the effectiveness of the therapy administered[73]. Indeed, any such scoring systems are not widely used outside of clinical trials, even if they do not seem time-consuming or difficult to use[74-77].

Can a diagnosis of IBS be made only using only symptom-based criteria? The evidence from the literature seems reassuring in this respect, because the probability of organic disease arising in patients fulfilling the IBS criteria is very low[78]. Nevertheless, the nature and severity of the symptoms themselves, or of the patient's concerns and fears, sometimes compel the physician to perform unnecessary, useless, and/or expensive diagnostic tests, transforming IBS into a diagnosis of exclusion.

Indeed, in the differential diagnosis, the conditions reported in Table 3 will have to be considered with greater or lesser probability[68].

Unfortunately, there are no available biological markers that clearly identify IBS patients.

Some recent studies have examined faecal lactoferrin and calprotectin, which seem quite suitable to differentiate between infectious bursal disease and IBS but are not able to provide a certain diagnosis of IBS[79,80].

Recent studies have investigated some biomarkers involved in the pathophysiology of IBS[45,81]. A recent systematic review and meta-analysis examined the placebo response rate in treatment trials for IBS and demonstrated a high placebo response[82].

In the case of a patient with IBS-like chronic recurring abdominal symptoms, the presence of alarming symptoms should first be assessed[68,69,83,84]. In the presence of alarming symptoms, further investigation should be undertaken. On the contrary, in the case of Rome Ⅲ criteria positivity and in the absence of alarm symptoms, possible comorbidities (which are part of the IBS management) should be considered. Serological screening for celiac disease and a few basal blood tests have to be performed; if a negative result is returned, it is usually sufficient to reassure the patient and to offer advice on drug therapies, lifestyle habits and diet. A check-up after 8-12 wk should be offered, and in cases with sustained improvement, the patient will enter into a follow-up program (Figure 1).

In the case of a patient with symptoms in any way compatible with irritable bowel syndrome but that did not satisfy the Rome criteria, or in the case of a patient with a poor response to the therapy, depending on the prevailing symptoms (constipation, diarrhoea, abdominal pain/bloating), different options should be considered (Figure 1).

In the case of constipation, dietary habits and behaviours, as well as the use of laxatives, should be checked. In the case of the ineffectiveness of these measures, if not already performed, an assessment of the thyroid function, routine blood tests and screening for celiac disease are recommended. In the case of diarrhoea and abdominal pain/distention, lactose breath test (LBT) (or simply lactose withdrawal), a faecal blood test, faecal Calprotectin or Lactoferrin, stool culture, test for ova and parasites, a chemico-physical examination to test for Clostridium difficile toxins and an abdominal ultrasound aimed at studying the enteric loops should be considered.

If signs of a specific disease emerge from the investigation or from specific treatments, further investigation should be initiated. In the case of a negative outcome, it will become mandatory to proceed to the next steps, as follows (Figure 1): (1) in the case of constipation, the possibility arises of performing a colonoscopy, anorectal manometry, defecography, intestinal transit time and, in carefully selected cases, colonic and gastrojejunal manometry; (2) in the case of diarrhoea and abdominal pain, it will become appropriate to check and eventually change the patients' drugs; (3) in the case of a failed colonoscopy, biopsies may be useful; and (4) in the case of a negative outcome of a colonoscopy, the further investigations reported in Figure 1 should be considered.

Still, it is mandatory to emphasise that none of these investigations, even those that are costly and unusual, should be performed to achieve the diagnosis of IBS, which is essentially based on the Rome Ⅲ criteria, as reported above. On the contrary, these tools are to be taken into account only in a patient with abdominal symptoms that are IBS-like but Rome Ⅲ criteria-negative or -equivocal. They may also be used in IBS patients with very severe symptoms that require a careful reassessment of the clinical situation.

In IBS, the follow up should be tailored to the patient, because the disease is characterised by variable remissions and relapses, with symptoms waxing and waning over time, often oddly and sometimes in coincidence with stressful events, anxiety, the intake of certain foods, *etc.* IBS patients usually tend to avoid fixed controls, although, at least at the beginning, a clinical visit 2-3 mo after the diagnosis is advised to assess the patient's adher-

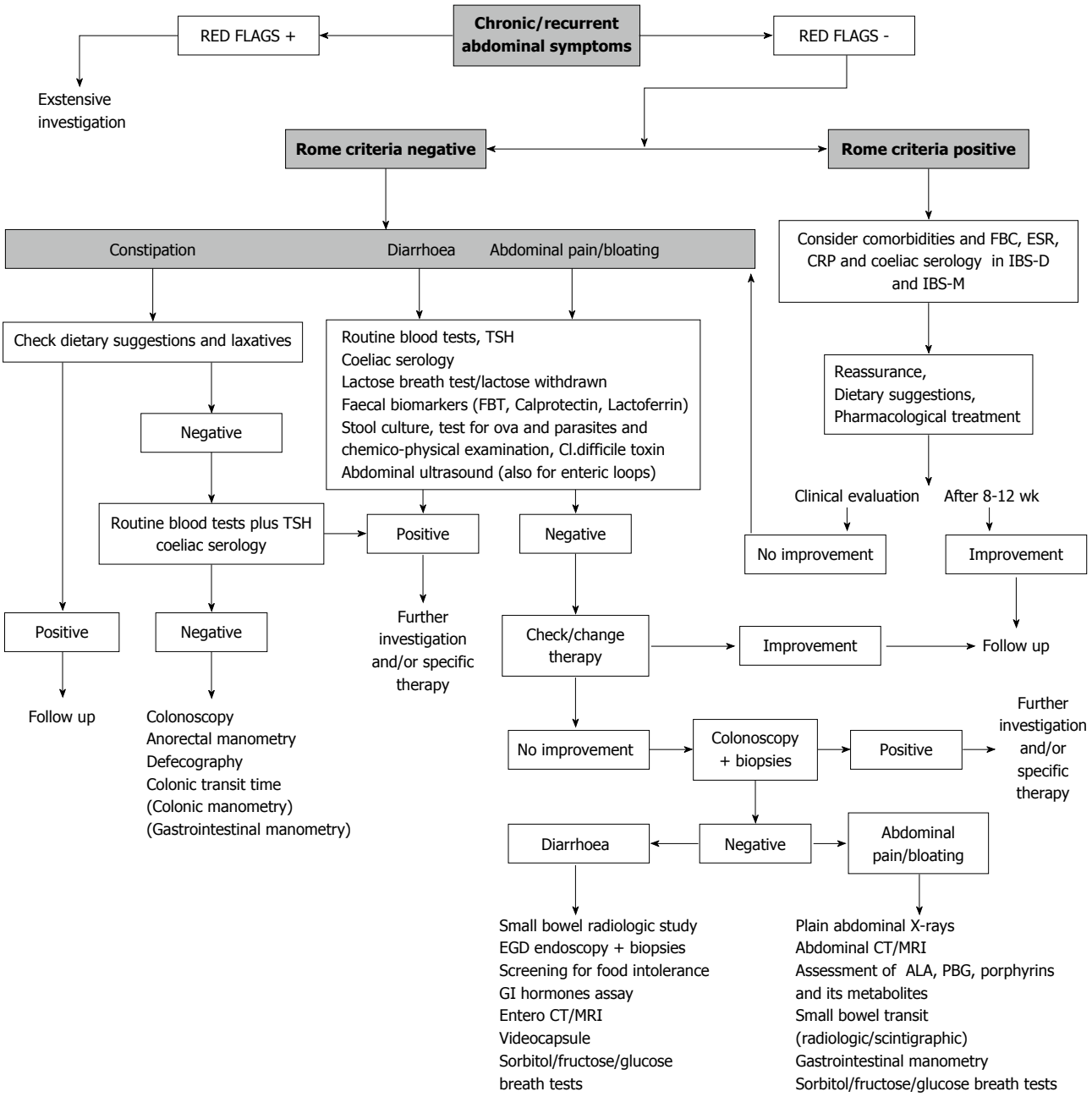

Figure 1 Diagnostic-therapeutic algorithm in a patient with abdominal symptoms possibly related to irritable bowel syndrome. FBT: Faecal blood test; FBC: Full blood count; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; PBG: Porphobilinogen; IBS: Irritable bowel syndrome; CT: Computed tomography; MRI: Magnetic resonance imaging.

ence to therapy and the dietary and behavioural recommendations.

The aim will be to help IBS patients perceive their symptoms as part of a chronic, intermittent disorder, learning to live with them. Thus, these patients can rejoin that "silent majority" of IBS patients who perceive her/his symptoms as no more than a nuisance and do not seek further special care, doctor visits, or additional diagnostic tests.

THERAPEUTIC PERSPECTIVES

Treatment strategies for IBS are based on the nature and severity of the symptoms, the degree of functional impairment of the bowel habits, and the presence of psychosocial comorbidity. In general, milder symptoms relate primarily to visceral hypersensitivity and are commonly treated symptomatically, with pharmacological agents directed at the gut. However, more severe symptoms are associated with greater levels of psychosocial problems and often require psychological and antidepressant medications.

There is limited evidence for the efficacy, safety and tolerability of the therapies currently available for the treatment of IBS. Overall, there is a limited availability of pharmacological agents licensed specifically for the treat-

Table 4 Indication of pharmacological agents in individual irritable bowel syndrome symptoms

Constipation	Diarrhoea	Pain
Soluble fibre	Opioid agents	Antispasmodics
Osmotic Laxative	5-HT$_3$ antagonists	Peppermint oil
5-HT$_4$ agonists	Probiotics	Serotoninergic drugs
Secretagogues	Antibiotics	Antidepressants
Probiotics	Mesalazine	Herbal therapy
SSRI	Colestyramine	Acupuncture
	Tricyclic antidepressants	

SSRI: Selective serotonin reuptake inhibitors.

ment of IBS subtypes, and new agents are eagerly awaited. In any case, it is difficult to achieve a significant therapeutic improvement in global IBS symptoms[64,69,71,85,86].

There is some evidence for improvements in individual IBS symptoms with the use of antidiarrhoeals, antispasmodics, bulking agents, laxatives, tricyclic antidepressants and behavioural therapy. Despite evidence that some pharmaceutical agents benefit the treatment of IBS in the short term, there is no medical intervention that has been proven to alter the long-term natural history of this condition. Further, there is no agreement on a gold-standard for the treatment of IBS. Finally, in functional GI disorders, in which the trial endpoints are likely to be less tangible than organic conditions, the placebo response rate may be very high (over 40%)[82]. Table 4 summarises the various drug categories and their relationships with individual IBS symptoms.

Education and reassurance

A strong physician-patient relationship should be the foundation for effective treatment and realistic expectations. Responding to all patient concerns and questions and spending time in the clinical visits validate their condition. A reassurance-based approach permits the patient to understand and accept his or her affliction and to participate in a care strategy. Using this approach, a decrease in the number of health care visits, a reduction in symptoms, and improved patient satisfaction can be easier obtained.

Diet

Patients with IBS commonly believe that specific dietary products contribute to their symptoms of abdominal discomfort, bloating, or alterations of bowel habits. The truth is that no specific food is likely implicated, as true food allergies and intolerances are rare. In many cases, IBS patients have an exaggerated gastric-colic reflex after eating certain foods.

Patients can associate with their complaints the ingestion of certain foods, such as fatty foods, caffeine, alcoholic beverages, carbonated foods, or gas-producing foods. Specifically, symptoms can be related to FODMAPs, such as fructans, galactans, lactose, fructose, sorbitol, xylitol, and mannitol[87]. Studies supporting this are limited and demonstrate a partial improvement in patients after the restriction of these foods. Otherwise, a lactose-restricted diet does not seem to produce a clear clinical benefit in IBS. Beyond this, recent evidence has shown that lactose intolerance was equally prevalent among IBS patients and the general population[64]. Finally, a recent study showed that patients with IBS but without celiac disease may reach satisfactory symptom control with a gluten-free diet but may suffer a symptom relapse after a gluten rechallenge[61]. Only a double-blind gluten challenge can discriminate between IBS and gluten-sensitivity patients. In any case, some care should be taken to avoid an unnecessarily restrictive diet with potentially serious nutritional consequences.

Fibre and bulking agents

Most physicians recommend the use of dietary fibre and bulking agents to regularise bowel function and to reduce meteorism and pain in patients with IBS. The quality of the evidence supporting this recommendation, however, is poor. Some randomised placebo controlled trials have compared the effectiveness of increasing the dietary content of soluble fibre (psyllium and ispaghula) or insoluble fibre (bran) in patients with IBS and constipation. There is some evidence that patients taking psyllium have significant symptom relief, whereas bran shows no clinical benefit and actually may worsen symptoms in many cases[64,69,71,73,85,86,88].

Antispasmodic agents

The rationale for using antispasmodic agents is to attenuate the postprandial abdominal pain seen in patients with IBS. The mechanisms of action of different antispasmodics can be divided broadly into those that directly affect the intestinal smooth muscle and those with anticholinergic/antimuscarinic effects[64,69,71,85,86]. The evidence for the effectiveness of these agents is not compelling.

One meta-analysis demonstrated an advantage of antispasmodics over placebo in terms of abdominal pain and distention[88]. Of all of the drugs studied, the most data were available for otilonium, trimebutine, cimetropium, hyoscine, and pinaverium. Trimebutine seemed to have no benefit over placebo in treating IBS, whereas the other four drugs all significantly reduced the risk of persistent symptoms after treatment. The anticholinergic side effects, including constipation, dry mouth, visual disturbances, and urinary retention, can lead to the discontinuation of these medications. Finally, there is evidence for the efficacy of some peppermint oil preparations (which may also act as antispasmodics) in IBS, but few data are available about the long-term results and adverse effects[88].

Anti-constipation agents

The presence or absence of abdominal pain should be more useful than other associated features for characterising IBS-C in comparison with chronic constipation. However, a clear clinical distinction is not always possible in clinical practice.

Traditional laxatives: Consistent with recent reviews, a therapeutic trial of traditional laxatives (*i.e.*, osmotic laxatives, stimulant laxatives), which are effective, safe, and generally inexpensive, should be considered for managing chronic constipation before newer agents (secretagogues, serotonin 5-HT$_4$ receptor agonists) are used[70,88]. In particular, polyethylene glycol (PEG) is more effective than lactulose in increasing stool frequency and improving stool consistency; thus, it is considered the first choice of treatment for chronic constipation[70].

However, no placebo-controlled, randomised study of laxatives in IBS has been published. Laxatives do not show a significant effect in reducing abdominal pain in IBS. A single small sequential study with PEG in adolescents with IBS-C showed an improvement in stool frequency[89].

Serotonin HT$_4$ agonists: 5-HT$_4$ receptor agonists induce fast excitatory postsynaptic potentials in intrinsic neurons, release acetylcholine, and induce mucosal secretion by activating submucosal neurons.

Tegaserod has been approved by the Food and Drug Administration (FDA) for the treatment of IBS-C in women. Tegaserod is also the only 5-HT$_4$ agonist that has been evaluated in an IBS-mixed population and showed an improvement of global symptoms. However, this drug was removed from the market in 2007 because cardiovascular events were found to be more frequent in tegaserod-treated patients than in placebo-treated patients[89,90].

Among the 5-HT$_4$ agonists for chronic constipation, the most evidence in humans is available for prucalopride[70,90]. The European Agency of Medicinal Products approved this medication for chronic constipation in women for whom laxatives fail to provide an adequate relief of their bowel habits. Prucalopride accelerates GI and colonic transit in constipation, but no placebo-controlled studies have been published, and no conclusive clinical evidence is available for IBS patients[90].

Intestinal secretagogues: By stimulating the efflux of ions and water into the intestinal lumen, secretagogues accelerate transit and facilitate defecation. Both lubiprostone and linaclotide increase intestinal chloride secretion by activating channels on the luminal enterocyte surface[90]. Lubiprostone works by activating apical CIC-2 chloride channels and does not affect colonic motor activity in healthy subjects. It is approved by the FDA for the treatment of women with IBS-C[91,92]. Linaclotide is a guanylyl cyclase C agonist that accelerates colonic transit in patients with IBS-C and chronic constipation[93]. In a recent randomised double-blind trial, linaclotide was shown to improve abdominal pain and discomfort in IBS-C, compared with placebo, over 12 and 26 wk[94]. In the same trial, diarrhoea was the most common adverse effect (19%), although few patients (5.7%) discontinued the drug as a result of this symptom. As of 2012, linaclotide is approved both by the FDA and also by the European Agency for the treatment of IBS-C.

Antidiarrhoeal agents

Opioid analogues: The opioid analogues loperamide and diphenoxylate stimulate inhibitory presynaptic receptors in the enteric nervous system, resulting in the inhibition of peristalsis and secretion. Loperamide has been shown to be effective in decreasing stool frequency and improving stool consistency across all studies[64,69,71,85,95], although it provided no significant improvement in global IBS symptoms (in particular, abdominal pain and distension) compared with placebo.

The simultaneous μ opioid agonist and δ opioid antagonist eluxadoline could reduce abdominal pain and diarrhoea in patients with IBS-D, compared with placebo, in a phase 2 study awaiting publication[96].

Serotonin HT3 antagonists: The 5-HT$_3$ receptor antagonists have been studied in IBS-D because they slow GI transit and decrease discomfort during the distension of the colon[64,69,71,85,86]. Ondansetron is the only 5-HT$_3$ receptor antagonist available in Europe and is licensed as an antiemetic, although it is not approved for use as a treatment for IBS[86]. The selective 5-HT$_3$ receptor antagonist alosetron was currently indicated for the treatment of women with severe IBS-D who had chronic symptoms of IBS[64,69,86,97].

Although it was originally approved by the FDA in 2000, alosetron was withdrawn from the market following reports of serious complications, including constipation, ischemic colitis, and bowel perforation, being associated with its use. Some evidence is available regarding other 5-HT$_3$ antagonists, such as cilansetron and ramosetron. In a recent double-blind randomised trial of 539 IBS-D patients, a positive response to ramosetron treatment was reported compared to patients receiving a placebo[98].

Bile acid binder: Some studies have indicated that a significant number of IBS-D patients can have mild to severe bile acid malabsorption. Several studies have shown a dose-response relationship between the severity of malabsorption and treatment with colestyramine, a bile acid binder[99].

Mesalazine: Mesalazine has intestinal anti-inflammatory properties, including cyclooxygenase and prostaglandin inhibition. A recent study showed that Mesalazine can reduce key symptoms of postinfectious IBS and noninfective IBS-D[100]. The results of an ongoing randomised trial of mesalazine in a group of IBS-D patients will be soon available[101].

Antibiotics and probiotics

Treatments aimed at altering or modifying the gut microbiota, including antibiotics and probiotics, have been the focus of a large number of recent studies on IBS patients[5,97,102,103].

Rifaximin is a semi-synthetic derivative of rifamycin with an additional benzimidazole ring that prevents its systemic absorption. A number of recent clinical trials

have evaluated the efficacy and safety of rifaximin in IBS patients (generally IBS-D). A recent systematic review and a meta-analysis[102,103] found rifaximin to be more efficacious than placebo for global IBS symptom improvement. The most common adverse events with rifaximin were headache, upper respiratory infection, diarrhoea, and abdominal pain. Serious side effects, however, were rare, and their prevalences were similar between rifaximin and placebo. Few data are available regarding other antibiotics. A subanalysis of a double-blind, randomised, placebo-controlled trial demonstrated that treatment with neomycin improved global symptoms in individuals with IBS-C compared with placebo[103].

Probiotics have demonstrated benefits for some symptoms, notably bloating and flatulence, and involve a variety of probiotic agents, including lactobacilli, bifidobacteria and streptococcus. Lactobacilli alone had no impact on symptoms, whereas probiotic combinations improved symptoms in IBS patients. Furthermore, there was a positive trend indicating that bifidobacteria improves IBS symptoms[71,85,86,96]. In a recent systematic review[104], probiotics appeared to be efficacious for IBS, but the magnitude of their benefit and the most effective species have not yet been completely established. Finally, probiotics have no serious side effects, and there is no significant difference in the observed adverse events between probiotics and placebo.

Psychological therapies

Among patients with IBS, the majority have anxiety, depression, or features of somatisation. Good patient compliance is necessary to achieve a successful clinical result after a psychotherapeutic approach or after the administration of antidepressants.

Psychotherapy: Among various psychological therapies, there is evidence for a benefit from cognitive behavioural therapy, dynamic psychotherapy, and hypnotherapy, but not from relaxation therapy[105-107]. The abnormal processing and enhanced perception of visceral stimuli in IBS can be normalised by psychological interventions. Psychotherapy is particularly successful in patients who reported a history of sexual abuse. Psychological therapies are not documented to have any serious adverse effects.

Tricyclic antidepressants: Tricyclic antidepressants (TCAs) are drugs with anticholinergic and non-selective serotonin reuptake inhibitor effects. Antidepressants could theoretically provide a benefit in IBS by both central and peripheral mechanisms[64,71,85,86,97]. Five tricyclic agents have been studied formally (amitriptyline, trimipramine, desipramine, clomipramine, and doxepin), and the effects of these agents are primarily related to pain. It has been suggested that patients with IBS-D obtain the greatest benefit from this approach[67]. The side effects of constipation, dry mouth, drowsiness, and fatigue occur in over one-third of IBS patients treated with TCAs, which often precludes good patient compliance.

Selective serotonin reuptake inhibitors, antidepressants: Physicians often prefer selective serotonin reuptake inhibitors (SSRIs) over TCAs because of their lower side-effect profiles. SSRIs, such as paroxetine and fluoxetine, can accelerate whole gut transit and are considered potentially effective in the treatment of IBS-C. A large trial[71] showed that a standard dose of an SSRI antidepressant led to a significant improvement in the health-related quality of life in patients with IBS, but no significant effects were observed in bowel habits or pain. However, in a double-blind randomised trial, fluoxetine was effective in decreasing global symptoms in the short-term therapy of a group of IBS-C patients[104].

Alternative approaches

Chinese herbal preparations have also been the subject of several trials[108]. By combining the effects of Iberis amara on smooth muscle tone with the spasmolytic effects of other plants, Iberogast, a popular combination of nine herbal plants, exerts a dual action on smooth muscle, stimulating or spasmolytic, depending on functional baseline conditions. These plant preparations have been shown to improve overall IBS scores and abdominal pain, but it is unclear which component is the active ingredient. A longer study of 16 wk with Chinese herbal preparations reported significant symptom improvement[109]. No conclusive data are available regarding any toxicity, especially regarding liver failure, of any Chinese herbal mixture.

Another popular alternative treatment concerns the use of acupuncture in IBS. A Cochrane review of six trials with a median sample size of 54 found insufficient evidence to determine whether acupuncture is an effective treatment for IBS[110]. In a recent open randomised trial, acupuncture for IBS provided an additional benefit over the usual care alone in a primary care experience[111].

Further studies are needed before any final recommendations on acupuncture or herbal therapy can be made.

CONCLUSION

Even though there is some evidence that changes in the digestive motility and secretion, visceral hypersensitivity, abnormalities of enteroendocrine and immune systems, genetic factors, infections, alterations of the intestinal microbiota and inflammation could play a role in IBS, its pathogenesis remains only partially understood. Thus, in clinical practice, its management is quite difficult. Because no biological markers are available, diagnoses can be made only on the basis of the symptoms described by the Rome Ⅲ criteria, for example. Unfortunately, many physicians do not use these criteria in their clinical practice and instead, driven by their own concerns or the concern of their patients, often prescribe many unnecessary diagnostic tests.

Furthermore, IBS therapy is far from satisfactory. The cornerstone for any effective treatment strategy should

be a solid patient-physician relationship; indeed, this relationship should be individualised for each patient. To achieve this goal, the use of combination drug therapies may be suggested. The data reviewed here indicate that there is limited evidence to support the individual efficacy of any of the agents currently available.

In conclusion, the pathogenesis, diagnosis and treatment of IBS remain subjects of much ongoing research. Further well-structured studies are needed to improve our knowledge about IBS and its management.

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Methodological issues in the study of intestinal microbiota in irritable bowel syndrome

Valentina Taverniti, Simone Guglielmetti

Valentina Taverniti, Simone Guglielmetti, Division of Food Microbiology and Bioprocessing, Department of Food, Environmental and Nutritional Sciences, Università degli Studi di Milano, 20133 Milan, Italy
Author contributions: Taverniti V and Guglielmetti S performed the literature search and drafted the manuscript.
Correspondence to: Simone Guglielmetti, PhD, Division of Food Microbiology and Bioprocessing, Department of Food, Environmental and Nutritional Sciences, Università degli Studi di Milano, Via Celoria 2, 20133 Milan, Italy. simone.guglielmetti@unimi.it
Telephone: +39-2-50319136 Fax: +39-2-50319292
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Abstract

Irritable bowel syndrome (IBS) is an intestinal functional disorder with the highest prevalence in the industrialized world. The intestinal microbiota (IM) plays a role in the pathogenesis of IBS and is not merely a consequence of this disorder. Previous research efforts have not revealed unequivocal microbiological signatures of IBS, and the experimental results are contradictory. The experimental methodologies adopted to investigate the complex intestinal ecosystem drastically impact the quality and significance of the results. Therefore, to consider the methodological aspects of the research on IM in IBS, we reviewed 29 relevant original research articles identified through a PubMed search using three combinations of keywords: "irritable bowel syndrome + microflora", "irritable bowel syndrome + microbiota" and "irritable bowel syndrome + microbiome". For each study, we reviewed the quality and significance of the scientific evidence obtained with respect to the experimental method adopted. The data obtained from each study were compared with all considered publications to identify potential inconsistencies and explain contradictory results. The analytical revision of the studies referenced in the present review has contributed to the identification of microbial groups whose relative abundance significantly alters IBS, suggesting that these microbial groups could be IM signatures for this syndrome. The identification of microbial biomarkers in the IM can be advantageous for the development of new diagnostic tools and novel therapeutic strategies for the treatment of different subtypes of IBS.

Key words: Intestinal dysfunction; Irritable bowel syndrome; Intestinal microbiota; Bifidobacteria; New generation DNA sequencing

Core tip: Irritable bowel syndrome (IBS) is the intestinal functional disorder with the highest prevalence in the industrialized world. The intestinal microbiota (IM) plays a role in its pathogenesis. Since the methodological aspects of the research on IM in IBS have never been considered in detail before, we carried out a revision of 29 original research articles. We reviewed the scientific microbiological message in light of the experimental method adopted. The analytical revision of the studies referenced in our review leaded to the identification of microbial groups whose relative abundance resulted significantly altered in IBS. Such microbial groups are potential IM signatures of IBS.

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INTRODUCTION

Irritable bowel syndrome (IBS) is a functional intestinal

disorder with the highest prevalence in the industrialized world[1,2]. Due to the absence of an evident pathogenesis, IBS is exclusively diagnosed based on the absence of mucosal, structural and biochemical diseases and the evaluation of specific symptoms according to Rome Ⅲ criteria[3,4]. The main symptoms that characterize IBS include abdominal pain and discomfort, accompanied by diarrhea (IBS-D), constipation (IBS-C), or a combination of the two (alternating IBS, IBS-A). The frequency and intensity of these symptoms largely varies, thereby affecting the quality of life of the patients[5].

The etiopathogenesis and pathophysiology of IBS are ambiguous and likely include many different factors, such as improper immune activation, visceral hypersensitivity, colon dysmotility, history of gastrointestinal infections, and psychological conditions[6-9]. In addition, many studies have also investigated a potential role for intestinal microbiota (IμB) in IBS.

Experimental observations showed that in IBS (1) toll-like receptor genes are upregulated[10]; (2) fecal levels of defensins are increased[11]; and (3) short chain fatty acid concentrations are frequently augmented[12-16]. Furthermore, it was shown that probiotics and antibiotics treatments could reduce IBS symptoms[17-19]. These data suggest that changes in the IμB are not only a consequence of IBS, but could also be a plausible causative factor. Nonetheless, current research efforts have not identified any definitive microbiological signatures of IBS and the experimental results are occasionally contradictory. The heterogeneity of the results on the role of IμB in IBS primarily reflects the high variability among various manifestations of IBS and marked differences in the IμB composition among subjects[20]. Moreover, the experimental methodologies employed and the specific protocols adopted to investigate complex ecosystems, such as the IμB, drastically impact the quality and significance of the results. To examine the methodological aspects of the research on the role of IμB in IBS, we reviewed 29 relevant original research articles obtained through a PubMed search using three combinations of keywords: "irritable bowel syndrome + microflora", "irritable bowel syndrome + microbiota" and "irritable bowel syndrome + microbiome". For each study, we reviewed the scientific evidence obtained with respect to the experimental technology adopted. The collected data from each study were compared among all considered studies to detect potential inconsistencies and explain contradictory results.

METHODOLOGIES EMPLOYED TO INVESTIGATE THE INTESTINAL MICROBIOTA IN IBS SUBJECTS

The 29 original research studies considered in the present review address the microbial community structure in the intestine of IBS subjects using several different experimental techniques. Only a few of these studies used classical (culture-based) strategies, which have extensively been replaced with molecular techniques (*i.e.*, strategies based on the analysis of nucleic acids).

The molecular methods employed in the selected studies primarily included (1) fluorescence *in situ* hybridization (FISH); (2) DNA microarrays; and (3) polymerase chain reaction (PCR)-based methods. The PCR-based methods can be further divided into three main groups: Real-time quantitative PCR (qPCR); Genetic fingerprinting [denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP)]; PCR fragment sequencing.

In the following paragraphs, the advantages and limitations of the technologies employed to correlate IμB to IBS are discussed. In addition, the experimental results obtained using each methodological strategy are presented and compared.

Culture-based methods

The classical strategies of microbial ecology, based on the cultivation of microorganisms, have been demonstrated as inappropriate for the analysis of complex microbial ecosystems, such as the intestinal environment, because the vast majority of the microorganisms (between 80% and 99%) in any environment are not cultivable using standard culturing techniques[21,22]. A few studies in the last 10 years, however, have adopted culture-dependent approaches to characterize the IμB of subjects with IBS (Table 1). For example, Mättö *et al*[23] found a moderate increase in the coliform bacteria concentration and aerobe/anaerobe ratio in fecal samples obtained from IBS patients (26 subjects: 12 IBS-D, 9 IBS-C and 5 IBS-A) compared with healthy controls (HCs, 25 subjects), whereas the bifidobacterial concentrations did not differ. More recently, Enck *et al*[24] applied culture-based analyses to examine fecal samples from more than 34000 subjects, including 7784 people with IBS. In contrast to Mättö[23], among the few bacterial groups considered, only bifidobacteria were significantly decreased in IBS samples. The differences in these results, however, are plausible, considering that Mattö used the Beerens medium[25], containing propionic acid, as a selective agent for bifidobacteria, whereas Enck *et al*[24] used DIC agar (Heipha GmbH, Germany), a commercial medium containing gentamycin and vancomycin as selective agents. Although bifidobacteria are considered resistant to these antibiotics, sensitivity has been reported for stressed cells belonging, for example, to the species *Bifidobacterium longum* (*B. longum*)[26]; therefore, the use of antibiotics as selective agents compromises the cultivation of viable bifidobacterial cells in a fecal sample. Furthermore, the bifidobacteria concentration was not significantly different in 10 IBS-D subjects compared with 10 healthy controls in another study[27] in which a medium similar to Beerens agar was used for the isolation. On the contrary, Chassard *et al*[28] detected reduced bifidobacteria concentrations in the fecal samples of 14 IBS-C women compared with 12 sex-matched HCs. However, in this study, bifidobacteria were isolated using de Man Rogosa Sharp (MRS) agar medium (pH 7.0),

Table 1 Outcomes of the selected original research studies (see text for details), which have been carried out to characterize the intestinal microbiota composition in irritable bowel syndrome

Participants	Rome criteria	Results	Sample	Technique	Ref.
27 IBS (20 F/7 M) 12 IBS-D 9 IBS-C 6 IBS-A Age: 20-65 22 HCs (15 F/7 M) Age: 25-64	II	↑ *Ruminococcus productus - Clostridium coccoides* ↓ *Lactobacillus* (IBS-D *vs* IBS-C) ↓ *Bifidobacterium* (IBS-D *vs* HC, IBD-C, IBS-A) ↓ *Desulfovibrio* (IBS-D *vs* HC, IBD-C, IBS-A) ↑ *Veillonella* (IBS-C *vs* HC)	Fecal	qPCR (SYBR Green)	[16]
26 IBS (19 F/7 M) 12 IBS-D 9 IBS-C 5 IBS-A Age: 20-65 25 HCs (18 F/7 M) Age: 23-63	II	More temporal instability in predominant bacterial population in IBS subjects Slight increase of coliforms in IBS and higher aerobe/anaerobe ratio in IBS ↑ *Clostridium* spp. ↓ *Eubacterium* spp.	Feces	DGGE Cloning and sequencing of 5 IBS and 4 HC samples (29 IBS and 16 HC clones); ABI PRISM BigDye terminator Cycle sequencing kit v. 3.0 Culture method	[23]
20 IBS (14 F/6 M) Mean age: 47.8 20 HCs (13 F/7 M) Mean age: 46.2	II	Mucosal bacteria concentration higher than 10^9 cells/mL in 65% of IBS subjects (35% in HC) Prevalence of *Eubacterium rectale-Clostridium coccoides* in IBS biofilm	Ileal and colonic biopsies	FISH	[33]
16 IBS (11 F/5 M) 7 IBS-D 6 IBS-C 3 IBS-A Age: 24-64 16 HCs (12 F/4 M) Age: 26-63	II	More temporal instability of predominant microbiota only in RNA-DGGE profiles in IBS *vs* HCs (not in DNA-DGGE) ↓ *C. coccoides - E. rectale* in IBS-C *vs* HC No differences in *Clostridium* population stability between IBS and HC	Feces	DGGE Multiplexed quantification of clostridial 16S rRNA through multiple transcript analysis with the aid of affinity capture (TRAC)	[49]
24 IBS 10 IBS-D 8 IBS-C 6 IBS-A Age: 21-65 23 HCs (16 F/7 M) Age: 26-64	II	Significant differences in microbiota composition in different IBS subcategories pooled in 3 groups on the basis of %GC (7-10-13 fractions) In fraction group 7: ↓ *Lactobacillus* in all IBS subgroups *vs* HC ↑ *Ruminococcus* in IBS-C and IBS-A ↑ *Streptococcus* in IBS-D In fraction group 13: ↓ *Collinsella* in IBS-C and IBS-D	Feces	16S rRNA gene cloning and sequencing of 3753 clones	[37]
20 IBS 8 IBS-D 8 IBS-C 4 IBS-A Age: 24-64 15 HCs Age: 25-64	II	↑ *Clostridium thermosuccinogenes* (IBS-A *vs* IBS-D) ↑ *Ruminococcus torques* 94% phylotype (IBS-D *vs* HCs and IBS-A) ↑ *Ruminococcus bromii*-like phylotype (IBS-C *vs* HCs) ↑ *Bacteroides intestinalis*-like and *C. cocleatum* (IBS-A and HCs *vs* IBS-D) ↓ *Clostridium aerofaciens*-like (IBS-D *vs* other groups)	Feces	qPCR (SYBR Green)	[46]
41 IBS (29 F/12 M) 14 IBS-D 11 IBS-C 16 IBS-A Mean age: 42 26 HCs (18 F/8 M) Mean age:32		↓ *Bifidobacterium* ↓ *B. catenulatum*	Feces Feces and duodenal brushes	FISH qPCR (Taqman)	[31]
10 IBS-D (6 F/4 M) Age average: 46.5 23 HCs Age average: 45 12 IBS-D (7 F/5 M) Age average: 46.5	II	Decreased diversity in the intestinal microbiota of IBS-D *vs* HCs ↑ Proteobacteria and Firmicutes ↑ Lachnospiraceae ↓ Actinobacteria and Bacteroidetes No significant differences in Enterobacteriaceae and *Eggerthella lenta*-type (*Atopobium*) phylotype between IBS-D and HCs	Feces	Genomic DNA fractioning on the basis of %GC (35%-40%/40%-45%/50%-55%/55%-60%/60-65/65%-70%/70%-75%); amplification of 16S rRNA gene; sequencing of 3267 clones for IBS subjects qPCR (SYBR Green)	[47]

22 HCs Age average: 45					
47 IBS (47 F) Age: 24-66	II	Significant difference in DGGE profile between IBS and HC, less microbial variation in IBS	Feces	DGGE of V1-V3 region of the 16S rRNA	[70]
33 HCs Age: 21-38		No significant intra and inter-differences in IBS subjects between luminal and mucosal microbiota. IBS impacts equally on both communities	Feces and colonic biopsies	DGGE of V6-V8 Region of the16S rRNA	
26 IBS (13 F/13 M) 8 IBS-D 11 IBS-C 7 IBS-A Age: 21.7 ± 2.0 26 HCs Age: 21.9 ± 2.9		↑ *Veillonella* ↑ *Lactobacillus* spp.	Feces	qPCR (SYBR Green) Culture method	[12]
10 IBS-D (8 F/2 M) Age: 23-50 10 HCs (6 F/4 M) Age: 21-54	III	↓ Aerobic counts in fecal samples of IBS-D No difference in mucosal samples between IBS-D and HC ↑ *Lactobacillus* spp. in fecal samples of IBS-D *vs* HC No difference in mucosal samples between IBS-D and HC	Feces samples and colonic biopsy	Culture method qPCR (SYBR Green)	[27]
11 IBS (7 F/4 M) Age: 25-64 22 HCs (17 F/5 M) Age: 21-61	II	Reduced biodiversity in IBS subjects Significant differences in profiles between IBS and HC subjects ↓ *B. vulgatus, B. ovatus, B. uniformis, Parabacteroides sp.* in IBS *vs* HC	Feces	DGGE on universal and specific primers for *Bacteroides* Sequencing of V3 region of the 16S rRNA genes	[69]
22 IBS (8 F/14 M) 1 IBS-D 13 IBS-C 8 IBS-A Age: 7-12 22 HCs (11 F/11 M)	Pediatric Rome III	No differences in total bacterial load between IBS and HCs Profile differences in IBS subtypes among each other, and between IBS and HCs In IBS: ↑ Proteobacteria ↑ γ-Proteobacteria ↑ *Haemophilus parainfluenzae* ↑ *Veillonella* ↑ *Dorea* ↓ *Eubacterium* ↓ *Anaerovorax* ↓ *Bacteroides vulgatus*	Feces	16S Metagenomics 454 Pyrosequencing (V1-V3 and V3-V5 regions of 16S rRNA) PhyloChip Microarray Hybridization on purified 27F and 1492R regions of 16S rRNA (on 28 IBS and 27 HC)	[35]
62 IBS (57 F/5 M) 25 IBS-D 19 IBS-C 19 IBS-A Age: 22-66 46 HCs (34 F/12 M) Age: 23-58	II	↑ Firmicutes/Bacteroidetes ratio ↑ *Bacillus* ↑ *Steptococcus* ↑ *Dorea* ↑ *Ruminococcus* ↑ *R. gnavus* ↑ *Blautia* ↑ *Clostridium* ↓ *Faecalibacterium* ↓ *Bacteroides* ↓ *B. vulgatus* ↓ *Prevotella* ↓ *Bifidobacterium* ↓ *B. gallicum* ↓ *B. pseudocatenulatum* ↓ *Methanobrevibacter* in IBS *vs* HC, particularly in IBS-C subgroup	Feces	HITChip phylogenetic microarray	[36]
11 IBS (5 F/6 M) 8 HCs (2 F/6 M) Age: 18-74	II	Greater biological variability of predominant bacteria among IBS subjects *vs* HC and higher microbial diversity (especially Bacteroides and lactobacilli) in IBS *vs* HC In IBS, Exclusive detection of *Eubacterium biforme* (absent in HC) ↑ *Bacteroidetes* ↑ *Lactobacillus* ↓ *Bifidobacterium* ↓ *C. coccoides*	Feces	qPCR (SYBR Green) DGGE on V3-V5 region of 16S rRNA gene qPCR (SYBR Green)	[63]
37 IBS (26 F/11 M) 13 IBS-D 13 IBS-C 13 IBS-A Age: 21.7 ± 2.0	II	No evident difference in predominant microbiota from profiles of both sample sites between IBS and HC ↑ *P. aeruginosa* in all subgroups if IBS and in both body niche samples	Duodenal brushes and feces	DGGE on V6-V8 region of 16S rRNA gene, generation of 51 clones and sequencing qPCR (Taqman)	[57]

20 HC (15 F/5 M) Age: 21.7 ± 2.0					
16 IBS-D (11 F/5 M) Age: 23-52	Ⅲ	Lower biodiversity in IBS-D *vs* HCs in fecal samples, no biodiversity differences in mucosal samples ↓ Clostridiales	Feces samples and colonic biopsy	T-RFLP	[71]
21 HCs (17 F/4 M) Age: 21-60		↓ Planctomycetaceae			[62]
81 IBS (69 F/27 M) 15 IBS-C Age: 20-73	Ⅰ and Ⅱ	*Staphylococcus aureus* detected only in IBS subjects, with higher prevalence in IBS-C Enterotoxin-encoding gene of *C. perfringens* detected only in IBS subjects	Feces	qPCR (SYBR Green)	
23 HCs (16 F/7 M) Age: 26-64		*Helicobacter pylori* detected in 3 IBS subjects, none in HCs		Sequencing of *S. aureus* amplicons	
23IBS-D (17 F/6 M) Age: 23-70	Ⅲ	Lower microbial richness in IBS-D Structural changes in IBS-D *vs* HC, from phylum to genus	Feces	454 Pyrosequencing of the V1-V3 and V6 regions of 16S rRNA gene	[54]
23 HCs (18/5 M) Age: 21-58		↑ Proteobacteria ↑ γ-proteobacteria ↑ *Enterobacteriales* ↑ *Enterobacteriaceae* ↓ *Faecalibacterium* ↓ *F. prausnitzii*		qPCR (SYBR Green)	
37 IBS (26 F/11 M) 15 IBS-D 10 IBS-C 12 IBS-A Age: 37 ± 12 20 HCs (13 F/7 M) Age: 39 ± 9	Ⅱ	Clustering by microbiota composition revealed subgroups of IBS patients: (1) a group (n = 15) with normal-like microbiota composition compared with HCs; and (2) a group (n = 22) with large microbiota-wide changes characterized by an increase of Firmicutes (mainly clostridia/Clostridiales) and a depletion of Bacteroidetes ↓ *Bacteroides* ↓ *Alistipes* ↑ *Lachnospiraceae incertae sedis* ↑ Butyrate-producing *Eubacterium halli* and *desmolans* ↑ *B. adolescentis*	Feces	Pyrosequencing of the V4 region of 16S rRNA gene	[77]
47 IBS 27 IBS-D 20 IBS-C Age average: 34.3 26 HCs Age average: 46.1	Ⅲ	Higher number of mucosa-associated bacteria in IBS ↑ *Bacteroides* ↑ *Eubacterium rectale-C. coccoides* ↓ *Bifidobacterium* in IBS-D than in IBS-C	Rectal biopsies	FISH	[32]
75 rural IBS Age: 4-18 20 Hurban HCs Age: 5-15	Ⅲ	↓ *Enterobacter* ↓ *Enterococcus* ↓ *Lactobacillus* ↓ *Bifidobacterium*	Feces	Culture-based analysis	[29]
22 IBS-D Age: 8-18 22 HCs Age: 11-18	Ⅱ	Higher variability among IBS subjects No difference between IBS-D and HCs at phylum level. No difference for *Clostridium* and *Faecalibacterium* ↑ *Veillonella* ↑ *Prevotella* ↑ *Enterobacter* ↑ *Lactobacillus* ↓ *Bifidobacterium* ↓ *Verrucomicrobium* Difference at species level in the genus *Bacteroides*: ↓ *B. fragilis* ↓ *B. thetaiotaomicron* ↑ *B. ovatus* ↑ *B. salyersiae* Positive abundance correlation between *Veillonella-Haemophilus* and *Streptococcus*; negative for *Ruminococcus* Confermation of data on Clostridia, Bacteroidetes, *Bifidobacterium* Confermation of data on *Bifidobacterium*, *Prevotella*, *Faecalibacterium*	Feces	Microbiota array Pyrosequencing (V1-V2-V3 region of 16S rRNA) FISH qPCR	[40]

14 IBS-C (14 F) 12 HCs (14 F) Age: 20-59	Ⅱ	No differences in total strict and facultative anaerobes between IBS-C and HCs No difference in hydrolytic bacterial communities ↑ Lactate utilizing sulphate-reducing bacteria (SRB) ↓ Lactate non SRB (butyrate-producing) ↑ H_2-utilizing SRB ↓ H_2-utilizing non SRB (acetogenic, methanogens) ↑ *Enterobacteriaceae* ↓ *Bifidobacterium* ↓ *Lactobacillus* ↓ *Bifidobacterium* ↓ *Roseburia-E. rectale*	Feces	Culture-based analysis FISH	[28]
19 IBS 24 HCs Age: 33.6 ± 9.1	Ⅲ	↑ Bifidobacteriaceae ↑ Lactobacillaceae ↑ *Clostridium* cluster Ⅸ ↑ *Eubacterium rectale* ↑ *Enterococcus faecium* ↑ *Clostridium difficile* ↑ *Bacillus cereus* and *B. clausii* ↑ *Campilobacter* spp. ↓ *Bacteroides/Prevotella* ↓ *Veillonella*	Feces	Microbiota Array	[42]
14 IBS-D(3 F/11 M) 18 HCs (7 F/11 M) Age: 18-65	Ⅲ	↑ *E. coli* ↓ *Clostridium leptum* ↓ *Bifidobacterium*	Feces	qPCR (SYBR Green)	[58]
16 IBS 9 HCs		Reduced microbial diversity in IBS In mucosal samples: ↑ Bacteroidaceae In fecal samples: ↑ Rikenellaceae ↑ Porphyromonadaceae ↓ Ruminococcaceae IBS-D: ↑ *Acinetobacter*, *Leuconostoc*, *Butyricimonas*, *Odoribacter* (fecal) ↓ *Desulfovibrio*, *Oribacterium* (biopsies) IBS-C: ↑ *Alistipes*, *Butyricimonas* (feces) and *Bacteroides* (biopsies) ↓ *Fusobacterium*, *Eubacterium*, *Coprococcus*, *Eubacterium*, *Haemophilus*, *Neisseria*, *Streptococcus*, *Veillonella*	Colonic biopsies and feces	Pyrosequencing (V1-V2 regions of 16S rRNA)	[48]
2 IBS-D 1 HCs Several sampling over 6-8 wk	Ⅲ	↑ *Alphaproteobacteria* ↑ Facultative anaerobe (Proteobacteria, *Streptococcus*) in days of acute diarrhea	Feces	Pyrosequencing (16S rRNA gene)	[89]

qPCR: Real time quantitative polymerase chain reaction; DGGE: Denaturing gradient gel electrophoresis; T-RFLP: Terminal restriction fragment length polymorphism; FISH: Fluorescence in situ hybridization; Ref.: Reference; IBS: Irritable bowel syndrome; IBS-D: Diarrhea-associated IBS; IBS-C: Constipation-associated IBS; IBS-A: Alternating symptoms IBS; HCs: Healthy controls. ↑: Increased presence in IBS; ↓: Reduced presence in IBS.

which is actually not a suitable selection medium for the isolation of these bacteria from feces.

In addition, Carroll *et al*[27] demonstrated a significant reduction in the concentration of aerobic bacteria in fecal samples from D-IBS patients compared with healthy controls. This result is not consistent with the results obtained by Mättö *et al*[23]. However, to determine the number of aerobes, Mättö *et al*[23] used nutrient agar, which is a particularly poor medium compared with the brain heart infusion agar, containing L-cysteine (0.05%) and hemin, adopted by Carroll *et al*[27]. Thus, the aerobic plate counts obtained from these two studies cannot be compared.

The inconsistencies in bacterial counts reflect the primary intrinsic flaw in culture-based methods: obtaining an appropriate selection medium for all members of a genus (or superior taxa).

The genus *Lactobacillus* is another microbial group often examined in microbiology. Tana *et al*[12] reported an increase in lactobacilli in fecal samples obtained from IBS patients (26 IBS subjects compared with 26 healthy controls). However, Chassard *et al*[28] reported that this same microbial group was reduced in IBS samples using MRS agar medium adjusted to pH 5.5 and incubation in aerobic

conditions, whereas Mättö *et al*[23] and Enck *et al*[24] reported that the lactobacilli concentrations were not significantly different using the same selective medium as Tana *et al*[12] (Rogosa agar) for the cultivation of these microbes. Therefore, the use of different culture media cannot explain the contradictory results concerning lactobacilli.

Moreover, culture-based analyses were used to characterize the fecal samples from 75 IBS children and adolescents living in rural areas in chernobyl compared with 20 healthy controls living in urban areas[29]. In this study, the researchers reported a lower abundance of all bacterial groups investigated, *i.e.*, the genera *Enterobacter*, *Enterococcus*, *Lactobacillus*, *Bifidobacterium*, in the IBS group. Thus, the choice of the selection medium profoundly affects the significance of the results obtained from analyses of the microbial ecology of a biological sample. The results of studies based on culture-dependent strategies suggest that changes in bifidobacteria, lactobacilli and the total aerobic count are typically associated with IBS. However, the intrinsic limitations of culture-based techniques, which do not examine a large majority of intestinal microorganisms, severely reduce the significance of these experimental data.

FISH

FISH in microbial ecology involves the detection of whole-microbial cells through the labeling of cellular rRNA using an oligonucleotide probe containing a fluorescent dye at the 5' end[30]. FISH probes, which commonly target 16S rRNA, are designed at various taxonomic levels, facilitating the *in situ* phylogenetic identification and enumeration of individual microbial cells. The FISH technique does not require PCR amplification; therefore, FISH does not have the potential problems associated with the nonspecific amplification of DNA during the PCR reaction.

Limitations: Similar to qPCR, FISH requires the preliminary selection of a target microbial taxonomic group (ribotype); therefore, only a limited number of previously known microbial groups can be analyzed. More importantly, FISH involves a labor-intensive protocol that includes intricate steps, such as the *in situ* acquisition of the target. Consequently, low signal intensity and background fluorescence are common problems.

In FISH experiments, reduced bifidobacteria concentrations have been detected in the fecal samples obtained from 41 IBS patients compared with 26 HCs[31], and in 14 IBS-C subjects compared with 12 HCs[28]. In addition, Parkes *et al*[32] showed reduced bifidobacteria concentrations in IBS-D patients compared with HCs and IBS-C patients.

FISH was also applied for the analysis of ileal and colonic biopsies, revealing a higher number of mucosa-associated bacteria in IBS patients (n = 20) compared with HCs (n = 20)[33]. This same study revealed the prevalence of *Eubacterium rectal* (*E. rectal*)-*Clostridium coccoides* (*C. coccoides*) in IBS. Similarly, higher mucosa-associated bacteria and increased numbers of *E. rectal*-*C. coccoides* were detected in rectal biopsies from IBS patients (n = 47) compared with those from HCs (n = 26)[32]. Furthermore, FISH analyses revealed increased *Bacteroides*[32] and reduced butyrate-producing bacteria, such as *Roseburia*-*E. rectal* (belonging to the family Lachnospiraceae), in IBS[28].

DNA microarrays (PhyloChip)

DNA microarray methods are based on the direct hybridization of PCR products amplified from total environmental DNA[34]. Therefore, the PCR amplicons are initially fluorescently labeled, and after hybridization, the signal intensity, which is directly proportional to the abundance of hybridization (*i.e.*, the amount of a specific sequence in the sample), is monitored through confocal laser scanning microscopy. The DNA microarrays used in microbial ecology are commonly based on the analysis of a pool of 16S rRNA gene fragments amplified through PCR from the total environmental DNA (PhyloChip). This technology facilitates the rapid high-throughput analysis of hundreds of microbial species in an environmental sample.

Limitations: Similarly to the binding of a primer to a nonspecific DNA target in PCR, cross hybridization is a major limitation of microarray technology. In addition, only those taxa included in the microarray can be analyzed; therefore, similar to qPCR and FISH, the ecological importance of a taxon that has not been previously selected could be erroneously omitted. Moreover, the results obtained solely through microarray are not considered sufficiently reliable, unless confirmation of these data is provided through other techniques, particularly qPCR.

Saulnier *et al*[35] did not detect a difference in the microbial richness between groups using high-resolution Phylochip Microarray on 28 IBS children and 27 HCs and the majority of taxa in IBS belonged to γ-Proteobacteria, particularly *Haemophylus parainfluenzae*. The results of the Phylochip Microarray analysis also showed the prevalence of the genera *Dorea* and *Veillonella* in IBS, similar to the results obtained for the same samples using 454 Pyrosequencing (see paragraph 2.4.3 for more details). Moreover, IBS children harbored lower levels of *Bacteroides*, including *B. vulgatus*. A previous study based on HITChip Phylogenetic Microarray showed reduced *Bacteroides* spp., including *B. vulgatus*, in IBS patients[36]. In this study, significant differences in the microbiota composition between 62 IBS patients and 46 HCs based on 129 phylotypes were revealed; specifically, IBS subjects presented a higher Firmicutes/Bacteroidetes ratio and increased numbers of *Bacillus*, *Streptococcus*, *Dorea*, *Blautia*, *Clostridium* and *Ruminococcus*. A significant abundance in the phylotype *Ruminococcus gnavus* (*R. gnavus*), including the species *Ruminococcus torques* (*R. torques*) (now reclassified as *Blautia torques*), was also detected. These findings suggested that *R. torques* and *R. gnavus* are potential IBS biomarkers. In addition, other phylotypes related to the genus *Ruminococcus* (*e.g.*, *R. productus*) were increased in IBS. However, IBS

patients presented reduced levels of *Faecalibacterium*, *Prevotella* and *Bifidobacterium*, with high significant differences in *B. gallicum* and *B. pseudocatenolatum*. Interestingly, the authors also showed a positive correlation with IBS symptoms, thus confirming the results of previous data[37-39].

To characterize the IμB of young IBS-D patients, Rigsbee *et al*[40] used the Microbiota Array Affymetrix, a platform containing sets of phylogenetic 16S rRNA gene probes, for the detection of 775 bacterial phylotypes in the human IμB[41]. In this study, IBS-D samples contained lower levels of the genus *Bifidobacterium* and higher levels of the genera *Veillonella*, *Prevotella* and *Lactobacillus*. Although there was no difference in the abundance of the complete *Bacteroides* genus between IBS patients and HCs, significant differences were observed for certain species, such as reduced *B. fragilis* and *B. thetaiotaomicron* and increased *B. ovatus* and *B. salyersiae*.

Similarly, Maccaferri *et al*[42] detected higher amounts of Lactobacillaceae in 19 IBS subjects compared with HCs using a fully validated high taxonomic fingerprint microbiota array. In the same study, a higher Bifidobacteriaceae concentration and a lower *Veillonella* concentration were detected in the IBS samples. Notably, the enrichment of several pathobiont bacterial species[43], such as *E. rectal*, *Enterococcus faecium*, *Campilobacter* spp. and *C. difficile*, was also reported in this study.

Culture-independent, PCR-based methods

Most culture-independent methods include PCR for the amplification of a specific DNA region from the total (metagenomic) DNA isolated from an environmental sample (*e.g.*, feces or intestinal biopsies). Although alternative genes are available, nearly all of the molecular methods used in these studies include an analysis of the gene encoding the ribosomal RNA subunit 16S (16S rRNA). The 16S rRNA gene is a conserved region of the bacterial chromosome that has been extensively used in microbial ecology research, as this gene is present in all bacterial genomes and contains both highly conserved and variable regions[22]. The highly conserved sequences, therefore, can be used as target regions for universal oligonucleotide probes (named universal primers) in the PCR amplification of the 16S rRNA gene from virtually all bacteria. Except for FISH, which is based on the direct *in situ* hybridization of an oligonucleotide probe onto rRNA targets, all the molecular methodologies reported here include the initial PCR amplification of the 16S rRNA gene using specific or universal primers. Consequently, all molecular biology protocols described herein inevitably require the extraction of nucleic acids from an environmental sample, which are subsequently used as templates for the characterization of microorganisms.

The protocol employed for DNA extraction affects the results of the downstream reactions. An efficient DNA extraction, producing high-quality genomic DNA, is essential to properly reflect the actual microbial diversity of a complex ecosystem and detect less represented microbial populations[44,45]. In the studies reviewed herein, different DNA extraction protocols have been adopted, including home-made methods[46,47] and commercial kits, such as the QIAamp DNA Stool Mini Kit (Qiagen)[48], the Fast DNAII spin kit (BIO 101)[23], the FastDNA Spin Kit (*QBIO* gene)[49], the ZR Fecal DNA Isolation kit (Zymo Research Corporation)[40], and the AccuPrep Genomic DNA Extraction Kit (Bioneer)[50]. Different kits generate diverse results in terms of DNA yield, purity and integrity, significantly affecting the microbial profiles[51] and differently impacting microbial diversity scores detected on the basis of the downstream techniques employed[52]. Understanding how an extraction protocol affects an analysis is difficult and outside of the scope of this review. However, other studies have addressed this technical issue[53,54].

qPCR: Specific oligonucleotides for the quantification of particular taxa *via* PCR (qPCR method) have been extensively used to overcome the problems of microbial cultivation.

The qPCR technique has clear advantages, such as the high sensitivity (*i.e.*, also limited concentrations of bacteria can be detected). Furthermore, qPCR facilitates the analysis of a large number of samples in a short time. Another important feature of qPCR is the design of primers that potentially target genes at any taxonomic level; thus, the identification of unique genetic signatures also facilitates quantification at the strain level, which is important when analyzing particular microbial behaviors, such as the fate of a probiotic strain in the gastrointestinal tract[50,55].

Limitations: However, the specificity of primers, particularly those targeting conserved ubiquitous genes, such as 16S rRNA, significantly varies depending on the experimental conditions of the assay. In other words, the protocol for a pair of primers targeting a specific group of microorganisms could lose specificity when using a different thermocycler[56] because even small changes in the reaction conditions could lead to the amplification of the genes from related taxa. Specificity problems can be drastically reduced using TaqMan fluorophore-quencher probes. However, with only two exceptions[31,57], the studies considered in this review exclusively used intercalating fluorescent dyes, such as SYBR Green, to measure the accumulation of amplicons in real time during each PCR cycle for the analysis of the IμB in IBS. The main limitation of qPCR is that this technique can only analyze one microbial group per reaction. Furthermore, the microbial groups are selected in advance, thereby limiting the potential identification of microbial groups that were not initially considered but might play an important role.

We selected 13 manuscripts published in the last 10 years that employed qPCR to characterize the IμB associated with IBS. Malinen *et al*[16] considered 20 different microbial groups ranging from the species and genus levels to supra-generic groups. This study showed several significant differences among IBS and HCs. Particularly, these authors showed a higher concentration of *Ruminococcus productus*/*C. coccoides* in IBS patients (n = 27) than in

the controls (n = 26). Several other differences were exclusively observed for diarrhea-predominant IBS patients (IBS-D, n = 12), including a reduced concentration of *Lactobacillus* spp., compared with IBS-C subjects (n = 9), and diminished *Bifidobacterium* spp. and *Desulfuvibrio* ssp., compared with controls and IBS-A subjects. Moreover, the Clostridiales genus *Veillonella* was more abundantly represented in IBS-C patients than in controls[16]. The qPCR analysis also showed a significant increase in the *Veillonella* spp. concentration in 26 young IBS patients (Age: 21.7 ± 2.0; 8 IBS-D, 11 IBS-C, 7 IBS-A) compared with age-matched HCs (n = 26)[12]. A significant decrease of bifidobacteria in diarrhea-predominant IBS patients was also observed in other studies using qPCR (22 IBS-D *vs* 22 HCs[40]; 14 IBS-D *vs* 18 HCs[58]). In another study, qPCR with Taqman technology was used to detect differences in the abundance of four different *Bifidobacterium* species in adult IBS patients (n = 19) and age-matched HCs (n = 19)[31]. These analyses revealed a significant reduction in the abundance of *B. catenulatum* in fecal specimens and duodenal mucosa brush samples obtained from IBS subjects. Although differences among the bifidobacterial species have been shown[59,60], the study of Kerckhoffs *et al*[57] is one of the very few that investigated bifidobacteria at intra-genus level in IBS (another example is[36]). Bifidobacteria are frequently analyzed in qPCR experiments, as these microbes are univocally recognized as health-promoting bacteria[61]. Thus, the available data obtained from bifidobacterial research, and reported herein, support the idea that a reduction of bifidobacteria is associated with IBS.

Interestingly, based on a previous study[37], Lyra *et al*[46] used qPCR to quantify 14 phylotypes in the fecal samples obtained from 20 IBS patients (8 IBS-D, 8 IBS-C, 4 IBS-A) and 15 healthy controls. Specifically, the abundance of several phylotypes, including the Clostridiales genera *Clostridium* and *Ruminococcus*, significantly differed among these subjects (Table 1). Moreover, in this study, the authors proposed *C. thermosuccinogenes* and *R. torques*-like phylotypes as potential biomarkers for IBS[38].

Rinttilä *et al*[62] used qPCR on samples obtained from IBS subjects (81 patients) to detect the presence of pathogens, such as *S. aureus* (with higher prevalence in IBS-C), *C. perfringens* and *H. pylori*, which were not identified in any of the control subjects (23 HCs).

Lactobacilli have often been included in qPCR analyses for the characterization of the IμB associated with IBS. In contrast to data concerning bifidobacteria, studies concerning lactobacilli have generated less convincing results, as previously shown for the culture-dependent studies described above. Malinen *et al*[16] reported reduced concentrations of *Lactobacillus* spp. in IBS-D patients (n = 12) compared with IBS-C patients (n = 9) but no differences were observed when compared with HCs (n = 22). In contrast, more recent studies have shown that lactobacilli were increased in the fecal samples of IBS-D patients (n = 10[27]) and IBS (n = 11[63]) compared with HCs (n = 10 and 8, respectively). Notably, in these studies, the same qPCR chemistry (SYBR Green) and primers[64] were used for the quantification of lactobacilli. Therefore, the observed differences might more accurately reflect actual differences in microbiota composition rather than methodological biases. The limited number of recruited subjects should also be considered to analyze these results.

Most studies have exclusively considered microbial groups belonging to the Bacteria superkingdom (also called "Eubacteria"). Experiments based on qPCR, however, have also revealed potential differences in the IμB associated with IBS in Archeabacteria. For instance, the reduced abundance of the genus *Methanobrevibacter* was reported in IBS subjects[36], particularly the IBS-C subgroup, consistent with the results of a previous study[65].

DGGE/T-RFLP: DGGE and T-RFLP are molecular techniques that produce an electrophoretic profile of microbial communities. Specifically, in DGGE, PCR products are obtained from environmental DNA using primers for a specific molecular marker (most commonly the 16S rRNA gene) and subsequently electrophoresed on a polyacrylamide gel under denaturing conditions using a chemical denaturant (*e.g.*, urea and formamide[66,67]).

In T-RFLP, the DNA fragments are obtained through PCR using a fluorescently labeled primer, followed by digestion with one or more restriction enzymes, and separated on an automated DNA sequencer[68] that only detects terminal fluorescently labeled restriction fragments, thereby simplifying the banding pattern and facilitating the analysis of complex microbial communities.

DGGE and TGGE are rapid and inexpensive techniques. These methods facilitate the simultaneous analysis and comparison of multiple samples. Different from qPCR, DGGE and TGGE facilitate the examination of different microbial groups in the same analysis.

Limitations: DGGE and T-RFLP are based on the PCR amplification of a specific genetic target; therefore, these methods have the same limitations concerning primer specificity as described for qPCR. Furthermore, DGGE does not provide direct taxonomic identification and involves the separation of DNA bands (excision from electrophoretic gel), cloning and sequencing. The separation of all DNA amplicons, however, is practically impossible because the PCR amplification of a target gene, such like the 16S rRNA gene from DNA isolated from an environmental sample, such as human feces, generates numerous DNA fragments. Consequently, only the most represented amplicons can be visualized in electrophoresis, and several DNA fragments might have similar melting points. Finally, the abundance of a specific microbial group can be exclusively estimated on the basis of the band intensity in electrophoresis. Thus, only those microbial groups represented with dominant bands in electrophoresis and showing markedly different abundance between the two conditions investigated can be identified as significant in DGGE. In T-RFLP, the separation of DNA amplicons through the amplification of the 16S rRNA gene is facilitated using an automated DNA sequencer; however, no more than approximately

100 fragments can be resolved per analysis, and more importantly, the taxonomic identification and quantification of the detected ribotypes can be deeply distorted by the fact that different bacterial species can share the same terminal restriction fragment length.

Concerning the characterization of the IμB associated with IBS using DGGE, an increase in *Clostridium* spp. and *Eubacterium* spp. and a decrease *Parabacteroides* spp. and several *Bacteroides* species in IBS samples was reported[69]. Furthermore, Kerckhoffs *et al*[57] showed the augmented presence of *Pseudomonas* spp. in duodenal mucosal brush and fecal samples from 37 IBS patients compared to 20 healthy subjects. Subsequent qPCR experiments confirmed the increased abundance of *Pseudomonas aeruginosa* in the same samples. In addition, DGGE technique displayed reduced biodiversity in IBS subjects, consistent with the results obtained by Noor *et al*[69]. In contrast, a Korean study showed that IBS subjects (n = 11) had a significantly higher diversity of total bacteria than HCs (n = 8)[64]. Maukonen *et al*[49] and Kerckhoffs *et al*[57] detected no significant differences in the microbiota variability between IBS patients and HCs. However, Codling *et al*[70] showed higher variability in HC subjects compared with IBS patients. The results of the DGGE analysis concerning microbial biodiversity in IBS are contradictory. In these studies, however, the general biodiversity was calculated according to the numbers and relative intensities of the bands detected among individual samples. Thus, this analysis has intrinsic technical limitations. Indeed, many taxa could be present at low levels and could be therefore only marginally amplified, generating bands that cannot be easily visualized on the electrophoretic gel. Therefore, DDGE profiles are not adequate for the determination of the biodiversity of a complex microbial ecosystem. Thus, the use of primers for the amplification of a specific group of bacteria (*e.g.*, genus-specific primers), generating a reduced number of taxa, could improve the significance of the evaluation of microbial diversity using DGGE. Indeed, Ponnusamy *et al*[63] used group-specific and detected the increased diversity of Bacteroidetes and lactobacilli and the decreased diversity of bifidobacteria and *C. coccoides* in IBS samples.

T-RFLP fingerprinting of the bacterial 16S rRNA gene was used to analyze the microbiota in fecal and mucosal samples from 16 IBS-D patients and 21 HCs, revealing lower biodiversity and the reduced abundance of Gram-positive Clostridiales and Gram-negative Planctomycetaceae in the IBS-D fecal samples[71]. These data are partially inconsistent with the results of the studies cited above, which showed an increase in certain taxa belonging to Clostridiales in IBS using qPCR. This inconsistency might reflect the fact that T-RFLP potentially included all taxa belonging to the Clostridiales, whereas qPCR analyses only quantified selected genera. Furthermore, the intrinsic limitations of T-RFLP fingerprinting distort the results.

16S rRNA gene library (clone library method): The preparation of a clone library containing microbial DNA fragments derived from an environmental sample is the "gold standard" for microbial community analyses. The most widely used methods include the PCR amplification of the 16S rRNA genes from an environmental sample, followed by cloning and sequencing of the individual DNA fragments[72]. The obtained sequences are subsequently compared with known sequences database, such as GenBank or the Ribosomal Database Project. For the data analysis, each clone sequence is assigned to a taxonomic lineage according to sequence similarity cut-off values (*e.g.*, cut-off values of 80%, 85%, 90%, 92%, 94%, and 97% for phylum, class, order, family, subfamily, and species, respectively)[72].

16S rRNA clone libraries facilitate the initial survey of the microbial diversity in an environmental sample, and differently from the methodologies described above, these libraries contribute to the identification of novel taxa.

Limitations: Environments characterized by complex microbial ecosystems, such as soil or feces, might require more than 40000 clones to document 50% of the richness[73]. However, until recently, 16S rRNA clone libraries rarely contained numbers of sequences of this magnitude. Therefore, these studies only revealed a small portion of the microbial biodiversity present in an environmental sample. This problem directly reflects the fact that the clone library method was, until recently, a time-consuming, labor-intensive and particularly expensive microbial ecology strategy.

Consistent with the limitations described above, the quality of the first studies employing clone libraries to characterize the IμB in IBS was drastically affected by the limited number of sequenced clones. Indeed, Mättö *et al*[23] sequenced the partial 16S rRNA gene from only 45 amplicons (29 amplicons from 5 IBS patients and 16 amplicons from 4 HCs), revealing the increased prevalence of *Clostridium* spp. and reduced prevalence of *Eubacterium* in IBS patients. Kerckhoffs *et al*[57] also evaluated a limited number of clones (n = 51) and did not detect significant differences between in the microbiota composition of both duodenal biopsies and fecal samples from IBS patients and HCs, except for an increase of *Pseudomonas aeruginosa* in IBS.

Kassinen *et al*[37] made an important contribution to the field of microbial ecology in IBS through 16S rRNA cloning and sequencing using a conventional sequencer (ABI PRISM® BigDye™ Terminator Cycle Sequencing, Applied Biosystems), generating 3753 sequences from the analysis of the fecal samples obtained from 24 IBS patients (10 IBS-D, 8 IBS-and 6 IBS-A patients) and 23 HCs. This study overcame the intrinsic problem inherent in most experimental approaches using PCR with universal primers, such as the 16S rRNA amplification, for the preparation of a clone library. Indeed, biases in favor targets with low guanine and cytosine (%GC) contents are observed in PCR amplification from a pool of 16 rRNA gene targets containing different se-

quences[74]. Therefore, the numbers of bacteria characterized by higher %GC in the 16S rRNA gene, such as bifidobacteria, might be underestimated. To overcome this problem, Kassinen *et al*[37] used cesium chloride gradient centrifugation to separate the genomic DNA from IBS and HC samples into three fractions based on %GC: fraction 7 (with a %GC between 25% and 30%), fraction 10 (%GC: 40%-45%), and fraction 13 (%GC: 55%-60%). Using this strategy, significant differences in the microbiota composition were detected among different IBS subcategories. In fraction 7, the members of the genus *Lactobacillus* were reduced in all IBS subgroups, whereas the *Ruminococcus* was higher in IBS-C and IBS-A patients, and *Streptococcus* was higher in IBS-D patients. Furthermore, in fraction 13, the high %GC bacterium *Collinsella*, phylum Actinobacteria (similar to bifidobacteria), was less abundant in IBS-C and IBS-D patients. This research group used a similar strategy to separate the genomic DNA obtained from 10 IBS-D subjects into 7 fractions based on %GC[47]. The sequences of 3267 clones were subsequently compared with an analogous HC library of 23 subjects, revealing an increase in Proteobacteria and *Firmicutes* (in particular, the family *Lachnospiraceae*) and a decrease in *Actinobacteria* and *Bacteroidetes* in IBS-D patients; decreased diversity in IBS-D was also observed.

Despite these efforts, studies based on the use of the clone library method have not completely overcome the problem of the limited bacterial diversity observed in intestinal samples, as only a limited number of clone sequences are observed. Thus, next-generation DNA sequencing technologies, such as the pyrosequencing, have made significant advancements.

Pyrosequencing: Pyrosequencing is a sequencing strategy based on the production of light from luciferase for the detection of individual nucleotides added to the nascent DNA; the resulting data are subsequently used to generate sequence read-outs. The rapid technological development of this strategy facilitates massive parallel high-throughput sequencing, which is applied to microbial ecology to sequence the hypervariable regions of 16S rRNA genes in large numbers. The use of pyrosequencing technology generates at least 100 times higher coverage of microbial diversity in a sample compared with typical Sanger sequencing. With this technology, the sequences of the hypervariable regions are generally short (100-350 bases) but provide sufficient phylogenetic information to determine the taxonomic level of genus.

In recent years, 454 Pyrosequencing has been used to study the microbial ecology of IBS. Carroll *et al*[75] used this technology to characterize the fecal DNA isolated from 23 IBS-D patients and 23 HCs. To this aim, the variable regions V1-V3 (an average of 8232 reads per sample) and V6 (an average of 6591 reads per sample) of the 16S rRNA gene were sequenced, revealing less microbial richness and a higher presence of the phylum Proteobacteria (particularly the class γ-Proteobacteria and the family Enterobacteriaceae) in the IBS-D population. Furthermore, the genus *Faecalibacterium* was less abundant in IBS-D samples, consistent with a significant reduction of the anti-inflammatory species *Faecalibacterium prausnitzii*[76], determined through qPCR. Saulnier *et al*[35] obtained analogous results concerning increased γ-Proteobacteria[35]. In this study, the 16S rRNA gene fragments from the fecal samples of 22 pediatric IBS patients and 22 HCs were sequenced through pyrosequencing, generating an average of 54287 reads per sample. The data analysis showed an abundance of γ-Proteobacteria and particularly, the species *Haemophilus parainfluenzae*. In addition, the Firmicutes genera *Dorea* and *Veillonella* were significantly represented in IBS patients. Similarly, Rigsbee *et al*[40] showed that the genus *Veillonella* was increased in pediatric IBS-D patients.

Moreover, Durbán *et al*[48] used pyrosequencing to study the microbiota population in feces and colon mucosa samples obtained from 16 IBS patients and 9 HCs. In this study, DNA was extracted from three types of samples per subject: biopsies of the ascending and the descending colon mucosa, and feces. Prior to pyrosequencing, the 16S rRNA genes were amplified from the extracted DNA, and equal amounts of the PCR products from different samples were pooled. The analysis of approximately 268000 reads showed reduced microbial diversity in the IBS samples and significant differences in the representation of several microbial taxa between IBS patients and HCs. Particularly, the families *Rikenellaceae* and *Porphyromonadaceae* were increased and *Ruminococcaceae* spp. were decreased in the fecal samples of IBS subjects. Furthermore, the family *Bacteroidaceae* was more abundant in mucosal samples. Several other taxa were diversely represented in IBS-D and IBS-C samples compared with HCs. This study, therefore, indicated several potential microbial signatures for IBS and IBS subtypes. However, these results were based on a limited number of sequence reads per subject (approximately 3500).

CONCLUSION

Intestinal microbiota plays a role in the pathogenesis of IBS and is not merely a consequence of the disorder[77]. A number of factors profoundly influence the identification of specific microbial modifications etiologically associated with IBS: The etiology of this disorder is heterogeneous and might profoundly vary among individuals. There is great variability among different subgroups of IBS (diarrhea, constipation-predominant and alternating IBS). The technologies adopted to characterize the IμB have intrinsic pitfalls associated with particular biases.

Despite these limitations, the analytical revision of the studies referenced in the present review resulted in the identification of microbial groups whose relative abundance, consistent with different studies using diverse methodological approaches, significantly altered IBS. These results suggest that the following microbial groups are potential IμB signatures of IBS, as briefly summarized below.

Bifidobacterium

Lower levels of members of the genus *Bifidobacterium*

have predominantly been identified in studies on IμB in IBS. Indeed, almost all of the studies analyzed in the present review (with only one exception[42]) suggest that bifidobacteria are underrepresented in IBS, particularly in the diarrhea-predominant type. Interestingly, most probiotic preparations shown as effective in managing IBS symptoms contain bifidobacteria (particularly, the species *B. animalis* subsp. *lactis*, *B. bifidum*, *B. breve* and *B. longum* subsp. *B. infantis*)[17,78], suggesting a preventing role for these microorganisms in IBS.

A mechanism underlying the beneficial role of bifidobacteria in IBS might depend on the presence of serine protease inhibitors (SERPINs) in these bacteria[79]. Indeed, supernatants obtained from IBS biopsy samples have high levels of these proteases (derived from the host or potentially produced by certain members of the phylum Firmicutes[80]). Such proteases have been implicated in the observed over-stimulation of sub-mucosal neurons in IBS subjects[81]. Therefore, the SERPINs from bifidobacteria might act on extra-cellular proteases to suppress the activity of these enzymes.

Veillonella

Different studies have shown an increase in the Firmicutes genus *Veillonella* in IBS patients[16,12,35,40] using different techniques (qPCR, Pyrosequencing and Microbiota Array Hybridization). Particularly, Tana *et al*[12] showed higher levels of *Veillonella* in IBS-C patients and demonstrated a correlation with severity of pain and increased levels of acetate and propionate in the feces of subjects. Interestingly, it has been demonstrated that *Veillonella* is abundant in jejunal samples of IBS patients and this bacteria might be involved in small-intestine bacterial overgrowth (SIBO)[82]. SIBO is defined as a malabsorption syndrome resulting from the presence of abnormal bacterial load in the small intestine (greater than 10^5 CFU per mL of intestinal aspirate and/or colonic-type species). Several studies have reported the prevalence of SIBO in IBS patients, although conflicting data have also been reported[83-85].

Furthermore, Rigsbee *et al*[40] showed a positive correlation among *Veillonella*, *Haemophilus* and *Streptococcus*, suggesting that *Veillonella* forms co-aggregation complexes with other bacteria present in the small intestine, such as *Streptococcus* and *Haemophilus*[12,86,87]. Higher proportions of *Haemophilus* and *Veillonella* have also been observed in microbiomes associated with esophagitis[88]. Thus, these data suggest that *Veillonella* might play a role in the onset of gastro-intestinal disorders, such as IBS.

γ-Proteobacteria

The studies described in the present review have presented non-controversial data concerning the increased prevalence of members the phylum Proteobacteria in IBS subjects[35,47,54,89]. Some studies have reported a significant increase in the abundance of the class γ-Proteobacteria in IBS[35]. Notably, *Haemophilus* was most represented among γ-Proteobacteria, and *Haemophilus parainfluenzae* was the predominant species.

The class γ-Proteobacteria comprises several families that include pathogenic bacteria (*e.g.*, Enterobacteriaceae, Legionellaceae, Aeromonadaceae, Vibrionaceae). Particularly, Enterobacteriaceae were increased in IBS[54]. Thus, it is likely that these bacteria are among those (potential) pathogens (also known as pathobionts) that contribute to the onset and maintenance of IBS.

Clostridiales/Blautia

Clostridiales is a wide and heterogenic Firmicutes order that includes several bacterial groups differently represented in IBS. Clostridiales also include the family Lachnospiraceae, a group of microorganisms that normally occur in the gut of humans and animals. This family comprises the genus *Blautia*, which comprises several misclassified species belonging to the *Clostridium* cluster XIVa, including *C. coccoides* and several *Ruminococcus* species related to *R. gnavus* (that also include *R. torques*)[90]. In several studies described herein, the increased presence of these bacteria has been demonstrated in IBS patients[16,32,33,36,46].

Clostridia abundantly colonize mucin[91], and it was proposed that an increase in these bacteria might reflect the increased production of rectal mucus in both IBS-C and IBS-D patients[92]. Particularly, *Clostridium* cluster XIVa has previously been associated with IBS[93]. More specifically, Jeffery *et al*[77] showed that the butyrate-producing clostridia of cluster XIVa are associated with IBS. Butyrate has been shown to cause visceral hypersensitivity[94]; thus, it is likely that an increase in butyrate-producing bacteria might promote sensory dysfunctions typical of IBS[77].

Faecalibacterium

Reduced levels of *Faecalibacterium* spp. has been shown in two studies reported in this review. Rajilić-Stojanović *et al*[36] showed that *Faecalibacterium* was the only microbial group within the phylum Firmicutes that was significantly underrepresented in both IBS-C and IBS-A subjects. Interestingly, *Faecalibacterium prausnitzii* possess anti-inflammatory properties[77], suggesting that the presence of this bacterium might modulate inflammatory conditions associated with IBS.

The available experimental data indicate modifications in the IBS IμB composition at the phylum level. Specifically, a general increase in *Firmicutes* and Proteobacteria with a concomitant reduction of *Bacteroidetes* and *Actinobacteria* has been associated with IBS.

Concluding remarks

The progress in DNA sequencing technologies offers promise to microbial ecology studies, facilitating the adequate detection and quantification of less represented microorganisms within the large microbial biodiversity in the intestinal ecosystem. Thus, sufficient research studies for the investigation of the IμB should include the following basic elements: New generation DNA sequencing technologies, such as 454 Pyrosequencing and Ion Tor-

rent[95], to obtain a high number of reads to satisfy the biodiversity requirements specified through rarefaction curves. Confirmation of the results using other methods, preferentially qPCR. An investigation of the microbiota components other than eubacteria, such as archaebacteria, fungi, yeasts and viruses.

The identification of microbial biomarkers in the IμB will contribute to the development of new diagnostic tools and novel therapeutic strategies for the treatment of different subtypes of IBS.

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Irritable bowel syndrome and food interaction

Rosario Cuomo, Paolo Andreozzi, Francesco Paolo Zito, Valentina Passananti, Giovanni De Carlo, Giovanni Sarnelli

Rosario Cuomo, Paolo Andreozzi, Francesco Paolo Zito, Valentina Passananti, Giovanni De Carlo, Giovanni Sarnelli, Department Of Clinical Medicine and Surgery, Federico Ⅱ University Hospital, School of Medicine, 80131 Naples, Italy
Author contributions: Cuomo R and Sarnelli G ideated the project; Cuomo R wrote the first draft of the manuscript; Andreozzi P, Zito FP, De Carlo G and Passananti V critically reviewed the manuscript; all authors approved the final version of the manuscript.
Correspondence to: Rosario Cuomo, MD, Associate Professor of Gastroenterology, Department of Clinical Medicine and Surgery, Federico Ⅱ University Hospital, School of Medicine, Via S. Pansini 5, Building 6, 80131 Naples, Italy. rcuomo@unina.it
Telephone: +39-81-7463892 Fax: +39-339-7221830
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Abstract

Irritable bowel syndrome (IBS) is one of the most common gastrointestinal disorders in Western countries. Despite the high prevalence of this disorders, the therapeutic management of these patients is often unsatisfactory. A number of factors have been suggested to be involved in the pathogenesis of IBS, including impaired motility and sensitivity, increased permeability, changes in the gut microbiome and alterations in the brain-gut axis. Also food seems to play a critical role: the most of IBS patients report the onset or the exacerbation of their symptoms after the meals. Recently, an increasing attention has been paid to the role of food in IBS. In this review we summarize the most recent evidences about the role of diet on IBS symptoms. A diet restricted in fermentable, poorly absorbed carbohydrates and sugar alcohols has beneficial effects on IBS symptoms. More studies are needed to improve our knowledge about the relationship between food and IBS. However, in the foreseeable future, dietary strategies will represent one of the key tools in the therapeutic management of patients with IBS.

Key words: Irritable bowel syndrome; Fermentable, poorly absorbed carbohydrates and sugar alcohols; Gut microbiota; Food intolerance; Gluten

Core tip: The most of irritable bowel syndrome patients reported food as a trigger of gastrointestinal symptoms and self-referred intolerance to certain food items. However, it is difficult identify which items are involved in symptoms triggering because food is a complex milieu of several chemicals, almost all potentially able to induce symptoms *via* several ways. It has been proposed three pathogenic mechanisms by which food items might induce symptoms: *via* immune activation (food hypersensitivity), *via* direct action of bioactive molecules (food chemicals) and *via* luminal distension.

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INTRODUCTION

Irritable bowel syndrome (IBS) is a functional gastrointestinal (GI) disorder characterized by abdominal pain or discomfort associated with abnormal bowel habit. Since the absence of reliable biomarkers, Rome Ⅲ diagnostic criteria define IBS as recurrent abdominal pain or discomfort for at least 3 d per month in the past 3 mo, associated with 2 or more of the following: improvement with defecation, onset associated with a change in the frequency of stool or onset associated with a change in the form (appearance) of stool. Based on stool form, IBS is

classified in IBS with constipation (IBS-C), IBS with diarrhea (IBS-D), mixed IBS (IBS-M) and unsubtyped IBS (IBS-U)[1]. Prevalence of IBS in the industrialized world is approximately 10%-15%, which makes IBS one of the most common GI disorders[2]. The pathogenesis of IBS is not completely understood, but several factors seem to play a role in the pathogenesis of IBS, including dysregulation of the brain-gut axis with impaired gut motility and sensibility, psyco-social factors, genetic factors, impaired gut barrier function and changes in the gut microbiome[3].

Food plays a key role in IBS: more than 60% of patients with IBS report the onset or worsening of symptoms after meals, within 15 min in 28% and within 3 h in 93% of these patients[4]. The most of IBS patients (84%) reported meal-related symptoms to at least one food item. In addition, self-reported food intolerance is associated with higher symptoms severity score and reduced quality of life[5-7]. In line with this, patients try to identify and remove the food items they do not tolerate: a cross-sectional study showed that 62% of IBS patients limited or excluded food items from the diet[8].

The role of food as trigger of GI symptoms in functional disorders is well-known, while it is much more difficult to pinpoint what food groups or items are involved in symptoms onset or worsening in IBS. For this reason, dietary recommendations for functional gastrointestinal disorders (FGIDs) are limited and largely based more upon empiricism or pathophysiology knowledge rather than randomized clinical trials or guideline consensus. The lack of a specific nutritional training and scientific evidences explains the skepticism of most primary care practitioners and gastroenterologists about dietary advices, that often are limited to change fiber intake or to reduce lipids consumption.

In the last years, the potential role of food in the management of IBS has been revisited[9]. Searching PubMed (MeDLINe) database using the terms "food" and "irritable bowel syndrome", we have found that the number of published papers increased from 7 in 1997 to 108 in 2011. This renewed interest has led to new advances in the pathophysiology and management of IBS, but also to new sources of confusion. For example, increasing attention has been paid to the role of wheat in GI symptoms. Recent studies have supported the existence of a subgroup of IBS patients with undiagnosed non-celiac gluten sensitivity, defined as a condition of morphological, immunological, or functional disorder that responds to gluten exclusion[10]. However, the existence of a objective clinical entity is controversial and recent evidences seem to reappraise the role of gluten in GI symptoms in these patients, focusing the attention on fermentable, poorly absorbed, short-chain carbohydrates present in wheat[11].

In this paper, we performed a literature review about the putative pathogenic mechanisms of food in IBS as well as the recent evidences supporting the role of food as a means of therapeutic strategies in the management of IBS. Since a great number of papers have been published in the last years, we focused mainly on high-quality works.

PUTATIVE MECHANISMS

It should be remembered that food is a complex milieu of nutrients. On the other side, the ingestion of food activates a complex response of GI tract that allows the transfer of nutrients from the intestinal lumen to the systemic circulation through the processes of digestion, absorption and expulsion of needless elements. The great complexity of food composition and GI physiology explain why it is difficult to identify single food items involved in IBS symptoms triggering or worsening.

Several mechanisms have been proposed to explain how food triggers GI symptoms in IBS. Gibson propose at least three pathogenic mechanisms by which food items might induce GI symptoms in functional bowel disorders: *via* immune activation (food hypersensitivity), *via* direct action of bioactive molecules (food chemicals), and *via* luminal distension[12].

A long-standing debate is whether or not immunological mechanisms are involved in the pathogenesis of IBS. In the last decades, it has been suggested that increased epithelial barrier permeability leads to immune activation and low-grade inflammation, that could play a crucial role in the pathogenesis of IBS[13]. Since the gut is the gatekeeper that controls nutrients access, it is not difficult to imagine that food antigens in definite conditions could trigger low-grade inflammation that would change the motor and sensory function of the gut in a group of susceptible individuals[14].

Adverse food reactions may play an important role in GI symptoms triggering, as many patients report an exacerbation of symptoms after food ingestion[15]. There is no international consensus for the terms of "food intolerance". This expression should be referred to non-immunological non-toxic adversion to food[16]. Chemicals with potential bioactivity such as salicylates, amines and glutamates are natural, pharmacologically active substances that are believed to cause adverse reactions, such as anaphylactoid reactions, urticaria, and asthma in susceptible individuals by a non-immune direct effect on mast cells to produce cysteinyl leukotrienes. However, bioactive chemicals would be able to trigger GI symptoms including nausea, vomiting, abdominal pain, bloating or diarrhea[17] and a line of evidences support the role of these molecules in IBS[15]. Although several mechanisms have been proposed to explain the pathogenesis of these symptoms, it has been hypothesized that chronic exposure to food chemicals may induce visceral hypersensitivity to luminal stimuli through the activation and overexpression of TRP channels on enteric nervous system neurons. In addition, some evidences in murine model suggest that salicylate intolerance may involve mast cells production of cysteynil leukotriens, which promote smooth muscle contraction and increase vascular permeability[18]. Salicylates, glutamates and amines have been the principal targets of elimination diet treating groups, with

Table 1 Estimated food allergy rates in North America[21]

Prevalence	Infant/child	Adult
Milk	2.5%	0.3%
Egg	1.5%	0.2%
Peanut	1.0%	0.6%
Tree nuts	0.5%	0.6%
Fish	0.1%	0.4%
Shellfish	0.1%	2.0%
Wheat, soy	0.4%	0.3%
Sesame	0.1%	0.1%
Overall	5.0%	3%-4%

Table 2 Pathophysiologic classification of allergic reactions to food[19]

Immunopathology	Disorder
IgE dependent	Urticaria and atopic eczema
	Rhinitis or asthma
	Oral allergy syndrome (angioedema of lips and tongue)
	Nausea, abdominal pain, diarrhea or constipation
Non IgE dependent	Atopic dermatitis
	Celiac disease
	Eosinophilic esophagitis, gastritis, gastroenteritis, enterocolitis and proctitis

contrasting results[15].

Luminal distension is another mechanism by which food induces GI symptoms. It is well-known the presence of visceral hypersensitivity in the majority of patients with IBS, resulting in a lower painful threshold of gut wall stretching. The presence of certain nutrients in food, in particular short chain carbohydrates, could induce or worsen GI symptoms in IBS patients *via* two main actions. First, these small molecules are osmotically active and increase luminal water volume in distal ileum and colon. Secondly, short chain carbohydrates are substrates for colonic bacterial fermentation, resulting in the production of gas. The increase of intra-luminal water and gas volume leads to luminal distension that induces GI symptoms in subjects with lower pain threshold or impaired motility pattern such as patients with functional GI disorders[19].

FOOD HYPERSENSITIVITY

The term food allergy is used to describe an adverse immune response to food. Food allergy can be classified on the basis of immunopathologic mechanisms in IgE-mediated (considered type I hypersensitivity) and non-IgE-mediated reactions (including type Ⅲ and Ⅳ hypersensitivity)[20]. In the Table 1 are reported estimated rates of food allergies in North America[21].

Classic IgE-mediated food allergies are classified as type- I immediate hypersensitivity reaction. These allergic reactions have an acute onset (from seconds to one hour) and may have extremely heterogeneous clinical manifestations[20]. Although it is relatively easy to recognize the allergic manifestations of skin, such as urticaria and atopic eczema, and respiratory tract, such as rhinitis or asthma, the GI tract can be affected by food allergies in several ways: oral allergy syndrome (angioedema of lips and tongue), nausea, abdominal pain, diarrhea or constipation. Rarely, food allergy manifested as signs and symptoms that can occur in IBS (diarrhea associated to abdominal pain)[22]. The spectrum of food allergies also includes delayed-onset diseases, that can be mediated by intestinal mucosal mechanisms involving not only IgE but also T cells, mast cells and eosinophils that produce proinflammatory mediators. Belong of this kind of disease: atopic dermatitis, celiac disease or eosinophilic GI diseases, such as esophagitis, gastritis, gastroenteritis, enterocolitis and proctitis (Table 2).

The increased prevalence of atopic conditions in patients with diarrhea-predominant IBS[23] and the positive response to oral sodium cromoglycate treatmentin these patients[24]. suggest that food hypersensitivity could play a role in pathogenesis of IBS.

An equivalent of prick test in the gut mucosa, the so-called colonoscopic allergen provocation test (COLAP), showed promising initial results. Food antigens selected according to the patients' history of food intolerance and the presence of specific IgE in serum were injected into the mucosa of the cecum during colonoscopy in seventy adult patients with chronic abdominal symptoms and suspected gastrointestinal food allergy and in five healthy volunteers. COLAP test was positive in response to at least one food antigen in 77% of patients, whereas no reaction was detected in the five healthy volunteers. Moreover, in the clinical follow up over a period of at least 6 months, a food eliminiation diet induced a significant improvement of symptoms in 29 of 35 patients (83%) with positive COLAP test. The researchers concluded that allergic reactions may play a part in a subgroup of patients with irritable bowel syndrome and COLAP test may improve the clinical management of these patients, supporting this "intestinal prick test" as a valuable diagnostic tool of GI food allergy[25].

Several researchers focused on the role of food-specific IgG and IgG4. Although dietary antigens physiologically induce the production of IgG4, the hypothesis that these immunoglobulin are involved in IBS stems from the observation that IBS patients had higher IgG4 titers to certain antigens, such as wheat, beef, pork and lamb, compared to controls[26]. Moreover, two studies revealed that a food elimination diet based on serum IgG/IgG4 antibodies is able to improve overall symptoms in IBS[26,27]. Despite the initial promising results of COLAP test and elimination diet based on serum IgG/IgG4 antibodies, there have been no further published reports of these tests[28].

In conclusion, the role of hypersensitivity in IBS remains uncertain. Clinical trials, *in vitro* and epidemiological studies have suggested a potential role of allergic mechanisms in the pathogenesis of IBS, but further studies are needed to elucidate this relationship. To date, food allergy and IBS should be considered as two distinct clini-

cal entities. Food allergy should be considered in case of uncontrolled symptoms in patients with IBS-like symptoms and when a clear allergic response to a specific food has been identified. Unfortunately, there is not a gold standard procedure for food allergy diagnosis. At present, skin prick tests and the radioallergosorbent test, the most used tests to investigate IgE-mediated allergy, suggest only individual sensitization but they are not sufficient *per se* to diagnose food allergy. For this reason, suspected food allergy needs to be confirmed by a double-blind, placebo-controlled food challenge[20].

Fat hypersensitivity

Lipids are a complex group of chemical substances including triglycerides, and its constituent fatty acid, as well as cholesterol, phospholipids and sterol. Fat is not a simply nutrient, in fact, lipids are able to modulate the responses of the gut to various stimuli. In patients suffering from FGIDs, such as irritable bowel syndrome, some of these modulatory mechanisms, being abnormal, may lead to the onset of gastrointestinal symptoms[29]. In fact, it has been hypothesized that in irritable bowel syndrome, as well as in other FGIDs, patients display intestinal hypersensitivity and exaggerated reflexes after normal stimuli, for example after fat ingestion[30]. These patients complain symptoms such as fullness, bloating and nausea after lipids intake much more frequently and at lower fat load than healthy subjects. It has been described that lipids through the inhibition of small bowel motility and the delaying of intestinal transit may cause gas retention and, then, abdominal bloating[31]. On the other hand, many evidences show that lipids stimulate colonic motor activity through a mechanism known as "gastrocolonic reflex". Such reflex seems to be upregulated in IBS patients and may lead to post-prandial diarrhea. Simrén *et al*[32] have, also, demonstrated that duodenal lipid load increased rectal sensitivity and perception of rectal distension in IBS patients, inducing different symptoms in the constipated and diarrheal subtypes of IBS with the same mechanism. In fact, if C-IBS patients experience rectal distension as pain, D-IBS subjects report primarily rectal urgency. However, although association between lipids intake and gastrointestinal symptoms has been observed, only few studies report lower dietary fat consumption in IBS patients if compared to healthy subjects.

FOOD CHEMICALS

Salicylates

Although, aspirin and other non steroidal anti-inflammatory drugs are the best studied compounds belonging to salicylates, salicylic acid and its derivates are present in many foods in different concentrations[33]. Salicylate intolerance is defined as a nonspecific antigen-induced pseudoallergic hypersensitivity reaction characterized by systemic and local manifestations[15,18-33].

Symptoms of acetylsalicylic acid intolerance are caused by overproduction of leukotriene metabolites (leukotrien B4, C4, D4, E4) and a reduction of prostaglandin, prostacyclin and thromboxan as consequence of cyclooxygenase inhibition. It has been hypothesized that in patients intolerant to salicylate the inhibition of these enzymes may be higher than in healthy subjects[34].

The typical triad of intolerance to salicylic acid comprises the occurrence of polyposis nasi, nonallergic asthma and angioedema as well as laryngeal edema following contact with substances containing acetylsalicylic acid. Further clinical manifestations of salicylate intolerance may include gastrointestinal symptoms such as abdominal pain, swelling, meteorism, colitis and diarrhea.

However, the presentation of such gastrointestinal symptoms, accompanied or not by typical systemic manifestations, may create diagnostic difficulties; in fact, the diagnosis of salicylate intolerance may be considered once other causes have been excluded.

The variety of symptoms of salicylate intolerance is linked to the different expression and concentration of cytokines in the different tissues. In fact, for example, leukotriene B4 is primarily involved in inflammation, leukotriene C4 is responsible of typical pseudo allergic mechanisms while leukotrienes C4, D4 and E4 cause bronchoconstriction, bronchial hyperreactivity, mucus production and vasodilatation[35]. These mechanisms have been used to explain intolerance to acetylsalicylic acid and, although it is possible to hypothesize that other salicylate derivates may induce symptoms sharing similar pathogenesis, further studies are needed.

At the moment, elimination diet represents the best way to diagnose and manage salicylate as well as other food chemicals intolerance, but, considered the widespread presence of these substances in foods (Table 3), too severe alimentary restrictions should be avoided for the risk of unpalatable diets and malnutrition. Moreover, all studies, which report that dietary manipulation may be a valid treatment choice in IBS patients, have important limitations in their trial designs, including inadequate patient selection, appropriateness and duration of exclusion diets, and methods of food challenge[15].

LUMINAL DISTENSION

Milk

The enzyme activity of lactase, a β-galactosidase present on the apical surface of enterocytes in the small intestinal brush border, physiologically starts to decline within the first few months of life in most of mammalian. In humans, approximately 70% of the adult population has a decreased lactase activity[36]. In people with lactase deficiency, lactose is not hydrolyzed and absorbed in the small bowel, but passes through the gastrointestinal tract into the colon into where bacterial fermentation produces gas and short-chain fatty acids and other products that can cause luminal distension and induce GI symptoms[37].

The typical symptoms of lactose intolerance are similar to those in IBS and include abdominal pain, bloating, flatus, diarrhoea, borborygmi. Conversely, patients with

Table 3 Food sources of salicylate reported in literature[15,18,33]

Food	State	Significant source of salicylate
Pepper (red chili)	Fresh	1.20
Sweet potato (white)	Fresh	0.50
Apricot	Fresh	2.58
Apricot	Canned	1.42
Apricot	Nectar	0.14
Orange	Fresh	2.39
Pineapple	Fresh	2.10
Almonds	Fresh	3.0
Raspberries	Fresh	3.14
Dates	Fresh	3.73

IBS more frequently report perceived intolerance to milk or dairy products compared to healthy individuals[8].

Despite the similarity between IBS and lactose intolerance, the prevalence of lactose intolerance in IBS patients is similar compared to controls[38] and testing patients for lactose intolerance or the use of lactase supplementation is not justified[39].

On the other side, subjective perception of intolerance for milk is not a useful criteria to identify people with lactose malabsorption. Vernia *et al*[40] tried to define the relationship between self-referred perception of milk intolerance and lactose intolerance. In this study, 475 consecutive IBS patients underwent a hydrogen breath test after an oral load of lactose. Data analysis of 201 age- and sex-matched pairs of IBS patients classified according to self-reported milk tolerance/intolerance showed that the prevalence of positive HBT was similar in milk "tolerant" (68.6%) and "intolerant" patients (75.6%), confirming that self-reported milk intolerance does not help in identifying lactose intolerance in IBS patients.

However, it is plausible to hypothesize that not lactose but milk-specific component may play a role in IBS symptoms and reducing milk and dairy products in the diet could represent an appropriate strategy in the management of IBS.

Fermentable oligosaccharides, disaccharides, monosaccharides and polyols

In the last couple of years, increasing evidences support the efficacy for the management of IBS of a diet with lower amounts of fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs)[41]. Scientific evidences showed that they are individually involved as a trigger for symptoms in patients with functional disorders[42-44]. At the base of the concept of enclosing these sugars into one group would be the common pathogenetic mechanism by which they contribute to symptoms burden in IBS: when FODMAPs are poorly absorbed through the small intestine, they pass in the bowel and increase intestinal luminal water content through their osmotic activity and induce gas production due to fermentation by gut bacteria. The increased content of water and gas causes luminal distension that induces GI symptoms in IBS patients. In addition, products of fermentation such as short-chain fatty acids could be involved in symptom generation[12].

The low FODMAP diet approach involves the reduction, not complete avoidance, of these sugars in the diet. Based on the knowledge of the FODMAP status of foods, foods are classified into high and low FODMAP content and the latter food consumption is encouraged (Table 4). In a first period of 6-8 wk, all known or suspected types of food with high content in FODMAP groups are strictly restricted from the diet, in order to determine the benefit of FODMAP restriction. Subsequently, individual FODMAPs are reintroduced to test their individual tolerance of each FODMAP *via* a series for food challenges[44]. As the authors rightfully acknowledge, restricting the intake of FODMAPs excludes a wide variety of foods from the diet with the potential risk to affect nutrient intake.

Several studies supported the potential benefits of restricting a spectrum of FODMAPs in the diet in IBS[45,46]. Recently, in a randomized double-blind controlled cross-over study, Halmos *et al*[47] demonstrated that a diet low in FODMAPs for a 3 wk period effectively reduced overall gastrointestinal symptoms -abdominal pain, bloating and bowel habit dissatisfaction- in a group of 30 unselected IBS patients, compared to a standard Australian diet.

In a non-randomized study, the low FODMAP diet was more effective than United Kingdom national dietary guidelines for symptom control in a series of consecutive patients with IBS who attended a follow-up dietetic outpatient visit for dietary management of their symptoms[45].

Other studies are needed to assess the long-term efficacy and safety of FODMAP restriction as well as to identify patient profiles that predict dietary response. However, low FODMAP diet represents the one of the most promising emerging strategies in the management of IBS.

Wheat

Many individuals complaining GI symptoms benefit from gluten withdrawal, although they cannot be classified as either celiac diseases or wheat allergy[48,49]. The hypothesis that gluten is able to induce IBS-like symptoms in non-coeliac people is not new[50,51]. Gluten has been considered the culprit of the causal relationship between wheat ingestion and GI symptoms. Indeed, recent literature has supported the existence of a subgroup of IBS patients with undiagnosed non-celiac gluten sensitivity, defined as a condition of morphological, immunological, or functional disorder that responds to gluten exclusion[10]. The existence of this condition is suggested by clinical trials showing that gluten-free diet was able to relieve GI symptoms in a randomized, double-blind, placebo-controlled, rechallenge trials[52]. Biesiekierski *et al*[52] confirmed the existence of gluten sensitivity in patients with IBS-D in a randomized, double-blind, placebo-controlled, rechallenge trial. In this study, 34 IBS patients who reported symptomatic relief after a GFD for at least 6 wk were enrolled. Nineteen patients received 16 g of non fermentable gluten per day *via* bread and a muffin, whereas the

Table 4 Food sources of fermentable oligosaccharides, disaccharides, monosaccharides and polyols[44]

	High FODMAP food source	Low-FODMAP food source
Excess fructose	Fruits (apples, pears, nashi pears, clingstone peaches, mango, sugar snap peas, watermelon, tinned fruit in natural juice) Honey Sweeteners (fructose)	Fruit (banana, blueberry, carambola, durian, grapefruit, grape, honeydew melon, kiwifruit, lemon, lime, mandarin, orange, passionfruit, paw paw, raspberry, rockmelon, strawberry, tangelo) Honey substitutes (maple syrup, golden syrup) Sweeteners (any except polyols)
Lactose oligosaccharides	Milk (cow, goat and sheep) Ice cream Yoghurt Soft cheeses	Milk (lactose-free, rice milk) Cheese (hard cheeses, camembert) Yoghurt (lactose-free) Ice cream substitutes (gelati, sorbet) Butter
Polyols	Vegetables (artichokes, asparagus, beetroot, Brussels sprout, broccoli, cabbage, fennel, garlic, leeks, okra, onions, peas, shallots) Cereals (wheat and rye when eaten in large amounts) Legumes (chickpeas, lentils, red kidney beans, baked beans) Fruits (watermelon, custard apple, white peaches, rambutan, persimmon)	Vegetables (bamboo shoots, bokchoy, carrot, celery, capsicum, choko, choy sum, corn, eggplant, green beans, lettuce, chives, parsnip, pumpkin, silverbeet, spring onion, tomato) Onion/garlic substitutes (garlic-infused oil) Cereals (gluten-free and spelt bread/cereal products)
Fructans and/or galactans	Fruits (apples, apricots, cherries, longon, lychee, nashi pears, nectarine, pears, peaches, plums, prunes, watermelon) Vegetables (avocado, cauliflower, mushrooms, snow peas) Sweeteners (sorbitol, mannitol, xylitol, maltitol, isomalt	Fruits (banana, blueberry, carambola, durian, grapefruit, grape, honeydew melon, kiwifruit, lemon, lime, mandarin, orange, passionfruit, paw paw, raspberry, rockmelon) Sweeteners (sucrose, glucose)

other 15 patients received gluten-free bread and a muffin. In the gluten group, 68% reported that symptoms were not adequately controlled compared with 40% in gluten-free group ($P = 0.0001$). Moreover, patients in the gluten-free group reported significantly greater improvements in GI symptoms such as pain, bloating, stool consistency and tiredness compared to patients in gluten group. Researchers suggested that gluten sensitivity may be a distinct clinical entity in a subset of patients with IBS.

Following studies failed to find a specific marker or pathogenetic mechanisms supporting the idea that gluten sensitivity is an objective clinical entity. Despite the lack of evidences, the mass media have publicized the advantages of GFD leading many patients to exclude gluten from diet. Two years later, the same group of researchers conducted a placebo-controlled, crossover rechallenge study in 37 subjects with gluten sensitivity and IBS. After a two weeks run-in on a gluten-free and low FODMAP diet test, subjects were placed on high-gluten (16 g gluten/d), low-gluten (2 g gluten/d and 14 g whey protein/d), or control (16 g whey protein/d) diets for 1 wk, followed by a washout period of at least 2 wk. Twenty-two participants then crossed over to groups given gluten (16 g/d), whey (16 g/d), or control (no additional protein) diets for 3 d. In all participants, gastrointestinal symptoms improved during reduced FODMAP intake and similarly worsened when their diets included gluten or whey protein. Participants were then rechallenged gluten (16 g/d), whey (16 g/d), or control (no additional protein) diets for 3 d and during this rechallenge symptoms increased by similar levels among groups, again regardless of the protein source. The researchers concluded that gluten sensitivity might not be a discrete entity and that gluten might not be a specific trigger of functional gut symptoms once dietary FODMAPs are reduced[11].

In conclusion, no clear evidences support that gluten may induce GI symptoms in individuals without CD. The observed effects of GFD in GI symptoms relief may be due to the fact that many gluten-containing cereals are high in fermentable, poorly absorbed,short-chain carbohydrates that seem to have a critical role in triggering IBS symptoms[12].

DIET AND GUT MICROBIOTA

Gut microbiota is individual-specific and is influenced by the genetic and environmental factors. In particular is well-known the role of nutrition in changes of gut bacteria[53,54]. In a recent study, researchers found that gut microbiota is able to rapidly switch between herbivorous and carnivorous functional profiles after a short-term macronutrient changes in diet[55].

Recently, the intestinal microbiota has been proposed as an etiological factor in physiopathology and pathogenesis of IBS[56]. Supporting the role of gut bacteria in IBS are studies that document the onset of IBS symptoms after an acute gastroenteritis and the qualitative and quantitative changes of bacteria composition that occur in IBS subtypes[57]. In a recent study, researchers aimed to assess the microbiota composition by molecular analysis of fecal samples from 62 patients with IBS patients and 46 healthy individuals. They found that gut microbiota of IBS patients differed significantly from that of controls. In particular, the microbiota of IBS patients had a 2-fold increased ratio of the Firmicutes to Bacteroidetes[58]. However, the role of microbiota is still unclear due to

methodological problems, influence of confounding factors and large differences between studies.

In agreement with this observation, we can speculate that diet-induced changes to the gut microbiota may contribute to the onset or worsening of IBS symptoms, as well as beneficial effects of certain nutrients on IBS symptoms could be, at least partially, mediated by changes in gut bacteria.

CONCLUSION

Food is able to trigger IBS symptoms in a great part of patients. Food related mechanisms involved in to trigger symptoms seem generally referred to food hypersensitivity, action of bioactive molecules and luminal distension. Intestinal microbiota aberration has a crucial role in luminal distension and considering that microbiota is often modified by dietary habits so we have closed the circle. The food changes the microbiota which in turn induces the abnormal fermentation of food ingested. A great attention is now directed to food containing FODMAP that are able to determine IBS symptoms both *via* microbiota aberration and luminal distension. Finally, studies oriented to define relationship between IBS and food could be a comprehensive strategy to improve medical therapy of IBS.

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Convergence of neuro-endocrine-immune pathways in the pathophysiology of irritable bowel syndrome

Maria M Buckley, Siobhain M O'Mahony, Dervla O'Malley

Maria M Buckley, Department of Physiology, Western Gateway Building, University College Cork, Cork, Ireland
Siobhain M O'Mahony, Department of Anatomy and Neuroscience and Alimentary Pharmabiotic Centre, University College Cork, Cork, Ireland
Dervla O'Malley, Department of Physiology and Alimentary Pharmabiotic Centre, University College Cork, Cork, Ireland
Author contributions: Buckley MM, O'Mahony SM and O'Malley D contributed equally to this work, reviewing the literature and writing the paper; O'Malley D reviewed the contributions and integrated the sections.
Correspondence to: Dervla O'Malley, BSc, DIS, PhD, Department of Physiology, Western Gateway Building, University College Cork, Western Rd, Cork, Ireland. d.omalley@ucc.ie
Telephone: +353-21-4205483 Fax: +353-21-4205370
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Abstract

Disordered signalling between the brain and the gut are generally accepted to underlie the functional bowel disorder, irritable bowel syndrome (IBS). However, partly due to the lack of disease-defining biomarkers, understanding the aetiology of this complex and multifactorial disease remains elusive. This common gastrointestinal disorder is characterised by alterations in bowel habit such as diarrhoea and/or constipation, bloating and abdominal pain, and symptom exacerbation has been linked with periods of stress, both psychosocial and infection-related. Indeed, a high level of comorbidity exists between IBS and stress-related mood disorders such as anxiety and depression. Moreover, studies have observed alterations in autonomic output and neuro-endocrine signalling in IBS patients. Accumulating evidence indicates that a maladaptive stress response, probably mediated by the stress hormone, corticotropin-releasing factor contributes to the initiation, persistence and severity of symptom flares. Other risk factors for developing IBS include a positive family history, childhood trauma, dietary factors and prior gastrointestinal infection. An emerging role has been attributed to the importance of immune factors in the pathophysiology of IBS with evidence of altered cytokine profiles and increased levels of mucosal immune cells. These factors have also been shown to have direct effects on neural signalling. This review discusses how pathological changes in neural, immune and endocrine pathways, and communication between these systems, contribute to symptom flares in IBS.

Key words: Stress; Corticotropin-releasing factor; Pro-inflammatory cytokines; Enteric nervous system; Vagus

Core tip: Irritable bowel syndrome (IBS) is a disorder characterised by symptoms such as diarrhoea and/or constipation, bloating and abdominal pain. However the underlying pathophysiology of this common disorder remains unclear. Nonetheless, a number of mechanisms have been proposed to contribute to the initiation, exacerbation and persistence of symptoms. Alterations in brain-gut communication, stress, previous infections, abnormal microbiota, altered cytokine profiles and increased intestinal permeability have all been proposed as contributors to IBS and indeed, we propose that complex interactions between neural, endocrine and immune factors underlie the heterogeneity of symptoms that is characteristic of IBS.

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INTRODUCTION

Irritable bowel syndrome (IBS) is the most common functional digestive condition with a worldwide prevalence rate of 10%-20% in the general population[1,2]. As with other functional disorders it is often difficult to identify an unequivocal organic cause, at least with the diagnostic tools available. This disorder accounts for approximately 3% all general practice consultation and up to 40% of gastrointestinal (GI) referrals[3] leading to a large economic burden. At the level of the individual, IBS significantly impinges on the quality of life of a patient causing recurrent abdominal pain or discomfort coupled with disturbed bowel habits[4]. IBS is subtyped according to bowel habit pattern, therefore patients are classified as diarrhoea-(IBS-D) or constipation-predominant IBS (IBS-C) or an alternating subtype (IBS-A), which fluxes between the two states[5]. Some reports suggest that IBS-D and IBS-A are more prevalent[6] while others show an equal distribution between all three subtypes[7]. Although little mortality is associated with IBS a satisfactory treatment still does not exist, primarily due to the fact that the aetiology and pathophysiology of IBS are incompletely understood. Nonetheless, dysfunctional brain-gut axis signalling is hypothesised to be at the heart of symptoms of IBS[8] and this incorporates three major systems, neural, endocrine and immune signalling. In this review we discuss the contribution of each system to IBS symptoms and how convergence and interplay between factors from each system may provide a better understanding of the heterogeneity of IBS.

NEURAL SIGNALLING IN IBS PATHOPHYSIOLOGY

Autonomic regulation of the gut

The two major persisting symptoms of IBS are visceral hypersensitivity and altered bowel habit[9] each of which are entwined within the nervous system. Functions of the GI tract are modulated by both intrinsic and extrinsic innervation[10]. Extrinsic innervation includes both branches of the autonomic nervous system, which are anatomically and functionally integrated within the brain-gut axis and are responsible for homeostatic regulation of GI function[11]. The parasympathetic nervous system stimulates smooth muscle and secretory actions while the sympathetic element inhibits motor and secretory activity of the GI tract. The parasympathetic afferent pathway runs primarily with the vagus and terminates in the nucleus solitary tact, which sends information regarding non-nociceptive information, including gastric accommodation and gastric-colic reflex, to corticolimbic structures[12]. The sympathetic afferent pathways mediates mainly nociceptive signals through spinal pathways primarily to the thalamus and then to the sensory cortex and pain matrix[13]. Information is also sent to specific brain regions such as the hippocampus, amygdala, prefrontal cortex[14] and the hypothalamus[15] for processing. These central regions which are capable of modulating gut function are also involved in emotional (*e.g.* mood, anxiety, pain) and cognitive behaviours (*e.g.* memory, decision making) and hence, in the development of coping strategies and general well-being[16]. A descending pain inhibitory pathway from the brainstem also exists in order to control the signals reaching the brain. The intrinsic or enteric nervous system works somewhat independently providing local reflexes, such as migrating motor complex and peristaltic reflexes, and y*et al*so receives input from the central nervous system (CNS) *via* the autonomic nervous system.

Autonomic dysfunction

A growing body of evidence suggests the existence of autonomic dysfunction in IBS[17-19] and some have shown correlations with symptoms[20]. Low vagal activity can lead to a reduction in bowel contractions, reduced motility, and constipation, while high vagal activity can result in increased contractions and diarrhoea[21]. The sympatho-vagal balance was found to be disturbed in IBS patients compared to healthy controls[22]. Furthermore, a study assessing female IBS patients with constipation and severe abdominal pain showed lower vagal activity than controls[23], which correlates with a study showing an increased parasympathetic tone in women with IBS-D compared to those with IBS-C[24]. Given the close association between the stress axis and the autonomic nervous system, increased sympathetic tone as seen with constipation may be due to the increase in corticotropin-releasing factor (CRF) expression[10], which is discussed in more detail in the next section. Indeed, the psychological disorders that often co-occur with IBS are also associated with altered autonomic balance[25].

Underlying neural causes of visceral hypersensitivity

Visceral hypersensitivity, as seen in a subset of IBS patients, is an exaggerated response to a stimulus such as colorectal distension, was first noted by Ritchie[26], 1973. Several neural theories have been proposed for this increased sensitivity, including sensitisation of primary afferent pathways, increased activity of endogenous pain facilitation and reduced engagement of endogenous pain inhibition[27]. IBS patients have significantly elevated levels of anxiety, interpersonal sensitivity, depression, hostility and somatization of effect, which can impact on pain perception[28]. Some studies indicate a difference in sensitivity to colon and rectal distension between diarrhoea and control subjects[29,30], while patients with constipation showed conflicting results[31,32]. However, a comparison between constipation and diarrhoea predominant IBS patients revealed no significant difference in pain threshold[33].

Peripheral mechanisms-sensitisation of primary afferents

Preclinical studies of acute gut inflammation have shown that sensitization of primary afferent pathways can result in visceral hyperalgesia[34-36]. A subset of IBS patients develop symptoms following an acute GI infection[37]. Usu-

ally peripheral sensitization is temporary and response properties of primary afferents return to normal state after resolution of the inflammation[27]. Evidence from human mucosal biopsies suggests neuroplastic remodelling in the epithelium[38]. Such plastic changes can affect the response properties of primary afferents which include spinal and vagal afferents[39]. Changes in afferent nerve terminals could affect responsiveness to visceral stimuli and interfere with the release of neuropeptides from these terminals resulting in neurogenic inflammation[27].

Central pain amplification

In turn then there are multiple mechanisms by which the CNS can modulate afferent signals from the viscera, including increased activation of endogenous pain facilitation and reduced engagement of endogenous pain inhibition[27]. Of course, these modulatory systems are also influenced by stress and mood[27]. Neuroimaging studies consistently support a role for altered neural processing of visceral stimuli[40]. Indeed, some sophisticated studies now incorporate the contribution of emotional factors and cognitive influences such as expectation, attention and learning, to their analyses of functional connectivity between brain regions and actual CNS structural changes[40].

It was noted that a thinning of the anterior mid-cingulate and insular cortices was evident in IBS patients[41], these areas being associated with perception of the internal state. Moreover, regional structural changes including decreased grey matter in the medial and ventrolateral prefrontal cortex, thalamus and periaqueductal grey are seen in IBS patients as compared to healthy controls[42]. These may point towards an impaired ability to activate the descending pain inhibition system. This hypothesis is supported by the observation that the reduction in grey matter in the ventrolateral prefrontal cortex was only found in the patients presenting with a high level of pain[42]. Central areas involved in the processing of the affective component of pain such as the pregenual anterior cingulate cortex and the orbital frontal cortex showed an increase in grey matter in IBS patients, which was abolished once data was corrected for anxiety and depression in these patients[40]. These findings further confirm the involvement of emotional systems in the processing of visceral pain. Consistent with this, Chen *et al*[43] showed that white matter aberrations are seen in the anterior cingulate cortex and the insula. However, as it is still not known whether these changes are present before symptoms emerge, or are actually acquired due to altered visceral signalling, these results should be interpreted with caution.

Enhanced CNS responses

A meta-analysis of functional magnetic resonance imaging studies in IBS patients reported differences in CNS response to colorectal distension[44]. The differences were seen in areas associated with visceral afferent signalling, attention and emotional arousal. The anterior cingulate cortex is one of the most commonly reported cortical areas that displays pain evoked activation during acute stimulation in patients[43]. Mertz *et al*[45] demonstrated that the anterior cingulate cortex, thalamus, the insula and the prefrontal cortex were more activated in IBS patients than controls and that the pattern of activation was dependent on previous experience. A greater activation of the thalamic, striatal and dorsolateral prefrontal cortex was seen in controls as compared to IBS patients during rectal distension indicating an abnormal descending modulation in IBS[46]. It has also been shown that female IBS patients have a greater engagement of the emotional arousal system during expectation of visceral pain than males[47]. These studies highlight the importance of the emotional status of patients in pain perception and that the female predominance may be in part due to the gender differences in the activation of circuits involved in stress and arousal[27]. Taken together these results indicate a role for both structural and functional abnormalities in the CNS in IBS pathophysiology.

ENDOCRINE PATHWAYS IN IBS PATHOPHYSIOLOGY

CRF

Stress is a pervasive condition that effects everyone and is defined as a "stereotyped body response to any demand"[48]. However, the high co-morbidity of stress-associated mood disorders such as anxiety and depression and altered bowel function in IBS patients[49], suggests that these individuals are more sensitive to the effects of stress. Indeed, the relationship between severe and chronic stress and symptom intensity in IBS patients[50] is linked to chronic stress, with the onset and duration of symptoms increased[51]. As noted above, this may mediated *via* altered autonomic signalling[52], however the key signalling factor initiated by stress is an endocrine hormone, CRF.

CRF is the vital hormone in the body's response to stress, activating the hypothalamic-pituitary-adrenal (HPA) axis in reaction to a variety of physical and psychological stressors. This results in enhanced levels of adrenocorticotropic hormone and cortisol in IBS patients as compared to healthy subjects[53,54]. CRF is secreted by the paraventricular nucleus (PVN) of the hypothalamus and its release is regulated by the amygdala, which is part of the limbic system[51].

CRF exerts its biological effects through activation of CRF1 and CRF2 receptors (CRFR1 and CRFR2), which are members of the seven transmembrane G-protein coupled receptor superfamily[55]. CRFR1 is prevalent in brain regions associated with affective, stress and nociceptive circuitries including the PVN, locus coeruleus and amygdala[56,57]. CRF neurons project from the PVN to the spinal cord, where they can alter the function of innervated organs[58].

CRF in the GI tract

Although much of the influence of CRF on GI function

is mediated centrally, the presence of CRF ligands and its receptors in the colon[55,59,60] suggests that organ-specific activation of these receptors may also be important for stress-induced changes in bowel function. CRFR1 is expressed on enteric neurons and in the mucosal layer[59] and is likely to be the focal mechanism by which stress induces changes in GI function including delayed gastric emptying[61], accelerated colonic transit[62] and motility[63]. The importance of CRF to these effects has been demonstrated using the non-selective CRF receptor antagonist, α-helical CRF[64]. Furthermore, the use of CRFR1 -/- mice has revealed the importance of the CRFR1 subtype in IBS like-symptoms, as these knock-out mice exhibit decreased visceral sensitivity[65], as well as decreased anxiety and an impaired stress response[66]. In addition CRF-evoked defecation in rats[67] is inhibited by blocking CRF1 receptors[68]. These results translate to IBS patients, where peripheral administration of CRF1 receptor antagonists reduces abdominal pain and anxiety[64]. In contrast to CRFR1-mediated increases in GI contractile activity, stimulation of CRFR2 is likely to result in inhibition of GI motility[69,70] and contribute to stress-induced colonic permeability dysfunction[71].

Effects of CRF on visceral hypersensitivity and colonic function

Some of the key symptoms of IBS, such as colonic motility, alterations in bowel habit and abdominal pain associated with gut hypersensitivity may be a consequence of CRFR1 signalling[72]. Consistent with this is increased thalamic expression of CRFR1 following colonic distension in the maternally separated rat model of IBS[73]. Moreover, central administration of CRF increases pain behaviours in response to colonic distension in rats[74] demonstrating the bidirectional signalling between the CNS and the gut. Activation of the CRFR1 signalling pathways causes increases in colonic motor activity and visceral pain[75,76], and conversely, activation of central and peripheral CRFR2 receptors delays gastric emptying[70]. Furthermore, activation of either CRFR1 or CRFR2 causes increased colonic permeability and inflammation[77]. The pathophysiology of stress-induced exacerbation of IBS symptoms may be due to central hypersecretion of CRF, as it has been shown that inhibition of CRFR1[78] as well as central inhibition with CRF antagonists decreases the response to water avoidance stress[79]. Also, stress increases intestinal permeability, visceral hypersensitivity, causes alterations in gastrointestinal motility and leads to profound activation of mast cells, resulting in the release of many pro-inflammatory mediators[80-82] which will be discussed in more detail later. Williams *et al*[67] illustrated that acute restraint stress increased large intestine transit rates and stimulated defecation and this was associated with mucosal mast cell activation[83,84], which was mediated by CRF[83]. These studies imply that the brain-gut axis of IBS patients has a magnified response to CRF. Thus, targeting CRF signalling molecules has been proposed as a potential treatment for IBS[70]. However, thus far, clinical trials using a CRFR1 antagonist have been disappointing[85].

Corticosteroids

Mineralocorticoids and glucocorticoids are steroid hormones which mediate the actions of the adrenal hormone, cortisol in the initiation and termination of the stress response, respectively[86]. Cortisol, which is the natural ligand for corticosteroid receptors, is elevated in IBS patients both at baseline and in response to stress[53,54]. In rodent studies, application of corticosterone to the amygdala induces colonic hypersensitivity and anxiety[87,88] and alters colonic transit[89], actions that are mediated through both mineralocorticoid and glucocorticoid receptors[90]. These studies demonstrate that central signalling by corticosteroids are potential targets for treating bowel dysfunction in IBS.

Glucagon-like peptide 1

A precipitating factor in symptom exacerbation is food ingestion, frequently in the form of abdominal pain and gas[91]. Although food intolerance has not been shown to cause IBS, ingestion of certain foods can result in abdominal pain, bloating, flatus and diarrhoea[92,93], especially carbohydrates, including gluten and lactose and fat-rich meals[92]. This appears to be more common in females and those who display increased anxiety levels again demonstrating the multi-factorial nature of IBS[93]. Prolonged and exaggerated colonic motor responses following a meal has been observed in IBS patients[94] and balloon distension in the jejunum demonstrated increased sensitivity in IBS patients following a meal[95]. Recent studies have reported the success of diets low in fermentable oligosaccharides, disaccharides, monosaccharides and polyols[96-98]. Reduction of poorly absorbed short-chain carbohydrates such as lactose, fructose and sorbitol, fructo-oligosaccharides, galacto-oligosaccharides and incompletely absorbed sugar polyols such as sorbitol and mannitol relieves symptoms of IBS, such as bloating, distension, abdominal pain, excessive flatus[98] and osmotic diarrhoea[99]. Although release of gas by fermentation is normal, the sensitivity of IBS bowels to distension results in visceral pain. The pathophysiological changes resulting in these symptoms are not yet clear. However, an important physiological response to the arrival of food in the GI tract is the secretion of incretin hormones such as glucagon-like peptide 1 (GLP-1) which is secreted by L-cells. The biological activities of GLP-1 include stimulation of glucose-dependent insulin secretion and insulin biosynthesis, inhibition of glucagon secretion and gastric emptying, and the inhibition of food intake. One report has related GLP-1 to IBS pathophysiology by demonstrating that a GLP-1 mimetic alleviated some of the pathophysiological symptoms of IBS with antispasmodic and pain-relieving properties[100]. Although, the molecular mechanisms by which GLP-1 achieves this outcome are not completely understood, it is thought to act in a neurocrine fashion. Indeed, GLP-1 has been found to increase firing rates in afferent vagal nerves[101]

and also decreasing neurally-evoked chloride secretion[102]. Interestingly, GLP-1 can also modulate GI secretion of cytokines and alter central CRF pathways that regulate stress-induced alterations in colonic transit[103]. GLP-1-expressing neurons are found in the enteric nervous system but also in brain regions such as the nucleus tractus solitarius and the ventrolateral medulla[104], revealing that the action of GLP-1 on gut function may be central or peripheral. GLP-1 activates the HPA axis through CRF neuronal stimulation, which may be important in the suppression of feeding behaviour[105]. Other GI hormones, such as motilin[106], which regulates the migrating motor complex in the fasting period and cholecystokinin[107] are elevated in IBS. In contrast, colonic expression of peptide YY[108] and circulating neuropeptide Y are lower in IBS patients[107]. Consideration must therefore be given to these and other GI factors in the pathogenesis of IBS.

ALTERATIONS IN IMMUNE FUNCTION IN IBS

Mounting evidence suggests that alterations in immune status such as elevations in mucosal mast cell numbers, pro-inflammatory cytokines and increased intestinal permeability are frequently noted in IBS patients[109]. Potential biomarkers of the disorder include alterations in cytokine profiles, mucosal and muscular infiltration of immune cells, changes in intestinal permeability and luminal microbiota which are discussed below.

Post-infectious IBS

Gross morphological evidence of inflammation is absent from IBS mucosal biopsies and other indicators of inflammation such as faecal levels of calprotectin and lactoferrin are not elevated[110,111]. Nonetheless, evidence is mounting on the important contribution of immune activation to the development of this syndrome. Indeed, one of clearest predictors of developing IBS is a prior history of bacterial or viral gastroenteritis[37,112], with one study showing a sevenfold increase in the risk of developing the functional bowel disorder following gastrointestinal infection[113]. Samples from individuals with post-infectious IBS show persistent increases in mucosal mononuclear immune cells[114], T-lymphocytes[115] and mast cells[116], which degranulate following stimulation releasing compounds such as histamine, tryptase and chymase. The extent of immune activation is an indicator of the severity of the infective gastroenteritis episode and the subsequent risk of developing IBS[114,117].

Immune cells and cytokines

Expression of lymphocytes and mast cells are elevated in IBS mucosal samples[118,119], although not all studies detected increased numbers of mucosal mast cells[118,120,121]. Nonetheless, soluble mediators released from degranulated mast cells were found to induce excitation of rat sensory neurons[121]. This has implications for GI sensory and motor function, with one study demonstrating that the colon is more susceptible to effects of stress on enteric nerve function following a prior bout of inflammation[122].

Evidence of immune activation in IBS includes elevated levels of pro-inflammatory cytokines such as interleukin (IL)-6 and IL-8[53,123-125], although not all studies detected such increases[120,126]. Furthermore, in peripheral blood mononuclear cells isolated from IBS patients, abnormal secretion of pro-inflammatory cytokines in response to immune challenges was observed[123,125,127]. Studies reporting changes in mucosal levels of pro-inflammatory cytokines in IBS biopsies varied with some studies describing an upregulation[116,128] but several others describing down-regulation of these cytokines[54,129]. That said, anti-inflammatory cytokines such as IL-10 and transforming growth factor β appear to be decreased in IBS colonic and rectal biopsies[54,128,129]. Expression of chemokines, including IL-8, CXCL-9 and monocyte chemoattractant protein-1, which are important in mucosal defence, were also decreased in IBS biopsies[129].

The source of these immune messengers are likely to be from mast cells, the numbers of which are elevated in IBS[128,130,131] and can secrete IL-6 and IL-1β[132] in addition to histamine, tryptase, chymase and proteases. Indeed, Buhner *et al*[131] described how excitation of non-IBS submucosal neurons with IBS biopsy secretions was dependent on serotonin, tryptase and histamine. Furthermore, the proximity of activated mast cells to colonic nerves was found to correlate with visceral pain severity[130].

Cytokines have been shown to have neuromodulatory effects with IL-6[133], IL-1β[134] and tumour necrosis factor (TNF) α[135] stimulating submucosal secretomotor neurons. This may result in changes in gut function including contractility[136], absorption and/or secretion[133]. IL-6 and IL-1β also influence mucosal ion transport and epithelial permeability and enhance cholinergically-mediated neurotransmission[133,137,138]. Furthermore, IL-6 has a potential role in neurogenic secretory diarrhoea[125] as this cytokine can suppress the inhibitory and anti-secretory effects of norepinephrine by blocking its release from sympathetic fibres[139]. Others have provided evidence that IL-6 attenuates the pre-synaptic inhibition of noradrenalin release, thereby releasing the sympathetic brake[134], which further contributes to a pro-secretory state. Aside from altered GI motility, the other main debilitating symptom of IBS is visceral pain sensitivity. Given the demonstrated effects of cytokines on enteric neuron excitability[133-135] and proven roles in nociception and sensory pain pathways[140], activation of enteric neurons and subsequent evocation of visceral pain make cytokines attractive candidates for mediating the visceral pain-related features of IBS.

Epithelial barrier integrity

The permeability of the epithelial layer which acts as a barrier between the external environment of the gut lumen and the body's internal milieu is an important consideration in immune activation in IBS. Indeed, some IBS patients have increased intestinal permeability[141], which

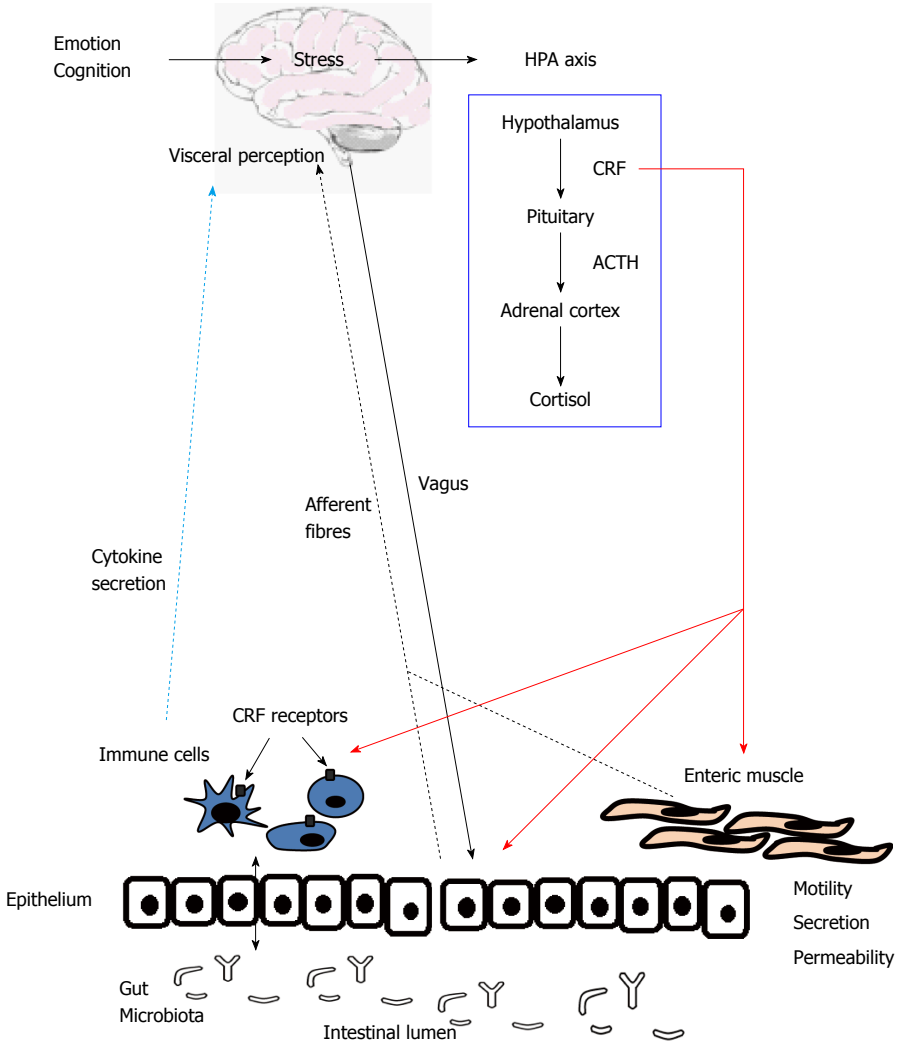

Figure 1 Convergence of neural endocrine and immune signalling pathways in bowel dysfunction. The figure illustrates the complex nature of functional bowel syndromes such as IBS. It illustrates interaction between three major bodily systems, neural pathways between the brain and gut, hormonal release, primarily from stress-induced activation of the HPA axis and secretion of immune factors such as cytokines. ACTH: Adrenocorticotropic hormone; CRF: Corticotropin-releasing factor; HPA: Hypothalamic-pituitary-adrenal; IBS: Irritable bowel syndrome.

may be due to proteasomal degradation of tight junction proteins[128,142]. Additionally, altered secretion of inflammatory cytokines may affect barrier function and permeability[129]. Breakdown of the mucosal barrier by IL-6 and other pro-inflammatory cytokines[137,143] may provide access for foreign proteins, thus initiating an immune response in the GI muscle layers resulting in changes in bowel function. In IBS, where circulating IL-6 levels are elevated and the HPA axis is hyper-activated[53], a coincident compromise of the mucosal barrier is observed. Thus, increased permeability of the mucosal barrier and the subsequent initiation of an immune response may contribute to the increase in sensitivity to visceral pain in IBS patients[144].

Microbiota

An additional factor contributing to brain-gut axis signalling in IBS currently gaining considerable attention is the importance of disrupting the luminal microbiota[145,146]. Indeed, microbiota dysbiosis, which may facilitate the adhesion of enteric pathogens in the human gut, has been reported in several IBS studies[147-150]. This virtual organ is integrated into the bi-directional communication in the brain-gut axis with studies demonstrating that microbiota dysbiosis exists in IBS patients and manipulation of the microbial environment with probiotics may lead to symptom improvement[151]. Probiotics have been shown to modulate the immune response in IBS, suppressing pro-inflammatory cytokines[152], maintaining intestinal barrier integrity[153], causing down-regulation of T cells and inhibition of nuclear factor kappa B[154]. Moreover, probiotics prevented adhesion of enteric pathogens to the wall of the GI tract[155]. However, more recent longer-term studies did not detect an improvement in symptoms[156,157]. Other members of the innate immune system that are altered in IBS include the pattern recognition receptors, toll-like receptors (TLRs), which recognise and respond to pathogens. Altered expression of TLR4, 5, 7 and 8 in mucosal biopsies from IBS patients further supports the importance of interactions between the luminal flora and

the host in this disorder[158].

CONVERGENCE OF PATHWAYS

The pathophysiology of altered bowel function in IBS patients remains unclear, however a number of mechanisms have been proposed to contribute to the initiation, exacerbation and persistence of symptoms. Alterations in brain-gut communication[159], stress[70], previous infections[57], abnormal microbiota[160], altered cytokine profiles[53] and increased intestinal permeability[142] have all been discussed. However, we believe that complex interactions between neural, endocrine and immune factors underlie the heterogeneity of symptoms that is characteristic of IBS as diagrammed in Figure 1.

For example, a perceived threat or stressor, which frequently precedes symptom flares, evokes responses from both the immune and stress systems. In healthy individuals this is a crucial response for the adaptation and ultimate survival of an organism. However, in the case of PI-IBS, co-morbidity with anxiety or depression and the occurrence of stressful life events around the time of exposure to the enteric pathogen have been independent predictors of risk for the development of IBS[114,161,162], although not all studies, including the Walkerton cohort[112], detected this association. IBS patients are more likely to be stress-sensitive, as measured by the Holmes and Rahe stress scale, and exhibit elevated numbers of colonic mucosal mast cells[130]. Moreover, acute stress causes increases in the numbers of white blood cells, natural killer cells and CD8+ T-lymphocytes, decreases B cell numbers and stimulates secretion of pro-inflammatory cytokines[163,164], whereas secretion of glucocorticoids and an associated decrease in secretion of pro-inflammatory cytokines is noted in cases of chronic stress[165]. Patients with IBS often exhibit concurrent increases in markers of a hyperactive stress response and immune upregulation such as CRF-stimulated HPA axis hyper-responsivity which is related to the elevation in IL-6 levels[53]. CRF also stimulates the recruitment and activation of granulocytes[166] and mast cells[167] to the gut mucosa.

Immune cells express receptors for several different stress-related peptides including CRF[168]. Indeed, we have detected both CRFR1 and IL-6 receptors on T-helper cells[169]. CRF peptides have potent immunomodulatory actions[170], including degranulation of mast cells[171] and secretion of cytokines[53,172], although it is not yet clear whether these effects are pro-[173] or anti-inflammatory[174,175].

In terms of crosstalk between the stress system and the neural response, many of the psychological disorders frequently found to be co-morbid with IBS also have the capacity to disrupt autonomic balance[52] and indeed, anxiety and depression are associated with depressed parasympathetic activity in IBS patients. Enteric neurons, which directly regulate absorpto-secretory function and gut motility have been shown to express both CRF receptors and IL-6 receptors[169]. Indeed, cytokines such as IL-6 can directly induce excitation of enteric neurons in animal models of IBS[133,176]. IL-6[133], IL-1β[134] and TNFα[135] can cause activation of submucosal secretomotor neurons thereby acting as neuromodulatory factors that can directly influence such gut functions as motility, absorption, secretion and blood flow. IL-6 and IL-1β also have effects on mucosal ion transport and epithelial permeability, in addition to enhancing cholinergically-mediated neurotransmission[133,137,138]. Indeed, soluble mediators released from mast cells in IBS biopsies were found to have excitatory effects on rat sensory neurons[121].

Although the pathophysiology of IBS still requires further elucidation, recent progress in the field has demonstrated the importance of molecular factors such as the stress hormone, CRF and cytokine release and their influence on neural communication between the brain and gut. Further research will hopefully reveal the aberrant signalling between endocrine, immune and neural systems in IBS patients and pave the way towards effective new therapies for this common bowel disorder.

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Microbiota-host interactions in irritable bowel syndrome: Epithelial barrier, immune regulation and brain-gut interactions

Niall P Hyland, Eamonn MM Quigley, Elizabeth Brint

Niall P Hyland, Eamonn MM Quigley, Elizabeth Brint, Alimentary Pharmabiotic Centre, University College Cork, 30 Cork, Ireland
Niall P Hyland, Department of Pharmacology and Therapeutics, University College Cork, 30 Cork, Ireland
Eamonn MM Quigley, Houston Methodist Hospital, Houston, TX 77030, United States
Elizabeth Brint, Department of Pathology, University College Cork, 30 Cork, Ireland
Author contributions: Hyland NP and Quigley EMM contributed equally to this manuscript; all the authors approved the final version.
Correspondence to: Elizabeth Brint, PhD, Department of Pathology, University College Cork, Coláiste Na Hollscoile, Bóthar an Choláiste, Co. Corcaigh, 30 Cork, Ireland. e.brint@ucc.ie
Telephone: +353-21-4205045
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Abstract

Irritable bowel syndrome (IBS) is a common, sometimes debilitating, gastrointestinal disorder worldwide. While altered gut motility and sensation, as well as aberrant brain perception of visceral events, are thought to contribute to the genesis of symptoms in IBS, a search for an underlying aetiology has, to date, proven unsuccessful. Recently, attention has been focused on the microbiota as a possible factor in the pathogenesis of IBS. Prompted by a number of clinical observations, such as the recognition of the *de novo* development of IBS following enteric infections, as well as descriptions of changes in colonic bacterial populations in IBS and supported by clinical responses to interventions, such as antibiotics and probiotics, that modify the microbiota, various approaches have been taken to investigating the microbiota-host response in IBS, as well as in animal models thereof. From such studies a considerable body of evidence has accumulated to indicate the activation or upregulation of both factors involved in bacterial engagement with the host as well host defence mechanisms against bacteria. Alterations in gut barrier function, occurring in response, or in parallel, to changes in the microbiota, have also been widely described and can be seen to play a pivotal role in generating and sustaining host immune responses both within and beyond the gut. In this manner a plausible hypothesis, based on an altered microbiota and/or an aberrant host response, for the pathogenesis, of at least some instances of IBS, can be generated.

Key words: Microbiota; Irritable bowel syndrome; Toll-like receptor; Epithelial barrier; Gut-brain axis

Core tip: Recent discoveries have kindled an interest in microbiota-host interactions in irritable bowel syndrome (IBS) and have led to new lines of research into this common and elusive disorder. It is clear that the microbiota is altered in IBS and that such alterations could well contribute to the pathogenesis of the disorder through, for example, increased permeability, an altered immune profile, effects on the central nervous system and modulation of gut neuromuscular function. This review will explore these host-microbe interactions and their relevance to the pathogenesis of IBS. This review will explore these interactions and their relevance to the pathogenesis of IBS.

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INTRODUCTION

The importance of the microbiota in the pathogenesis of irritable bowel syndrome (IBS) has only recently begun to be understood with alterations in the composition of the gut microbiota being increasingly investigated as a factor in the pathogenesis and pathophysiology of IBS. The human microbiota is a complex ecosystem which may contain as many as 1000 to 1150 bacterial species and between 10^{13} to 10^{14} microorganisms with the greatest density and diversity of bacteria being found in the distal small bowel and colon[1]. The number of bacteria within the gut is about 10 times that of all cells in the human body. While data remains limited, it is evident that IBS patients have an altered microbiota relative to healthy individuals. Bacterial diversity is reduced[2] and more detailed analyses have identified differences at species and strain level[3] among both children and adults with IBS. Not surprisingly, given the heterogeneity of the IBS phenotype, these results have not been consistent and the sizes of the study populations involved have not been large enough to encompass the entire symptom and demographic spectrum that is IBS. Other clinical evidence also supports a role for the microbiota in IBS, including the role of enteric infections as well as the well documented symptom responses to antibiotics, such as rifaximin, and certain probiotics[4].

IBS is one of the most common gastrointestinal ailments worldwide affecting anywhere from 5%-15% of adults in the general population[5]. Despite considerable effort, a biomarker(s) specific for IBS has not been identified[6] and its definition remains entirely clinical, based on the presence of abdominal pain/or discomfort associated with altered bowel habit, often accompanied by symptoms of bloating and distension[7]. The spectrum of symptom severity in IBS is broad with the majority of those affected never seeking medical advice but self-medicating or instituting dietary or life-style measures to control symptoms. At the other end of the spectrum are a smaller number of affected individuals whose symptoms are debilitating and impose a very significant impact on quality of life. IBS is commonly associated with other gastrointestinal ailments such as gastroesophageal reflux, functional dyspepsia and extra-intestinal disorders[8]. Over the years, altered motility, visceral hypersensitivity, immune alterations and, more recently, compromised epithelial barrier function have all been invoked to explain the genesis of symptoms in IBS. Whether considered individually or collectively, these factors undoubtedly play a role in the onset and exacerbation of symptoms in IBS, although none can satisfactorily claim to be a fundamental cause of IBS[9]. Indeed, one of the few true causes of IBS that has been identified is enteric infection; several large series attest to the de novo development of IBS following acute enteric bacterial, viral and parasitic infections[10]. This latter observation kindled an interest in microbiota-host interactions in IBS and has led to a new and surprising line of research into this common and elusive disorder. This review will explore these interactions and their relevance to the pathogenesis of IBS.

INTESTINAL EPITHELIAL BARRIER: AN INTERFACE FOR HOST-MICROBE INTERACTIONS IN IBS

Given the size of the intestine and the density of the commensal flora, the gut represents an enormous interface between the host and its' environment, and, thereby, functions as a barrier between the external environment and the internal milieu and is essential in maintaining health and preventing disease[11]. The intestinal epithelial barrier comprises a thick mucus layer and a single layer of intestinal epithelial cells (IECs) which separate commensal bacteria from the underlying submucosa and as such are a critical component of commensal-host interactions[12]. It is now well understood that IECs are not an inert component of this interaction but are both effected by, and themselves effect, the microbiota. The commensal flora has been shown to directly affect the epithelial barrier through its regulation of tight junction proteins. Examples of this include, increased expression and distribution of zonula ocludin-2[13] as well asupregulation of other gap junction proteins such as occudin2 and claudin-2 in response to a number of probiotic bacteria in several IECs[14]. Commensal flora also contribute to the production of mucus as the mucus layer is considerably reduced in the gut of germ-free mice, but recovers on exposure to bacterial products[15,16]. Given the influence of microbes on the integrity of the intestinal epithelium, this may be of relevance in the context of the compromised epithelial barrier and alterations in permeability observed in IBS[17,18]. The mechanisms underlying this increased permeability in IBS include alterations in tight junction protein expression, localisation or function, changes in the microbiota, presence of active inflammation and/or presence of pro-inflammatory cytokines and increased cell shedding[19]. In particular, reduction of the tight junction protein zonula ocludin-1 (ZO-1) and disruption of apical expression of claudin-1, occludin and ZO-1 have been observed in IBS[20,21]. In addition, single nucleotide polymorphisms in the gene encoding the tight junction protein E-cadherin (CDH1) are associated with an increased risk for the development of post-infectious IBS[22]. Of particular note, is the relationship between increased permeability and the severity of abdominal pain experienced by IBS patients[23]. Moreover, in IBS patients, Zeng and colleagues partially reversed changes in small intestinal permeability with a probiotic cocktail[24]. This increased permeability of the barrier seen in IBS patients may also contribute to the low-grade inflammation that characterises this syndrome, due to increased bacterial translocation[25].

COMMENSAL REGULATION OF IMMUNITY: RELEVANCE FOR IBS

The mucosal surface of the intestinal epithelium has evolved to allow the correct balance of responsiveness, being broadly unresponsive to the presence of the commensal bacteria in the gut lumen whilst still being able to mount an immune response to the presence of pathogenic bacteria[26]. How commensals and the immune system achieve this balance is an area of on-going investigation[27]. It seems likely that no single mechanism applies to all commensals; different strains or species employ different strategies. Nonetheless, a range of potential mechanisms have been identified[28,29]. For example, *Bifidobacterium infantis* prevents nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) and interleukin (IL)-8 activation and also inhibits the secretion of the chemokine CCL20 in response to *Salmonella typhimurium*, *Clostridium difficile*, *Mycobacterium paratuberculosis* and, even, bacterial flagellin[30,31]. Some strains, indeed, appear to exert potent anti-inflammatory effects: in an experimental animal (IL-10 knockout) model of colitis, both a *Lactobacillus* and a *Bifidobacterium* suppressed the production of the pro-inflammatory cytokines interferon-γ, tumor necrosis factor-α, and IL-12, while levels of the anti-inflammatory cytokine transforming growth factor-β were maintained[32]. Similar effects have been demonstrated for the probiotic cocktail VSL#3 in experimental models of colitis[33,34]. What is very exciting is the observation, again in an animal model, of the ability of orally administered probiotics to exert anti-inflammatory effects at sites well distant from the gut[35]. These differential cytokine responses to commensals and pathogens have also been demonstrated in man[36].

Immunological alterations are increasingly being reported in IBS with the hypothesis that there is a low-grade inflammatory state associated with this condition. Investigation of the role of the microbiota in mediating these immune alterations in IBS are in their infancy, but further study may provide some insight into the pathogenesis of IBS. Accumulating data support the presence of an immune engagement between the microbiota and the host in IBS; an interaction that involves both systemic and mucosal immunity that could generate a low-grade inflammatory response.

Toll like receptors, mucosal immunity and IBS

A number of factors may allow the epithelium to tolerate commensal organisms with the innate immune system and pattern recognition receptors (PPRs) playing a critical role. PPRs, such as toll-like receptors (TLRs), mediate the interaction between the host and the microbiota and, in doing so, facilitate both inflammatory and homeostatic processes[37]. Indeed commensal-TLR interactions in the intestine have been predominantly implicated in homeostatic events[38]. Commensal signalling through TLRs results in the inhibition of the NF-κB inflammatory pathway[39] and also in the upregulation of TLR inhibitory proteins such as PPARγ. For one commensal, *Bacteroides fragilis*, symbiosis with the host has been shown to be mediated through the activation of TLR2 on Foxp3$^+$ regulatory T cells by a PSA, produced by the bacterium, resulting in immunological tolerance[40]. The intimacy of the interaction between the microbiota and these PPR's is also illustrated by the observation that the microbiota determines expression of TLR2 in the colon[41].

Expression of TLRs has also been recently reported to be altered in IBS. Increased levels of TLRs 4 and 5 and decreased levels of TLRs 7 and 8 have been shown in colonic biopsy tissue of IBS patients[42]. The work of Belmonte *et al*[43] further characterised these changes according to IBS subtype and showed that only the IBS-mixed subgroup showed upregulation of TLRs 2 and 4. These authors also showed the alterations in expression were confined to epithelial cells. Similar alterations in expression of TLRs have been shown in a rat model of stress-induced IBS[44]. Work performed by Tattoli *et al*[45] has further demonstrated that TLR ligands can directly affect gastrointestinal motility possibly implying that disruptions in the composition of the microbiota may result in changes in gut motility, as observed in IBS patients. The colonic mucosal tissue from IBS patients also displays an altered cytokine profile possibly reflecting the alterations in TLR expression[46]. And, whilst evidence has been advanced to indicate that alterations in the microbiota are present in IBS[47], how such changes might directly affect TLR expression and cytokine production in these patients remains unclear. Moreover, the microbiota may also have the capacity to influence expression of non-TLR receptors, such as μ-opioid and cannabinoid receptors, in IECs which may be equally relevant in the context of IBS[48].

Commensals, systemic immunity and IBS

In addition to the ability of the microbiota to modulate local mucosal immune responses, extensive clinical and experimental data have been generated to indicate that commensal bacteria can also modify systemic immune responses[49]. Commensals may promote the development of T helper cells, including T_H17 cells and result in a controlled inflammatory response which is protective against pathogens, in part, through the production of IL-17[50]. Commensals, such as *Bifidobacterium infantis* and *Faecalobacterium prasunitzii*, differentially induce regulatory T cells (Tregs) and result in the production of the anti-inflammatory cytokine, IL-10[51]. Similarly, colonization of mice with *Bifidobacterium fragilis* resulted in the expansion of IL-10 producing Tregs and amelioration of the disease experimental autoimmune encephalomyelitis in a mouse model[52]. The regulation of immunity by commensals is likely to occur, not only *via* TLRs, but also through a variety of commensal-derived substances, ranging from relatively nonspecific fatty acids and peroxides to highly specific bacteriocins[53,54], which can inhibit or kill other, potentially pathogenic, bacteria[28]; meanwhile certain strains produce proteases capable of denaturing bacterial

toxins[55].

Systemic immune alterations have also been observed in IBS. B cells isolated from the blood of IBS patients display an amplified activation level[56]. Similarly, T cells isolated from both blood and colonic biopsies showed increased activation levels in IBS patients compared to healthy controls; evidenced by increased expression of the activation markers CD69 and HLA-DR[57]. Increased levels of antibodies to bacterial flagellin[58,59] and elevated levels of beta-defensin-2 in the faeces have also been demonstrated in IBS[60]. In addition, the ratio of IL-10 to IL-12 cytokines from peripheral monocytes is decreased in IBS patients compared to healthy controls; this ratio was normalised following treatment with *Bifidobacterium infantis*[61].

COMMENSAL REGULATION OF THE GUT-BRAIN AXIS: RELEVANCE FOR IBS

The ability of gut microbiota to communicate with the brain and thus modulate behaviour is emerging as an exciting concept in health and disease. Indeed, it has been proposed that the microbiota can influence the development[62] and function[63] of the central nervous system (CNS), thereby, leading to the concept of the microbiota-gut-brain axis[64,65]. Studies focusing on the impact of enteric microbiota on the host and, in particular, on the CNS are essential to our understanding of how the gut-brain axis may influence the pathogenesis of IBS[64]. Moreover, functionally, an association between psychological stress, intestinal transit and "dysbacteriosis" has been reported[66].

Influence of commensals on the central nervous system

There is clear evidence of communication between commensals and the CNS facilitated through neuroendocrine, neuroimmune, the autonomic nervous system and the enteric nervous system (ENS), collectively forming complex networks. This communication functions bidirectionally with the microbiota influencing CNS function and *vice versa*[67]. For example, oral administration of *Bifidobacterium infantis* 35624 influences the concentrations of 5-hydroxyindole acetic acid and dihydroxyphenylacetic acid in the frontal cortex and amygdala, respectively[68]. Moreover, *Bifidobacterium infantis* 35624 has been shown, in an animal model of depression and visceral hypersensitivity (the maternally-separated rat), to normalise immune responses, reverse behavioural deficits and restore basal norepinephrine concentrations in the brainstem[68]. A more recent study, describing the effects of *Lactobacillus rhamnosus* (*JB-1*) on behaviour and central expression of gamma aminobutyric acid receptors, demonstrated these effects to be vagal-dependent thereby establishing the vagus nerve as a key pathway in transducing microbe-gut to brain signals[69]. Germ-free models have also proven to be a useful tool in interrogating the influence of the gut microbiota on central nervous system function. For example, germ free mice display altered central expression of the neurotropic factor; brain derived neurotropic factor, as well as serotonin. Moreover, the latter was resistant to restoration of the microbiota in adulthood[70], implicating a role for the microbiota in early-life development and its absence with persistent long-term effects on hippocampal gene expression. The first clinical study demonstrating the influence of commensal organisms on brain activity, using a probiotic cocktail, is of particular relevance to IBS. Healthy female subjects, who consumed the probiotic cocktail containing *Bifidobacterium animalis* subsp *Lactis*, *Streptococcus thermophiles*, *Lactobacillus bulgaricus*, and *Lactococcus lactis* subsp *Lactis* twice daily for four weeks, exhibited altered activity in brain regions that control central processing of emotion and sensation[71], areas of particular relevance in the context of IBS. Collectively, these latter observations could address some of the proposed pathophysiological mechanisms associated with symptom development in IBS, namely, disturbances in the brain-gut axis.

Influence of commensals on the enteric nervous system and neuromuscular function

The ENS and human smooth muscle cells, key regulators of intestinal motility, express the machinery necessary to respond directly to commensals[72,73]. Therefore, commensals have the capacity to influence neuromuscular function, indicating a role for this interaction in IBS. Direct influence on the ENS can be inferred by studies examining peripheral TLR expression. TLR-4 and TLRs-3 and -7 are expressed in the ENS of both the murine and human intestine and colon[72]. Moreover, studies on human smooth muscle cells suggest that a direct interaction between these and the microbiota is possible, as stimulation of TLR4 induced inhibition of smooth muscle contractility[73]. While other studies have demonstrated that commensal organisms may influence neurotransmitter release and production of gamma-aminobutyric acid[74]. Of more direct relevance to IBS, manipulating the host-microbiota interaction to improve neuromuscular function was demonstrated in a study using *Lactobacillus paracasei,* in which the bacterium attenuated gut muscle hypercontractility in an animal model of post-infectious IBS[75]. This effect was strain-dependent and appeared to be mediated, in part, through a modulation of the immunological response to the initial infection and, in part, through the direct effects of the organism, or a metabolite thereof, on gut muscle. Additionally, studies interrogating the effects of several microbes on intestinal motility in germ-free animals highlight the selective and divergent effects of individual strains on intestinal motor function, with some, but not all strains, influencing transit[76].

Indirect interactions are mediated through commensal-derived factors including methane (CH_4), hydrogen sulphide (H_2S) and short-chain fatty acids. Noteworthy, in the context of IBS, levels of *Methanobrevibacter smithii* in the stools of constipation-predominant IBS patients correlate with levels of CH_4 production[77], suggesting that, in a subgroup of constipation-predominant IBS patients,

bacterial-derived CH_4 contributes to the pathophysiology of the disorder. Moreover, CH_4, produced mainly by *Methanobrevibacter. smithii* in humans, has been associated with alterations in intestinal motility. In an animal model, CH_4 significantly reduces intestinal transit following *in vivo* infusion[78], and *in vitro* recordings suggesting that one of the mechanisms by which CH_4 influences the ileal contractile response is *via* regulatory control of sensory neurotransmission[79]. Like CH_4, H_2S also exerts an inhibitory effect on intestinal neuromuscular function[80,81]. Sulphate reducing bacteria, responsible for the disposal of H_2, and subsequent generation of H_2S, are also relevant in the context of IBS[82]. H_2S exerts an inhibitory effect on neuromuscular activity[83]. Further studies confirmed an inhibitory role for H_2S on motor complexes and also indicated that this effect was independent of the ENS[84]. Moreover, H_2S-induced inhibitory responses were sensitive to potassium (K) channel and, in particular K_{Ca}^+ channel, blockade in the presence of neural inhibition *in vitro*[84].

One of the principal roles of the colonic microflora is to salvage energy from carbohydrates that have not been digested in the upper gastrointestinal tract, the major end-products of which are short-chain fatty acids (SCFA), in addition to the gaseous end-products H_2, CO_2 and CH_4[85,86]. The SCFAs include acetate, propionate and butyrate, the latter of which has multiple effects in the gastrointestinal tract, including impacts on visceral perception, motility and secreto-motor function[87]. Noteworthy, faecal bacteria from diarrhoea predominant IBS patients produce less SCFA in an *in vitro* fermentation system. Differences in SCFA production by colonic bacterial flora in patients with diarrhoea predominant IBS may be related to the development of gastrointestinal symptoms and, in particular, neuromuscular dysfunction[88]. SCFAs may also influence ENS plasticity through monocarboxylate transporters[89]. However, these plastic changes in the ENS display a level of SCFA specificity, as neither acetate nor propionate alter the neurochemical make-up of the myenteric plexus[89]. Moreover, butyrate also appears to directly influence intracellular calcium concentrations in myenteric neurons[90] as well as activating the G protein coupled receptors, GPR41 and GPR43 which are widely expressed in rat and human colon[91]. However, altering the activity of the microbiota, with prebiotics for example, supports the concept that it is not only the presence or absence of the microbiota that is capable of regulating intestinal motor physiology, but that qualitative changes in the microbiota can alter neuromuscular function[92]. The issue of differentiating between direct effects of the microbiota or its products and the secondary consequences induced by components of the microbiota is one that bedevils the interpretation of many studies in this area.

CONCLUSION

It is clear that the microbiota is altered in IBS and that such alterations could well contribute to the pathogenesis of the disorder through, for example, increased permeability, an altered immune profile, effects on the CNS and modulation of gut neuromuscular function. To date, however, there is a paucity of clinical studies in IBS patients evaluating the effects of selectively manipulating the microbiota based on preclinical evidence leading to a causality dilemma; whether changes in the microbiota are cause or effect in disorders such as IBS. It is quite unlikely in the context of IBS, given its comorbidities and variability in symptom presentation, that a single microbial alteration will be identified as causative for all IBS pathogenesis, or that one microbial intervention will universally improve all symptoms. Rather, several interventions may prove efficacious in ameliorating various subgroups or individual symptoms. Moreover, focus has moved from the description of qualitative changes in the microbiota in IBS to their metabolic activity. Such an approach has only recently been applied in the context of IBS, where the activity of the microbiota was assessed in relation to symptom presentation[93]. This approach now needs to be expanded with the expectation that data from such studies which will not only determine which microbes may be protective, or causative in IBS, but will also identify which metabolites may be effective therapeutically.

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Recent advances in pharmacological treatment of irritable bowel syndrome

Georgia Lazaraki, Grigoris Chatzimavroudis, Panagiotis Katsinelos

Georgia Lazaraki, Grigoris Chatzimavroudis, Panagiotis Katsinelos, Department of Endoscopy and Motility Unit, G. Gennimatas General Hospital, School of Medicine, Aristotle University of Thessaloniki, 54635 Thessaloniki, Greece
Author contributions: Lazaraki G mainly drafted and wrote the manuscript; Chatzimavroudis G is involved in writing and editing the manuscript; Katsinelos P is responsible for drafting and critical approval of the manuscript.
Correspondence to: Panagiotis Katsinelos, MD, PhD, Assistant Professor of Gastroenterology, Department of Endoscopy and Motility Unit, G. Gennimatas General Hospital, School of Medicine, Aristotle University of Thessaloniki, Ethnikis Aminis 41, 54635 Thessaloniki, Greece. gchatzimav@yahoo.gr
Telephone: +30-2310-963341 Fax: +30-2310-963341
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Abstract

Irritable bowel syndrome (IBS) is a highly prevalent functional disorder that reduces patients' quality of life. It is a chronic disorder characterized by abdominal pain or discomfort associated with disordered defecation in the absence of identifiable structural or biochemical abnormalities. IBS imposes a significant economic burden to the healthcare system. Alteration in neurohumoral mechanisms and psychological factors, bacterial overgrowth, genetic factors, gut motility, visceral hypersensitivity, and immune system factors are currently believed to influence the pathogenesis of IBS. It is possible that there is an interaction of one or more of these etiologic factors leading to heterogeneous symptoms of IBS. IBS treatment is predicated upon the patient's most bothersome symptoms. Despite the wide range of medications and the high prevalence of the disease, to date no completely effective remedy is available. This article reviews the literature from January 2008 to July 2013 on the subject of IBS peripherally acting pharmacological treatment. Drugs are categorized according to their administration for IBS-C, IBS-D or abdominal pain predominant IBS.

Key words: Irritable bowel syndrome; Irritable bowel syndrome constipation; Irritable bowel syndrome-diarrhea; Constipation; Diarrhea; Irritable bowel syndrome treatment; Irritable bowel syndrome-pain

Core tip: Irritable bowel syndrome (IBS) is a highly prevalent functional disorder that reduces patients' quality of life and imposes a significant economic burden to the healthcare system. This article extensively reviews the literature from January 2008 to July 2013 on the subject of IBS peripherally acting pharmacological treatment. Pathophysiology background and mode of action in IBS of each substance are also discussed.

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INTRODUCTION

Irritable bowel syndrome (IBS) is a highly prevalent (10%-20% of the United States adult population)[1] functional disorder that reduces patients' quality of life. IBS is defined in the Rome Ⅲ criteria as a chronic disorder characterized by abdominal pain or discomfort associated with disordered defecation [either constipation (IBS-C), diarrhea (IBS-D), or mixed/ alternating symptoms of constipation and diarrhea (IBS-M)][2]. Symptoms should begin at least 6 mo before and abdominal pain or discomfort should be present at least 3 d per month for 3

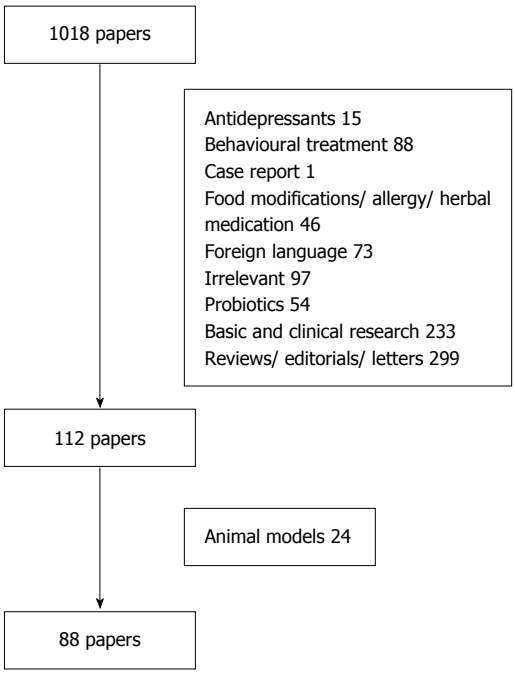

Figure 1 Flowgram of the selected studies for the review.

mo during last 6 mo and should be associated with two or more of the following: improvement with defecation, onset associated with a change in stool frequency and/or change in stool form. Bloating and abdominal distention are also frequently reported by IBS patients reflecting sensitivity to normal amounts of intestinal gas. By definition, no disease that could explain the symptoms should be present[2].

IBS represents important costs for the healthcare system. One should look carefully for alert signs [*i.e.*, anemia, unintentional weight loss, gastrointestinal (GI) bleeding, nausea/vomiting, family history of cancer] of a serious underlying disorder to differentiate functional symptoms from organic disorders. Thus, for younger patients who meet criteria for IBS with normal physical examination and no "red flags", an extensive laboratory work up should not be considered[3].

It is likely that the definition of IBS represent an auspice of different conditions/disease states for which we lack specific biomarkers. Alteration in neurohumoral mechanisms and psychological factors, bacterial overgrowth, genetic factors, gut motility, visceral hypersensitivity, and immune system factors are currently believed to influence the pathogenesis of IBS[4-6]. It is possible that there is an interaction of one or more of these etiologic factors leading to heterogeneous symptoms of IBS.

Since IBS is not a single disease entity, but rather likely consists of several different disease states, IBS treatment is predicated upon the patient's most bothersome symptoms. Specifically, our treatment strategy seems to target constipation, diarrhea, bloating or pain[7]. A wide range of medications (prokinetiks, antispasmodics, sedatives, tranquilizers, laxatives, fecal bulking agents, probiotics and antibiotics) along with life style and diet modifications have been proposed for this highly prevalent condition; however to date there is no definite effective cure for this state[7].

In the present review, we report the results of our search in PubMed, Scopus, and Google Scholar databases from January 2008 to July 2013 on the subject of IBS peripherally acting pharmacological treatment. MeSH terms "irritable bowel syndrome treatment" and "IBS treatment" were used as search terms. English-written articles only were included. Data from metanalysis and clinical studies were included. Abstracts, case reports, comments/reviews, *in vitro* studies, animal studies and pharmacogenetic studies were excluded from the review. The search resulted in 1018 papers after omission of duplicate articles; finally 86 papers were included after omission of non-relevant articles. Flowgram of the search is presented in Figure 1. Drugs are categorized according to their administration for IBS-C, IBS-D or abdominal pain predominant IBS.

IBS-C

The evaluated studies in each category are reported in Table 1. Below is a list of available treatment methods based on the findings.

Laxatives

Several clinical observations have reported a decrease in bowel motility and a prolonged transit time in patients with IBS-C compared with controls[8,9]. Also, some IBS-M patients report an alternation in bowel habits with extended periods with small, hard bowel movements or no bowel movement followed by periods with loose stools. Osmotic agents, stimulants, and stool softeners are all comprised in the category of laxatives. Polyethylene glycol (PEG) is the only laxative that has been evaluated in the treatment of IBS. The first study published in 2006 assessed the effects of PEG 3350 in patients with IBS-C (Rome Ⅱ criteria)[10]. Mean bowel movement frequency was significantly increased; however, there was no change in mean pain level for the group with the PEG therapy. In the last 5 years 2 new studies evaluated the efficacy of PEG in IBS-C. The first study[11], a randomized, double-blind, placebo-controlled trial used fasting and postprandial (PP) perception of rectal distension as measurements. Symptoms were also recorded. Forty two patients with IBS-C (Rome Ⅱ criteria) and with a pain threshold of < 32 mmHg participated. Patients received either oral PEG, 3.45 g t.i.d. orally for 30 d or placebo. PEG improved consistency of faeces. Both, PEG and placebo increased bowel movements per week ($P < 0.001$), and relieved symptoms without significant side-effects. However, there were not significant differences in fasting and PP rectal tone and thresholds for first sensation, gas sensation, urge to defecate, and pain between PEG and placebo. The investigators concluded that changes in rectal tone and sensation were not related to PEG 3350 and placebo effects. Patients with IBS-C gained some

Table 1 Pharmacological treatment irritable bowel syndrome-C studies and clinical efficacy during last 5 years

Category/No. of studies/ Ref.	No. of patients	*vs* Placebo	Abdominal distention/ pain	SBMs	Stool consistency	Recommendation *vs* placebo
Laxatives/2						
Awad *et al*[11] 2010		Yes	NS	NS	SS	Equal
Chapman *et al*[12] 2013		Yes	NS	SS	SS	Equal
Linaclotide/5						
Johnston *et al*[19] 2010		Yes	SS	SS	SS	Superior
Chey *et al*[20] 2012		Yes	SS	SS	-	Superior
Rao *et al*[21] 2012		Yes	SS	SS	-	Superior
Quigley *et al*[22] 2013		Yes	SS			Superior
Videlock *et al*[23] 2013		Yes	SS	SS	SS	Superior
5-HT4 agonists						
Renzapride/2						
Lembo *et al*[49] 2010		Yes	SS	SS	SS	Superior but AE
Ford *et al*[82] 2009	726	Yes	NS	NS	NS	Equal
Cisapride/1						
Ford *et al*[82] 2009	726	Yes	NS	NS	NS	Equal
Lubiprostone/4						
Johanson *et al*[57] 2008		Yes	SS (16/32/48 μg)	SS (16/32/48 μg)	SS (16/32/48 μg)	Superior
Fukudo *et al*[58] 2011		Yes	SS (48 μg)	SS (48 μg)	SS (48 μg)	Superior(48 μg)
Drossman *et al*[59] 2009		Yes	SS (16 μg)	SS (16 μg)	SS (16 μg)	Superior
Chey *et al*[60] 2012		No, extention study, comparison to inclusion	SS	SS	SS	Favourable profile of effectiveness, safety, tolerability
CDCA/1						
Rao *et al*[65] 2010		Yes	-	SS	SS	Superior

SBMs: Spontaneous bowel movements; SS: Statistically significant; NS: Not significant; 5-HT: 5-hydrodytryptamine; CDCA: Chenodeoxycholic acid.

relief from their symptoms both with PEG and placebo. In the second study[12], following a 14-d run-in period without study medication, 139 adult patients with IBS-C were randomized to receive PEG 3350+E or placebo for 28 d. The primary endpoint was the mean number of spontaneous bowel movements per day in the last treatment week. In both groups there was an increase in mean bowel movement frequency compared to run-in. The difference between the groups in week 4 from 4.40 (PEG 3350+E) to 3.11 (placebo) was statistically significant (95%Cl: 1.17- 1.95; $P < 0.0001$). However, although mean severity score for abdominal discomfort/pain was significantly reduced compared with run-in with PEG 3350+E, there was no difference *vs* placebo. Spontaneous bowel movements (SBMs), responder rates, stool consistency, and severity of straining also showed superior improvement in the PEG 3350+E group over placebo in the fourth week. The authors concluded that PEG 3350+E was superior to placebo for relief of constipation but resulted in no improvement to abdominal discomfort/pain compared to placebo in spite of the presence of a statistical significant improvement in abdominal discomfort/pain that was observed compared with baseline.

Guanylate cyclase-c receptor agonists

Linaclotide is a guanylin peptide. Guanylin peptides are a family of peptides with similar structure to the heat-stable enterotoxin produced by *Escherichia coli* and other enteric bacteria that cause secretory diarrhea. They have a conformation to bind with guanylate cyclase-c (GC-C) receptors. Binding of GC-C receptors, which are abundantly expressed on enterocytes lining the intestine, stimulates production of cyclic guanosine monophosphate[13]. This leads to a cascade of intracellular events resulting in the activation of the cystic fibrosis transmembrane conductance regulator (CFTR) and the subsequent transepithelial chloride (Cl^-) and potassium (K^+) ion efflux from enterocytes, with secondary passive water secretion into the intestinal lumen[14]. Linaclotide is minimally absorbed and therefore believed to act locally[15]. In animal models linaclotide has been shown to stimulate intestinal secretion, accelerate GI transit time and reduce visceral pain through GC-C dependent activation[13].

Clinical studies have investigated linaclotide in patients with IBS-C and chronic constipation (CC). In an earlier phase Ⅱa study[16] 36 women with IBS-C that received a 5-d course of linaclotide 1000 mg. The result was a significantly accelerated ascending colon ($P = 0.004$) and total colonic transit time at 48 h ($P = 0.01$). Linaclotide had no effect on gastric emptying or small bowel transit time; however, it accelerated the time to first bowel movement, decreased stool consistency, and enhanced ease of stool passage. Data from CC studies have demonstrated improvement of weekly SBMs and various other constipation-related clinical parameters, including stool consistency and straining in a dose-dependent fashion. In addition, patients treated with linaclotide experienced improvements in abdominal discomfort, bloating, and constipation severity. Constipation symptoms tended to return to baseline, without evidence of a rebound, after discontinuation of linaclotide[17,18]. The overall frequency of adverse events reported with linaclotide and placebo

were similar[18], with diarrhea the most common adverse event (AE) reported with linaclotide.

In the recent years 4 studies and 1 meta-analysis were published regarding linaclotide efficacy in IBS. Specifically, a phase Ⅱb study[19] published in 2010 the efficacy, safety, and dose response of linaclotide administered at 75, 150, 300, and 600 μg once daily for 12 wk. Four hundred twenty patients with IBS-C were assessed. The study recorded changes from baseline in daily bowel habits and daily abdominal symptoms. There were also weekly global assessments. All doses of linaclotide significantly improved the frequency of SBMs and complete spontaneous bowel movements (CSBM). They also improved the severity of straining, stool consistency and abdominal pain compared with placebo. Mean changes in abdominal pain (assessed on a 5-point scale) from baseline were -0.71, -0.71, -0.90, and -0.86 for linaclotide doses of 75, 150, 300, and 600 μg, respectively, compared with -0.49 for placebo. Other abdominal symptoms and global measures of IBS-C were also improved compared with placebo. The drug presented effect within the first week that sustained during the 12 wk of treatment. Diarrhea was the only dose-dependent adverse event and was usually of mild or moderate severity. Although all linaclotide doses were associated with a statistically significant improvement compared with placebo for most end points, the higher doses of linaclotide (*i.e.*, 300 and 600 μg) were generally more effective across most parameters. Because the 300 and 600 μg doses provided comparable efficacy and the higher dose was associated with an increase in side effects, a dosage of 300 μg per day was selected for continued evaluation in phase Ⅲ trials. In 2012, 2 studies were published together. The first, a phase Ⅲ trial[20] included 804 patients with IBS-C (Rome Ⅱ criteria). Participants were randomized to linaclotide 290 μg orally or placebo once daily for 26 wk. The study had the rigorous end point to be a "responder" as recommended for IBS-C in the Food and Drug Administration guidelines for IBS clinical trials (May 2012); the percentage of responders was 33.7% in the linaclotide group compared with 13.9% in the placebo group ($P < 0.0001$). Significant differences in favor of linaclotide ($P < 0.0001$) were also observed for an even more rigorous end point which required that patients meet the ≥ 30 % of improvement in worst abdominal pain and both ≥ 3 CSBMs/wk and an increase of ≥ 1 CSBM/wk from baseline for a minimum of 9 out of 12 wk. The effects of linaclotide on abdominal and bowel symptoms were manifested within the first week of treatment and sustained over the entire 26-wk treatment period. The second study[21] randomized 800 patients with IBS-C to 290 μg linaclotide orally or placebo once daily, for 12 wk. This was followed by a 4-wk withdrawal period after randomization. The same FDA end points that were used in the former trial were used as primary end points. In the linaclotide group 33.6% of patients compared with 21% of patients in the placebo group ($P < 0.0001$) [number needed to treat (NNT) = 8.0, 95 %CI: 5.4-15.5] met the FDA end points. A statistically significant percentage of patients treated with linaclotide *vs* placebo met the rest of end points (primary and secondary, $P < 0.05$ and $P < 0.001$ respectively). During the withdrawal period, after randomization, patients remained improved as long as they were receiving linaclotide whereas those that were re-randomized to placebo presented relapse of symptoms. Symptoms did not become worse relative to baseline. In both studies AEs were generally comparable between linaclotide and placebo groups, with the exception of diarrhea, which occurred more commonly with linaclotide than with placebo, and was mostly mild or moderate in severity. Recently further analysis on the data of these 2 trials was performed[22]. Overall, 803 and 805 patients were randomized. A significantly greater proportion of patients in the linaclotide group *vs* placebo patients presented improvement in abdominal pain/discomfort during the 12 wk treatment period. Similarly, significantly more linaclotide-treated patients compared to placebo-treated patients were responders for ≥ 13 wk (abdominal pain/discomfort: 53.6% *vs* 36.0%; IBS degree-of-relief: 37.2% *vs* 16.9%; $P < 0.0001$). The proportion of sustained responders was also significantly greater with linaclotide *vs* placebo in both trials ($P < 0.001$). In these trials, treatment-emergent AEs were reported by more than half of those receiving linaclotide, with the most noteworthy being a greater incidence of diarrhea in one of five subjects. These observations are obviously related to the secretagogue mechanism of the drug.

Finally a meta-analysis to determine the efficacy of linaclotide, compared with placebo, for patients with IBS-C or CC was published in 2013[23]. The search identified seven trials of linaclotide in patients with IBS-C or CC with six finally included in the analysis. The relative risk (RR) for the response to treatment with 290 mg linaclotide, compared with placebo, was 1.95 (95%CI: 1.3-2.9), and the NNT was 7 (95%CI: 5-11). Linaclotide also improved the stool form and reduced abdominal pain, bloating, and overall symptom severity in patients with IBS-C or CC.

Therefore, linaclotide has the potential to offer relief for the multiple symptoms from which patients with IBS-C suffer.

Serotonin receptor modulators

Serotonin (5-hydrodytryptamine; 5-HT) is predominantly (90%-95% of the body's 5-HT) produced in the enterochromaffin (EC) cells in the intestinal mucosa, and also by a subpopulation of enteric neurons[24]. Acting as a signaling molecule through the intrinsic and extrinsic afferent nervous system of the GI tract, 5-HT plays an important role in various aspects of GI sensory, secretory, absorptive, and motility function[24]. Abnormal levels have been shown in individuals with IBS. Several studies describe increased serotonergic activity in association with IBS-D[25-28]. Similarly, a decrease in serotonergic activity has been observed in IBS-C[26,27]. Pharmaceutical agents acting on 5-HT receptors have, therefore, evolved to ameliorate the smooth muscle spasm, abdominal

pain, and change in bowel habit that IBS patients experience. Of the identified serotonin-receptor subtypes, the 5-HT1p, 5-HT3, 5-HT4, and 5-HT7 receptors seem to play an important role in GI tract functioning[29]. Intraluminal distension of the intestine (translated to abdominal pain in IBS patients) stimulates 5-HT release from EC cells and activates 5-HT3 receptors of primary afferent neurons. 5-HT3 receptors activation results in the release of various neurotransmitters, such as acetylcholine. This induces colonic transit acceleration and abnormal water transport, which in turn leads to defecation abnormalities. Receptor antagonists of 5-HT3 have been reported to slow small bowel and colonic transit, decrease intestinal secretion and colonic tone[29]. Of great relevance to IBS-C and CC are the 5-HT4 receptors. In the gastrointestinal tract, 5-HT4 receptors are located on enteric neurons and smooth muscle cells, and their stimulation leads to acetylcholine release causing prokinetic effects. Based on biochemical structure, 5-HT4 agonists can be broadly categorized as benzamides (metoclopramide, cisapride, renzapride, mosapride, clebopride, and ATI-7505), carbazimidamides (tegaserod), benzofurancarboxamides (prucalopride), and other agonists such as velusetrag[15].

5-HT4 agonists

Tegaserod is a selective 5-HT4 receptor partial agonist with promotility effects in the small and large intestine[30-32] and modulation of visceral sensation[33,34]. The efficacy and tolerability of tegaserod in the treatment of women with IBS-C was initially reported in 2 multicenter, double-blind, placebo-controlled trials. More than 2000 patients from the Western hemisphere were involved[35,36]. These clinical trials consistently reported the superiority of tegaserod over placebo in improving IBS symptoms (abdominal pain, stool frequency, stool consistency, straining, and bloating). Later, other trials have confirmed the safety and tolerability of tegaserod[37-40]. Side effects included headache, abdominal pain and diarrhea. Although there were no reports of ischemic colitis in the clinical trials, 26 events of possible colonic ischemia were identified during postmarketing surveillance. This was translated to an estimated incidence of 7 cases of colonic ischemia per 100000 patient-years of tegaserod use[41]. Cardiovascular and cerebrovascular events in the group receiving tegaserod were also reported later[42] in a pooled analysis[13] cardiovascular ischemic events in 11614 patients receiving tegaserod compared with 1 out of 7031 patients in the placebo group (0.1% *vs* 0.01% respectively, $P = 0.02$)]. A pathogenetic mechanism that was proposed was that tegaserod may induce platelet aggregation through 5-HT4 receptors located on platelets[43]. Later retrospective studies found no relationship between tegaserod and cardiovascular events; however the drug was definitely withdrawn from the market in 2009.

Mosapride has stimulatory effects on gastric and colonic motility[44]. Unlike cisapride, mosapride does not bind to K1 channels or D2 dopaminergic receptors. Mosapride was primarily developed for upper GI tract conditions, such as functional dyspepsia, gastroesophageal reflux disease, and nausea and vomiting[15]. Data from animal models show that mosapride accelerates colonic transit time[45], augments motility in the proximal and distal colon in a dose-dependent manner[45] and has a stimulatory effect on the defecatory reflex[46,47]. In humans a study showed that mosapride changes rectosigmoid motility and perception in patients with IBS[48].

In 2010, the efficacy and safety of renzapride were assessed in a study of 1798 women with IBS-C. Patients were randomized to a 4 mg daily dosage of renzapride, 2 mg *b.i.d.* or placebo for 12 wk[49]. The primary end point was global relief of IBS symptoms. A subset of patients ($n = 971$) were enrolled in a 12-mo, open-label study of oral intake of renzapride 4 mg daily. Relief of overall IBS symptoms was achieved at (mean ± SD) 0.55 ± 0.04, 0.60 ± 0.04 and 0.44 ± 0.04 in the renzapride 4 mg daily, 2 mg b.i.d. and placebo groups ($P = 0.027$ and $P = 0.004$ respectively). Stool consistency and frequency were statistically significantly improved in the renzapride group, as well as bloating and abdominal distension. Three episodes of ischemic colitis were reported. The authors concluded that due to the limited benefit of renzapride over placebo and the reported cases of ischemic colitis, no further study with renzapride as possible treatment of IBS-C should be conducted.

Lubiprostone (chloride channel stimulators)

Lubiprostone is a bicyclic fatty acid derivative of prostaglandin E1. The underlying mechanism of lubiprostone is stimulation of electrogenic chloride secretion by activating chloride channel type-2 (ClC-2)[50] and CFTR[51] in the intestinal epithelial cells apical membrane. Primary functions of ClC-2 channels include maintenance of the membrane potential of the cell, regulation of pH and cell volume, and regulation of chloride ion channel transport and fluid secretion. Dose-dependent ClC-2 activation of ClC-2 channels or CFTR chloride channels in intestinal epithelial cells produces an active secretion of chloride ions from cells into the intestinal lumen followed by a passive secretion of electrolytes and water which increases the liquidity of the luminal contents. The luminal distension increased by intestinal fluid promotes the GI tract motility which in turn increases the intestinal and colonic transit[41]. Besides this mechanism, lubiprostone enhances and stimulates contraction in colonic as well as gastric muscles through prostaglandin E receptors (EP1 or EP4)[52], suggesting the modulatory effects of lubiprostone on GI motility through the activation of prostaglandin receptors.

Previous work has demonstrated that lubiprostone accelerates small bowel and colonic transit and increases the frequency of bowel movement in healthy adults[53]; however, the thresholds for pain do not seem to be affected by lubiprostone. Multiple randomized controlled trials (RCTs) have demonstrated the efficacy of lubiprostone in idiopathic CC[54-56]. In these trials, lubiprostone was consistently found to be superior to placebo at increasing

the number of weekly SBMs as well as improving stool consistency, straining, constipation severity, bloating, and treatment effectiveness. The most commonly reported side effects included nausea, headache, and diarrhea. A pooled analysis of 91 patients meeting diagnostic criteria for IBS-C from the 2 phase Ⅲ constipation trials revealed significant improvements in constipation symptoms as well as abdominal symptoms due to lubiprostone as compared to placebo. This observation led to further evaluation of lubiprostone in the treatment of IBS-C[41].

The efficacy and tolerability of lubiprostone have been assessed in several RCTs. First, 195 IBS-C patients received daily doses of 16 (8 μg twice daily), 32 (16 μg *b.i.d.*) or 48 μg (24 μg *b.i.d.*) lubiprostone or placebo for 3 mo[57]. In the lubiprostone group mean abdominal discomfort/pain scores were significantly improved compared to placebo after 1 and 2 mo ($P = 0.023$ and $P = 0.039$, respectively). All 3 doses of lubiprostone were superior to placebo with regard to frequency of SBM ($P = 0.0499$), constipation severity ($P = 0.0056$), stool consistency ($P < 0.0001$), and straining ($P = 0.0094$) in each of the 3 mo of treatment. Treatment with lubiprostone showed significantly higher rates of GI AEs ($P = 0.020$), especially diarrhea and nausea. The 16 μg/d dose demonstrated the optimal combination of efficacy and safety and was therefore the dose selected for further study in subsequent phase Ⅲ clinical trials. Another Japanese trial[58] studied adequate dosing of lubiprostone for the treatment of constipation in CC or IBS-C patients. One hundred seventy patients (128 without IBS and 42 with IBS) randomly received a placebo or 16 μg, 32 μg, or 48 μg of lubiprostone daily for two weeks. There was a dose-dependent increase in weekly average number of SBM compared to baseline in the first week (placebo: 1.5; 16 μg: 2.3, 32 μg: 3.5; and 48 μg: 6.8, per week, $P < 0.0001$). The 32 and 48 μg dosage treatments had a significantly higher primary efficacy endpoint than the placebo treatment ($P = 0.0017$, $P < 0.0001$, respectively). The 16 μg treatment showed no significant increase in change in SBMs during the first week over placebo. The primary endpoint was significantly better only in patients with IBS treated with 48 μg of lubiprostone than those treated with placebo ($P = 0.0086$).

There was a combined analysis of two phase-Ⅲ RCTs of lubiprostone 8 μg twice daily *vs* placebo for 12 wk that reported data of 1171 patients with IBS-C [Rome Ⅱ criteria][59]. Patients responded with respect to relief of IBS symptoms over the past week. Patients were characterized monthly responders (moderate relief in 4/4 wk or significant relief in 2/4 wk) or overall responders (a monthly responder in 2/3 mo of the trial). The primary efficacy endpoint was the percentage of overall responders. Significantly more patients in the lubiprostone group were considered overall responders compared with the placebo group (17.9% *vs* 10.1%, $P = 0.001$). Lubiprostone was also superior to placebo in improving individual IBS symptoms (abdominal discomfort/pain, stool consistency, straining, constipation severity), and quality of life (QOL). A similar incidence of AEs to those treated with placebo and lubiprostone was observed. Another recent study[60] evaluated the long-term safety, tolerability and patient outcomes of lubiprostone in patients with IBS-C. This was an extension study analyzing the data of 476 IBS-C patients who had completed one of two randomized phase Ⅲ studies. Patients received placebo or lubiprostone orally for 36-wk (8 μg, twice daily). Those receiving lubiprostone during the initial 12-wk phase Ⅲ trial experienced an increase in response from 15% to 37% and those initially receiving placebo experienced an increase in response from 8% to 31% at the conclusion of the 36-wk extension period. The overall safety profile of lubiprostone during this study was similar to that observed in the preceding phase Ⅲ studies. AEs were diarrhea (11.0%), nausea (11.0%), urinary tract infection (9.0%), sinusitis (9.0%) and abdominal distention (5.8%). Diarrhea and nausea were the most common treatment-related AEs.

An evidence-based systematic review was performed by the ACG IBS Task Force that evaluated lubiprostone in the treatment of IBS-C[61] concluding that "Lubiprostone in a dose of 8mg twice daily is more effective than placebo in relieving global IBS symptoms in women with IBS-C." Regarding men with IBS-C, the ACG task force suggested a need for further studies before a recommendation for use in this population. Lubiprostone is contraindicated in patients with mechanical bowel obstruction and should be avoided in patients with preexisting diarrhea; there have also been postmarketing reports of dyspnea (typically resolves over several hours but sometimes reoccurs with subsequent dosing)[41].

Bile acid modulators

Bile acids have been used in the treatment of patients with gallstones and cholestatic liver diseases. Longterm treatment is generally well tolerated other than the consistent side effect of diarrhea[62], which mimics the chronic loose stools observed in patients with a disrupted enterohepatic circulation from ileal disease resulting in spillage of bile acid into the colon[63]. In the setting of bile acid-related diarrhea after ileal resection or disease, high concentrations of bile acids decrease net colonic fluid and electrolyte absorption and induce secretion[64]. The mechanisms involved in promoting secretion include intracellular activation of adenylate cyclase, increased mucosal permeability, and inhibition of apical Cl^-/OH^- exchange[65]. Furthermore, instillation of bile acids directly into the colon increases intracolonic pressure and motility index[66].

Chenodeoxycholic acid (CDCA), a primary bile acid previously used for dissolution of gallstones, elicited diarrhea at dosages of 750 to 1000 mg/d[67]. CDCA (with hydroxyl groups in the 3α, 7α positions) promoted colonic secretion in comparison to its 3α, 7β epimer, ursodeoxycholic acid[68]. Previous studies in healthy volunteers[69] and in patients with gallstones who had CC receiving CDCA demonstrated a significant increase in the frequency of

Table 2 Pharmacological treatment irritable bowel syndrome-C studies and clinical efficacy during last 5 years

Category/No. of studies/Ref.	*n*	*vs* Placebo	Abdominal distention/pain	QOL/patient satisfaction/ global improvement	Stool consistency/ bowel habits	Recommendation *vs* placebo
5-HT3 antagonists						
Alosetron, cilansetron/4						
Cremonini *et al*[79] 2012	705	Yes	-	SS	-	Superior
Rahimi *et al*[80] 2008	4.17 Metanalysis	Yes	SS	SS	-	Superior
Andresen *et al*[81] 2008	7487 Metanalysis	Yes or mebeverine	SS	SS	-	Superior
Ford *et al*[82] 2009	7216 Metanalysis	Yes	SS	SS	-	Superior
Ramosetron/3						
Matsueda *et al*[85] 2008	418	Yes	SS (5/10 μg)	SS (5/10 μg)	-	Superior
Matsueda *et al*[86] 2008	539	Yes	SS (5 μg)	SS (5 μg)	SS (5 μg)	Superior
Lee *et al*[87] 2011	343	Mebeverine 135 mg t.i.d	NS(5 μg)	NS (5 μg)	NS(5 μg)	Equal
LX-1031/1						
Brown *et al*[92] 2011	155	Yes	SS only the 1st week (1000 mg 4 times/d)	-	SS (1000 mg 4 times/d)	Superior
Crofelemer/1						
Angel *et al*[94] 2008	246	Yes	SS 500 mg b.i.d	SS	SS	Superior
Antibiotics						
Rifaximin/2						
Pimentel *et al*[105] 2011	1260	Yes	SS	SS	SS	Superior
Menees *et al*[106] 2012	1803 Metanalysis	Yes	SS	SS	-	Superior
5ASA compounds, mesalazine/3						
Corinaldesi *et al*[108] 2009	20	Yes	NS	SS	NS	Equal
Andrews *et al*[109] 2011	12	No Comparison to baseline	SS	SS	-	-
Tuteja *et al*[110] 2012	17	Yes	NS	NS	NS	Equal

QOL: Quality of life; ASA: Aminosalicylic acid; SS: Statistically significant; NS: Not significant.

bowel movements and loosening of stools[70]. CDCA also accelerated colonic transit time resulting in ease of stool passage, and sense of complete evacuation[69].

Recently a double-blind placebo-controlled study[65] evaluated pharmacodynamics (colonic transit, bowel function) and pharmacogenetics of CDCA in 36 female patients with IBS-C. Participants were randomized to treatment with delayed-release oral formulations of placebo, 500 mg CDCA, or 1000 mg CDCA for 4 d. Colonic transit and ascending colon emptying were significantly accelerated in the CDCA group compared to the placebo group ($P = 0.005$ and $P = 0.028$, respectively). Looser stool consistency ($P = 0.003$), increased stool frequency ($P = 0.018$), and greater ease of passage ($P = 0.024$) were noted with CDCA compared with placebo. The investigators also found a correlation between fasting serum 7 alpha-hydroxy-4-cholesten-3-one (7aC4), a biomarker of bile acid synthesis, and colonic transit time in the placebo group: subjects with an increased 7aC4 showed a faster overall colonic transit time. In the CDC group, 7aC4 showed a modest influence on colonic transit at 24 h ($P = 0.055$) and 48 h ($P = 0.019$).

IBS-D

The evaluated studies in each category are reported in Table 2. Below is a list of available treatment methods based on the findings.

Antidiarrheals

As mentioned above alterations in bowel habits in IBS are in part a result of altered GI motility. Accelerated small bowel and colon transit times as well as exaggerated motility patterns have been demonstrated in those with IBS-D compared with controls[8,9]. Consequently, antidiarrheals remain among the more commonly used gut-acting agents used in the treatment of patients with IBS-D.

Among the class of antidiarrheals, loperamide is the only substance that has been evaluated in RCTs for the treatment of IBS. In total, four studies have been published[71-74] showing an improvement in the number of bowel movements and stool consistency compared to placebo in IBS-D patients; however results were rather disappointing regarding pain. The ACG Task Force recently performed a systematic review of antidiarrheals in the treatment of IBS and concluded that "The antidiarrheal agent loperamide is not more effective than placebo at reducing abdominal pain or global symptoms of IBS, but is an effective agent for treatment of diarrhea, improving stool frequency and stool consistency. RCTs with other antidiarrheal agents have not been performed.

Safety and tolerability data on loperamide are lacking"[61].

5-HT3 antagonist (alosetron, cilansetron, ramosetron)

As already mentioned receptor antagonists of 5-HT3 have been reported to slow colonic and small bowel transit and decrease intestinal secretion and colonic tone[29]. Early, rigorous, large clinical trials with alosetron 1 mg *b.i.d.* have all demonstrated the efficacy of alosetron in the global and individual symptoms of IBS-D in women. Alosetron decreases urgency, reduces stool frequency, and increases stool consistency. Improvement is seen within 1 wk of therapy, which persists throughout the treatment period[75,76]. The use of alosetron also demonstrated improvement in 3 QOL domains (including food/diet, social functioning, and role-physical on the validated generic QOL instrument, the SF-36 75)[77] and in the global IBS symptoms[78]. Recently a total of 705 women (severe IBS-D, Rome II criteria) were randomized to alosetron 0.5 mg *q.d.*, 1 mg *q.d.*, 1 mg *b.i.d.*, or placebo for 12 wk[79]. IBSQOL, treatment satisfaction, daily activities, and lost workplace productivity were evaluated. The authors concluded that in women with severe IBS-D, alosetron treatment, including 0.5 mg *q.d.,* resulted in statistically significant and clinically relevant improvements in health-related QOL, restriction of daily activities and treatment satisfaction over placebo.

During the last 5 years 3 metanalyses have been published on this subject. The first[80] included 8 multicenter, randomized, placebo-controlled, 12-wk clinical trials with 4170 patients with IBS randomized to receive either alosetron or placebo. Alosetron was significantly more effective in global improvement in symptoms than placebo (RR = 1.60; 95%CI: 1.44-1.76; $P < 0.001$), in adequate relief of IBS pain and discomfort (RR = 1.31; 95%CI: 1.20-1.43; $P < 0.001$). In the alosetron group, there were 4 cases of ischemic colitis (0.16%) and 2 cases of serious complications of constipation (0.08%). The second[81] trial collected data from 14 RCTs [alosetron (n = 3024) or cilansetron (n = 1116) *vs* placebo (n = 3043) or mebeverine (n = 304)]. 5-HT3 antagonists were more effective than mebeverine and placebo in achieving global IBS symptoms improvement (pooled RR = 1.60; 95%CI: 1.49-1.72), abdominal pain and discomfort relief (pooled RR = 1.30; 95%CI: 1.22-1.39). Superiority of both agents was demonstrated in patients of either sex. Nine patients (0.2%) in the 5-HT3 antagonists group were reported with possible ischemic colitis *vs* none in control groups. The third meta-analysis[82] pooled the data from eight clinical trials of alosetron and three clinical trials of cilansetron. This analysis, which included a total of 7216 patients with IBS, found 5-HT3 antagonists more effective than placebo in treating IBS-D. The RR of IBS symptoms persisting with 5-HT3 antagonists was 0.78 (95%CI: 0.71-0.86) compared to placebo.

Severe complications of constipation and ischemic colitis have emerged as significant side effects with alosetron use and this led to the drug's withdrawal from the United States marketplace in 2000. An expert panel reviewed the postmarketing data[83] reporting similar incidence rates for ischemic colitis and constipation (0.95 and 0.36 cases per 1000 patient-years, respectively) to rates during the postmarketing cycle before alosetron withdrawal. No mesenteric ischemia, surgeries, transfusions, or deaths occurred in patients with ischemic colitis and no cases of contipation were associated with toxic megacolon, perforation, surgeries, transfusions, or deaths. AEs were typically of short duration and all improved on prompt withdrawal of alosetron.

Ramosetron, is also a selective serotonin 5-HT3-receptor antagonist that possesses a specific three dimensional chemical conformation able to bind long lastingly to 5-HT3 receptors. Traditionally it has been used in oncology as a medication for hyperemesis due to chemotherapy[84]. The first double-blind, RCT[85] randomized 418 IBS-D patients to ramosetron 5 μg, 10 μg or placebo. Significantly higher rates of patients treated with both doses of ramosetron reported relief of IBS symptoms compared to placebo; the outcome measure was "global assessment of relief of IBS symptoms" in a monthly basis with similar benefits in men and women. The second study was also double-blind RCT. Five hundred thirty nine IBS-D patients received 5 μg ramosetron or placebo once daily. Ramosetron was shown effective for discomfort, altered bowel habits (44% *vs* 24%, for ramosetron vs placebo respectively, $P = 0.001$) and abdominal pain (46% *vs* 33%, for ramosetron vs placebo respectively, $P = 0.005$), without any serious AEs[86]. Overall 47% of individuals treated with ramosetron reported a positive response to treatment compared to 27% of placebo-treated patients ($P = 0.001$). Ramosetron was compared to mebeverine in another study with male IBS-D patients[87]. Patients (n = 343) were randomized to receive 5 μg ramosetron once daily or 135 mg mebeverine t.i.d for four weeks. Adequate relief of IBS symptoms at the last week of treatment was the primary end point and this was measured as the proportion of patients reporting relief in an intention to treat analysis. Both in the ramosetron and mebeverine groups, responder rates for global IBS symptoms, altered bowel habits and abdominal pain significantly increased during treatment. Although abdominal pain/discomfort and urgency (severity scores), the stool form score, and the stool frequency in both treatment arms significantly improved compared to baselines, statistical significance was not reached. Furthermore, in the comparison between ramosetron and mebeverine groups, the responder rates were similar (37% *vs* 38% on ITT analysis) as well as AEs. Events of severe constipation or ischemic colitis were not reported. When the oral administration of 5 μg ramosetron was prolon data analysis of the postmarketing survey[88]. Further RCTs studies ged for a minimum of 28 wk (up to 52 wk) the responder rate was increased as well as the overall improvement of IBS symptoms. The rate was further increased subsequently in the to evaluate ramosetron are needed.

LX-1031

As already mentioned 5-HT is an important neurotransmitter in the GI tract released from EC cells and inter-

neurons[24]. 5-HT is synthesized through the actions of the rate-limiting enzyme tryptophan hydroxylase (TPH), of which 2 different types, TPH1 and TPH2, are expressed by EC cells and neurons. After release of 5-HT from EC cells or neurons, it is inactivated by uptake into enterocytes or neurons through the 5-HT reuptake transporter, followed by metabolization to 5-hydroxyindole acetic acid (5-HIAA), which is excreted in the urine. Abnormalities of serotonergic signaling, including altered expression of TPH-1 and 5-HT reuptake transporter, and altered release of 5-HT, have been implicated in IBS pathogenesis[24,89]. Specifically, patients with IBS-D have increased platelet-depleted 5-HT concentrations during fasting and postprandial conditions compared with healthy volunteers and patients with IBS-C[27].

LX-1031 is an orally administrable, TPH inhibitor, with poor systemic absorption and low penetration through the blood-brain barrier that decreases serotonin synthesis[90,91]. Among healthy volunteers, LX-1031 was well tolerated and dose dependently inhibited 5-HIAA levels, supporting the potential of the drug to inhibit 5-HT synthesis in the human GI tract upon oral administration[91]. Brown *et al*[92] reported the results of a phase IIa study with LX-1031 in patients with non-constipating IBS. A total of 155 patients were randomized to a 4-wk treatment with placebo or 250 mg or 1000 mg LX-1031 *q.d.* After 1 wk, a significantly greater number of patients obtained adequate relief of IBS symptoms with the high dose of LX-1031 compared with placebo (48% *vs* 22%, $P = 0.02$). In weeks 2-4, the response to LX-1031 was higher compared with placebo, but no statistical significance was reached. As a result, the therapeutic gain (adequate relief) decreased from 25% to 10%. Stool consistency measured with the Bristol Stool Form Scale improved significantly with the high dose compared with placebo during weeks 1, 2, and 4. In a subset of patients, urinary 5-HIAA was measured as a marker of 5-HT synthesis before and after 4 wk of treatment with LX-1031. Overall, the high dosage decreased 5-HIAA excretion by approximately 25%. In this subgroup, a significant correlation was found between the percent decrease in urinary 5-HIAA excretion and the adequate relief response at the end of the treatment, indicating that decreased 5-HT synthesis is the mechanism underlying the symptomatic benefit. This is supported further by a post hoc analysis that showed a significantly higher symptomatic benefit in those who achieved a > 15% decrease in urinary 5-HIAA excretion during treatment. LX-1031 was well tolerated and no safety issues were observed; however, more studies are needed to establish fully the safety and tolerance profile of this drug[89].

Crofelemer

Crofelemer is a proanthocyanidin oligomer. Crofelemer acts through an antisecretory mechanism by reducing excess intestinal chloride ion secretion. It exerts an antisecretory action on two distinct chloride channel targets on the luminal membrane of intestinal epithelial cells, namely the CFTR and calcium-activated chloride channel[93]. The drug is being investigated for the treatment of acute infectious diarrhea, chronic diarrhea associated with human immunodeficiency virus/acquired immunodeficiency syndrome, and IBS-D.

A randomized, double-blind, placebo-controlled, phase Ⅱ a 12-wk treatment study evaluated crofelemer for IBS-D. A total of 246 patients with IBS-D received either placebo or crofelemer at dosages of 125, 250, or 500 mg twice daily[94]. The primary end point was improvement in stool consistency. The study found that none of the doses of crofelemer improve d stool consistency, stool frequency, or urgency, or provided adequate relief of IBS symptoms. However, the 500-mg twice-daily dosage of crofelemer significantly increased pain- and discomfort-free days especially in women with IBS-D. Large clinical trials are necessary to evaluate the effectiveness and safety of crofelemer.

Antibiotics

The potential utility of antibiotics in IBS treatment has been supported by a growing body of evidence demonstrating the important role of bacteria in IBS pathogenesis. It has been proposed that small intestinal bacterial overgrowth (SIBO) might explain the physiological hallmarks of altered gut motility, visceral hypersensitivity, abnormal brain-gut interaction and immune activation seen in IBS[95]. This is supported by multiple lines of evidence; first, gas analysis is abnormal in 10%-84% of IBS patients undergoing lactulose breath testing[96,97]; second, the distribution of inflammatory mediators and /or inflammatory cells have been shown to be disturbed in some patients with IBS[98]. It is thought that SIBO may contribute to many of the clinical manifestations of IBS through bacterial fermentation and stimulation of a gut immune response, characterized by release of inflammatory mediators, such as interleukins and tumour necrosis factor-α, which may affect motility, secretion and sensation[95,99]. Postinfectious IBS, which occurs in 4%-31% of individuals assessed up to 12 mo after an episode of acute gastroenteritis[100], also supports an aetiological role of bacteria in IBS.

In earlier studies[97,101] the systemic antibiotic neomycin has been evaluated and was found to improve global symptoms compared with placebo. The non-absorbed (< 0.4%), oral antibiotic rifaximin is the most thoroughly studied antibiotic for the treatment of IBS. Rifaximin appears to be well suited for the treatment of IBS because of its broad-spectrum bactericidal activity *in vitro*, its efficacy for SIBO *in vivo*, its favorable tolerability profile and its lack of association with clinically relevant resistance or Clostridium difficile colitis[99,102]. Rifaximin has demonstrated its efficacy in RCTs evaluating IBS patients[103,104]. IBS trials utilized high doses of rifaximin: 400 mg three times daily for 10 d[104], 400 mg twice daily for 10 d[103], and 550 mg twice daily for 14 d[105]. Rifaximin, at these high doses, demonstrated statistically significant improvement in symptoms whereas patients reported at signifi-

cantly greater rate global improvement in IBS symptoms and/or bloating compared to patients treated with placebo. Pimentel *et al*[105] evaluated rifaximin as treatment for IBS in TARGET 1 and TARGET 2 studies. These were phase Ⅲ, double-blind, placebo-controlled trials, identically designed. Patients who suffered from IBS without constipation were included in the studies and were randomized to receive for two weeks 550 mg rifaximin or placebo, three times daily. Patients were then followed for an additional period of 10 wk. The study measured (weekly assessments) the proportion of patients that responded reporting adequate relief of global IBS symptoms and IBS-related bloating. A significantly higher rate of patients in the rifaximin group reported adequate relief of global IBS symptoms and bloating during the first 4 wk after treatment compared to patients in the placebo group (40.7% *vs* 31.7%, $P < 0.001$ and 40.2% *vs* 30.3%, $P < 0.001$, respectively). AEs were similar between the two groups. A metanalysis[106] that included 5 trials reporting data from 1803 patients was published in 2012. Rifaximin was found to be more efficacious than placebo for global IBS symptom improvement (OR = 1.57; therapeutic gain = 9.8%; NNT = 10.2). Rifaximin was significantly more likely to improve bloating than placebo (OR = 1.55; therapeutic gain = 9.9 %; NNT = 10.1). The authors noticed that studies with older patients and more females demonstrated higher response rates, which was consistent regardless of treatment group. Although therapeutic gain offered by rifaximin is modest, it was similar to that yielded by other currently available therapies for IBS.

The American Task Force systematic review[61] concludes that rifaximin has shown improvement of global IBS symptoms and bloating in trials included in their analysis. Rifaximin has mostly been offered in patients with IBS-D; therefore it seems as a reasonable option for IBS patients with bloating and patients with IBS-D. The suggested dose is 400 mg three times a day for 10-14 d; however symptoms may recur over three to nine months.

5ASA compounds

Mesalamine is an anti-inflammatory agent, effective in the treatment of inflammatory bowel disease. It has been proposed for IBS-D on the basis of treatment of the underlying chronic inflammation. Bowel infections, bacterial overgrowth syndrome, antibiotics, stress and unfavorable dietary habits can precede visceral hypersensitivity and lead to a clinical manifestation of IBS. Although there is no specific morphologic correlate of IBS, these predictors can affect the colon microbiota and the local immune system, decrease the protective properties of the bowel mucosa, impair mucus production, and may be caused by only minimal alterations on the cellular level. The detection of minor lesions is often accompanied by a decrease of proliferation and enhanced apoptosis of colonocytes[107]. Progression of the disease leads to more pronounced morphological changes of the colon mucosa epithelium: reduced frequency of serotonin-producing cells and mast cells and increased frequency of secondary cells and increasing number of cellular infiltrations by eosinophils, neutrophils, lymphocytes, plasmocytes and fibroblasts of stroma[107]. These morphological criteria are signs of inflammatory processes and activation of immune mechanisms. In this context mesalazine has been evaluated in a RCT trial in 20 IBS patients[108]. Patients received 800 mg mesalazine or placebo three times daily for eight weeks. The primary outcome measure was changes in the number of colonic immune cells on biopsies obtained at baseline and at the end of treatment. Symptom severity, changes in subsets of immune cells and inflammatory mediators were also evaluated. In the group of mesalazine the total count of immune cells and specifical the mast cells were reduced as compared with placebo ($P = 0.0082$ and $P = 0.0014$, respectively). General well-being was also improved in the group of mesalazine ($P = 0.038$), but did not seem to have an impact on abdominal pain ($P = 0.084$), bowel habits or bloating ($P = 0.177$). The drug was well tolerated with no serious AEs reported. In another study[109] 12 women with diarrhoea-predominant IBS received oral mesalazine (1.5 g *b.i.d.*) for four weeks followed by a 4-wk washout phase. Molecular profiling of stool bacterial communities and IBS symptoms were assessed before, during and after mesalazine treatment. Qualitative and quantitative effects of mesalazine on stool microbiota, mucosal proteolytic activity and IBS symptoms were assessed. Faecal bacteria decreased by 46% on mesalazine treatment ($P = 0.014$), but returned to baseline during washout. Eight of 12 (67%) patients responded favorably to mesalazine based on a global relief questionnaire, with significant decreases in the number of days with discomfort and increases in bowel movement satisfaction. In a recent trial[110] 17 patients who developed IBS-D after gastroenteritis were randomized to receive mesalamine 1.6 gm *b.i.d.* or placebo for 12 wk. Mesalamine was not associated with significant improvement in global symptoms, abdominal pain, bloating, stool urgency, frequency, or consistency (all $P \geqslant 0.11$) or QOL ($P \geqslant 0.16$). At this point, data from all these studies seem inconclusive. Further study of the bacteriological and anti-inflammatory properties of mesalazine in IBS is necessary.

ABDOMINAL PAIN

Antispasmodics

Exaggerated motility response of the small bowel and colon to environmental stimuli may be responsible for the symptoms, especially pain, experienced in IBS[111-113]. For this reason antispasmodics have been used for the symptoms of IBS. Antispasmodics encompass several different drug classes (smooth-muscle relaxants, antimuscarinics, anticholinergics) and unique agents (pinaverium, trimebutine). Given their mechanism of action, these agents are directed at those subgroups of IBS, with a predominant symptom of abdominal pain and stool patterns that are either mixed or more diarrheal in nature. The propensity of these agents to promote constipation

makes them a less attractive option for patients with IBS-C. The anticholinergic properties of these agents restrict their usefulness in clinical practice. Common side effects that often limit these drugs usefulness in the treatment of IBS are dizziness, dry mouth, confusion (particularly in elderly patients), blurry vision, urinary retention, and constipation[41].

A systematic review and meta-analysis of antispasmodics as a class was performed by the ACG IBS Task Force[61]. The Task Force identified 22 studies suitable for inclusion in their systematic review. Most of these clinical trials are dated, with only 3 of the studies performed in the last 10 years. Studies evaluated hyoscine, hyoscyamine, otilonium, cimetropium, pinaverium, trimebutine, alverine, mebeverine, pirenzipine, prifinium, propinox, and a trimebutine/rociverine combination. The 22 trials collectively included data from 1778 patients with IBS. The pooled analysis of these studies revealed a RR of symptoms persisting with antispasmodics compared with placebo of 0.68 (95%CI: 0.57-0.81) and a NNT of 5. The pooled analysis that was performed on the 13 studies, included 1379 patients in whom AEs were reported. There was significant heterogeneity among these patients; moreover these clinical trials were collectively fraught with methodological flaws, including diagnostic criteria used, inclusion criteria used, dosing schedule used, duration of therapy studied, study end points used to assess response, and study size (only three studies enrolled more than 100 patients). The review concluded that some drugs in the antispasmodics class (cimetropium, hyoscine, pinaverium) may be an option for relief of abdominal discomfort and pain in IBS-patients. Older systematic reviews have yielded mixed results regarding the efficacy of antispasmodics for IBS[114,115].

Mebeverine is an antispasmodic that has been successfully used in the management of IBS for many years. Mebeverine is a musculotropic agent that has antispasmodic activity and regulatory effects on the bowel function[116]. During oral administration at doses of 135-270 mg *t.i.d.*, it shows no typical anticholinergic side effects. There is no indication that the incidence of side effects caused by mebeverine is higher than that of a placebo[114]. In 2010, a metanalysis was published on the efficacy and tolerability of mebeverine in IBS in its usual dosages[117]. Eight randomized trials including 555 patients with all IBS subtypes, randomized to receive either mebeverine or placebo, met the metanalysis criteria. The pooled RR for clinical improvement of mebeverine was 1.13 (P = 0.7) and 1.33 (P = 0.12) for relief of abdominal pain. The efficacy of mebeverine 200 mg compared to mebeverine 135 mg indicated RRs of 1.12 (P = 0.168) for clinical or global improvement and 1.08 (P = 0.463) for relief of abdominal pain. Thus, mebeverine was shown to be well tolerated with no significant AEs; however, its efficacy in global improvement of IBS did not reach statistical significance. Recently the results of an exploratory RCT of mebeverine, methylcellulose, placebo and a self-management online (website) treatment method (cognitive behavior treatment) were published[118]. One hundred thirty-five patients, with IBS symptoms fulfilling Rome Ⅲ criteria were randomized to over-encapsulated mebeverine, methylcellulose or placebo for six weeks and to 1 of 3 website conditions. Mean IBS SSS (symptom severity scale) decreased by 35 points from baseline to 12 wk of treatment. There was no significant difference in IBS SSS or IBS-QOL score between medication and website groups. However, IBS SSS at six weeks was lower in the No-website group than the website groups (P = 0.037). In the end of the study, the global relief of IBS symptoms was significantly improved in the website groups compared to the non-website group at 12 wk of treatment (Enablement and Subjects Global Assessment of relief P = 0.001 and P = 0.035 respectively).

Otilonium bromide (OB) has been shown to reduce the pain severity in IBS patients effectively[61]. OB is an ammonium derivative with spasmolytic activity in GI smooth muscle by inhibiting the calcium ion influx through L-type voltage operated calcium channels. OB pharmacologically has been demonstrated to inhibit central/peripheral tachykinin-2 receptor; in this way it reduces the sensory signals afferent transmission from the periphery to central nervous system[119]. Additionally, OB binds with high affinity to muscarinic receptor subtypes M1, M2, M3, M4 and M5[120,121]. M3 sub-receptor is located in human colonic crypt cells to mediate secretion coupled with calcium channels. Due to its potent muscarinic blockade of M3, OB exhibits its antisecretory properties, thus improving stool consistency[121]. Among researches on the OB efficacy on IBS patients, early studies indicated that OB is effective for abdominal pain and bloating but there was a difficulty in demonstrating efficacy over placebo[122,123]. A review based on four OB trials was eventually conducted in 2008. Various antispasmodics were studied, but OB (four trials, 435 patients, RR of persistent symptoms 0.55, 0.31 to 0.97) showed consistent evidence of efficacy over placebo[124]. Subsequently, two RCTs were published. The first multi-center phase Ⅳ double-blind study[125] randomized 356 patients with various IBS subtypes to receive OB (40 mg *t.d.s.*) or placebo for 15 weeks, and follow-up was extended 10 additional weeks. The effect of OB was significantly greater than placebo in the reduction of weekly frequency of episodes of abdominal pain at the end of treatment period (P = 0.03); similarly OB was superior to placebo in the reduction of abdominal bloating (P = 0.02) and in the global efficacy by patient assessment (P = 0.047). However, no difference between the effect of OB and placebo was found in the intensity of abdominal pain, the proportion of patient responders, and the safety and quality of life scores. During follow-up, the therapeutic effect of OB remained greater than placebo in terms of withdrawal rate due to symptom relapse (P = 0.009), global efficacy of treatment and relapse-free probability (P = 0.038). Therefore, the study demonstrated superiority of OB *vs* placebo in the reduction of pain and bloating, and in protection from relapse as a result of the long-lasting

effect. These symptoms improved progressively during the study. It should be pointed out that IBS trials are subjected to high placebo effect, typically between 30% and 60% thus making difficult to detect the therapeutic gain and interpretation of the results[126]. The second trial was an Asian study[127] which randomized 117 participants to receive 40 mg OB or 100 mg mebeverine, thrice daily for eight weeks. The abdominal pain/discomfort frequency score (APDFS) and safety profile were assessed. Compared to baselines, the APDFSs in OB and mebeverine were significantly reduced (0.55; $P = 0.011$ and 0.37; $P = 0.042$ respectively). However, when the improved results of the two treatments were compared between them, statistical significance was not reached). One hundred eighteen AEs were reported (OB = 65 and mebeverine = 53); these comprised mostly dry mouth in both arms, followed by nausea and dizziness (particularly in OB).

Similarly, solifenacin, a muscarinic type 3 receptor antagonist, that is used to treat overactive bladder in adults has been evaluated in a recent study for the symptomatic relief of diarrhea in 20 IBS-D patients[128]. After a 2-wk observation period, all participants received solifenacin for six weeks. Subsequently, the administration of solifenacin was discontinued and ramosetron, a serotonin 3 receptor antagonist, was administered for four weeks. Two weeks after initiation of solifenacin, an overall improvement was observed in 16 out of 20 participants (80%). The efficacy of solifenacin in the treatment of IBS with diarrhea was not inferior to that of ramosetron. However, the study had the limitation of not being placebo-controlled.

In recent years, increasing attention has been given to the role of the nonadrenergic and noncholinergic (NANC) nervous system for the regulation of colonic motility. Nitric oxide (NO) has been identified as an important component of the NANC nervous system and as an inhibitory neurotransmitter in the colon[129]. NO mediates the relaxation of smooth muscle cells in the GI tract by production of intracellular guanosine 3,5-cyclic monophosphate (cGMP)[129] and is also involved in nociception[130]. Sildenafil is an orally administered drug that has been used to augment NO activity and is widely used as a treatment for erectile dysfunction. In an earlier study[131] stimulation of the NO-cGMP pathway by sildenaifil administration decreased rectal tone but did not influence rectal distensibility. Relaxation of the rectum was accompanied by an increase in rectal volumes to reach perception thresholds in healthy subjects and in patients with IBS, but no direct effect on rectal perception could be demonstrated. Recently, another small study[132] evaluated the effects of sildenafil tone inhibition on rectal sensitivity. Eight control subjects and 21 IBS patients (Rome Ⅱ) were enrolled in a double-blinded study, after dosing with placebo or sildenafil (50 mg *p.o.*). Sildenafil increased the first desire to defecate and the pain in the hypersensitive IBS patients. It also increased rectal compliance, but only in diarrhea-IBS. No trials regarding the effectiveness of sildenafil on the relief of the IBS symptoms and the quality of life are available.

Opioid receptor agonists

Opioid receptors, including m, d, and k, are expressed along the GI tract and play a key role in regulating GI motility, secretion, and visceral sensation. Recently, exogenous opioids have been shown to reduce GI transit through activation of m-opioid receptor (MOR) and they can treat diarrhea in acute situations. Agents that simultaneously activate MOR and antagonize d-opioid receptor (DOR) have differential GI effects and can possess increased analgesic potency compared to pure MOR agonists[133]. Eluxadoline is a locally active, mixed MOR agonist/DOR antagonist with low oral bioavailability that is being developed for the treatment of IBS-D. In vitro, eluxadoline reduces contractility in intestinal tissue and inhibits neurogenically mediated secretion[134]. In a recent phase Ⅱ study[135] 807 patients were randomly assigned to groups receiving twice daily 5, 25, 100, or 200 mg oral eluxadoline or oral placebo for 12 wk. The primary end point was clinical response at week four, defined by a mean reduction in daily pain score of more than 30% from baseline and of at least 2 points on 0-10 scale, as well as a stool consistency score of 3 or 4 on the Bristol Stool Scale (1-7) for at least 66% of daily diary entries during that week. The authors concluded that patients given eluxadoline were significantly more likely to be clinical responders, based on a combination of improvement in abdominal pain and stool consistency. Another selective, potent k-opioid agonist, asimadoline, which has been shown to improve pain and abnormal bowel function, has been evaluated in a trial[136]. Asimadoline has low permeability through the blood-brain barrier. In this trial, 596 patients with varying IBS subtypes were randomized to receive 0.15, 0.5, 1.0 mg asimadoline or placebo *b.i.d* for twelve weeks. Asimadoline (0.5 mg) significantly prolonged the total time (number of mo) with adequate relief of IBS pain or discomfort (46.7% *vs* 20.0%), adequate relief of IBS symptoms (46.7% *vs* 23.0%). It also significantly reduced pain scores (week 12: -1.6 *vs* -0.7), increased pain free days (42.9% *vs* 18.0%), and improved urgency and stool frequency (-2.3 *vs* -0.3). These positive results were observed in IBS-D patients with at least moderate pain in baseline. However, no significant difference was observed in the percentage of months with adequate relief. Asimadoline failed to show a benefit in IBS-C.

Drugs acting through the endocannabinoid system have also been studied. Two types of G-protein- coupled cannabinoid receptors, CB1 and CB2, have been identified and cloned[137]. CB1-immunoreactivity is located on the normal colonic epithelium, smooth muscle, and the myenteric plexus. Dronabinol, a nonselective CB receptor agonist, has been shown to inhibit and colonic motility in healthy humans[138]. In a recent study[139], the effect of dronabinol on colonic sensory and motor functions in 75 patients with mixed IBS subtypes who were cannabinoid naïve was assessed. Patients were randomly assigned to

groups that were given a single dose of placebo or 2.5 mg or 5.0 mg dronabinol. Single nucleotide polymorphisms CNR1 rs806378, fatty acid amide hydrolase (FAAH) rs324420, and MGLL rs4881 were also studied. In all patients, dronabinol decreased fasting proximal left colonic motility index compared with placebo and increased the colonic compliance. The effects of dronabinol were greatest in IBS patients with diarrhea or IBS alternating. Dronabinol did not alter sensation or tone but it affected fasting distal motility index in patients, regardless of FAAH rs324420 variant (CA/AA *vs* CC) ($P = 0.046$)

GLP-1 (Rose-10)

GLP-1(glucagon-like peptide 1) is normally released after food intake. It stimulates insulin release and reduces gastric emptying and small intestinal motility[140]. GLP-1 has been reported to inhibits small intestinal motility in IBS patients[141] and to prolong colonic transit[142]. The initial use of GLP-1 analogues was to normalize blood glucose levels in patients with diabetes; however, based on the aforementioned observation,s are now being studied to treat abdominal pain attacks in patients with IBS. The GLP-1 analog ROSE-010 has been demonstrated to reduce acute IBS pain in a RCT involving 166 IBS patients[143]. Participants were assigned to receive single subcutaneous injections of ROSE-010 100 μg, 300 μg and placebo in a cross-over design. Patient-rated pain relief and intensity were evaluated with a visual-analog scale. The primary outcome measure was the proportion of patients with a minimum 50% pain reduction from 10 to 60 min after treatment. A significantly higher proportion of patients reported greater than 50% of the maximum total pain relief response after 100 and 300 μg of ROSE-010 treatments than after placebo (23% and 24% *vs* 12%; $P = 0.011$ and $P = 0.005$, respectively). Times to meaningful and total pain relief were shorter for both doses of active drug *vs* placebo. A second single-center RCT evaluated safety, pharmacodynamics, and pharmacokinetics in women with IBS-C[144]. Patients were administered once daily 30, 100, or 300 μg ROSE-010 subcutaneously or placebo for three consecutive days as well as a single repetitive dose after 2-10 d. Validated scintigraphy was used to measure GI and colonic transit. Single-photon emission computed tomography was used to measure gastric volumes. The primary outcome measures were gastric emptying of solids half time, the colonic transit geometric center at 24 h, and the gastric accommodation volume. Gastric emptying was significantly retarded at the doses of 100 and 300 μg ROSE-010. Gastric volumes, small bowel or colonic transit at 24 h and bowel functions were not significantly altered by ROSE-010. Colonic transit at 48 h was accelerated with the 30 and 100 μg ROSE-010 doses. AEs were vomiting ($P = 0.008$) and nausea nausea ($P < 0.001$). Based on the observation that at the doses of 30 and 100 μg the drug accelerated colonic transit time, the authors concluded that it could be a candidate for relief of constipation in IBS-C. More in-depth assessments of the IBS pain attack characteristics are ongoing and future clinical trials with ROSE-010 are being planned[15].

Ketotifen

Experimental studies have shown that mast cells play an important role in IBS through visceral hypersensitivity[145]. Patients with IBS excibit an increased number of mast cells in the small intestine[146], large intestine[147,148] and rectum[149]. The number of mucosal mast cells and their proximity to sensory nerves in colonic tissue has also been studied and found positively correlated to abdominal pain[148]. Mast cells activation results in degranulation; thus mediators pre-stored in vesicles such as tryptase, histamine and several cytokines are rapidly released inducing an inflammatory response. Sodium cromoglycate and ketotifen are well known membrane stabilizers that act by blocking mast cell degranulation[145]. Klooker *et al*[145] conducted a RCT to assess the effect of ketotifen on IBS. Sixty patients with various IBS subtypes (Rome Ⅱ criteria) were included in the study. The idea was to evaluate whether increased number of mast cells and/or increased spontaneous mucosal tryptase release is associated with visceral hypersensitivity and whether mast cell stabilization with ketotifen had an impact on visceral perception; this was estimated by measurements of rectal distension in hypersensitive patients with IBS. Abdominal symptoms were also monitored. The trial consisted of two weeks of screening/observation, then a treatment period of eight weeks and a follow-up period of another two weeks. Barostat measurements were performed at baseline and then after eight weeks of treatment with ketotifen or placebo. Rectal biopsies were also collected before and after treatment. Ketotifen was shown to be superior to placebo in increasing the threshold for discomfort in patients with IBS with visceral hypersensitivity; it also significantly improved abdominal pain and quality of life. Mast cells and spontaneous release of tryptase were lower in patients with IBS than in healthy volunteers. However, ketotifen did not histamine and tryptase release. Further studies are needed to confirm the beneficial effect of ketotifen in IBS symptoms and clarify its way of action.

CONCLUSION

IBS is a highly prevalent functional disorder that reduces patients' quality of life. IBS is not a single disease entity, but rather likely consists of several different disease states; currently, treatment is predicated upon the patient's most bothersome symptoms. Various drug categories (antispasmodics, laxatives, dopamine antagonists, 5-HT3 antagonists and/or 5-HT4 agonists, sedatives, antibiotics, probiotics), modifications in diet and lifestyle, and complementary and alternative therapies have been proposed as symptomatic treatment. It is difficult to draw conclusions from previous studies since IBS trials are subjected to high placebo effect, typically between 30% and 60% thus complicating the detection of the thera-

peutic gain and interpretation of the results. For IBS-C, linaclotide and lubiprostone seem promising for the relief of multiple symptoms from which patients with IBS-C suffer. Regarding IBS-D, although the 5-HT3 antagonist alosetron was shown to be superior than placebo at relieving global IBS symptoms in male and female with a high level of evidence, it was withdrawn from the market due to complications (ischemic colitis). Newer 5-HT3 antagonists (cilansetron, ramosetron) have emerged; however there is lack of consistent data demonstrating whether the drug is superior over placebo. In the category of antibiotics, rifaximin has been presented as efficacious in RCTs evaluating IBS patients. It has emerged as a strong option for the treatment of IBS because of its broad-spectrum bactericidal activity *in vitro*, its efficacy for SIBO *in vivo*, its favorable tolerability profile and the lack of association with clinically relevant resistance or Clostridium difficile colitis. Among the antispasmodics, OB showed consistent evidence of efficacy over placebo. Other molecules, *i.e.* NO donors, Opioid Receptor Agonists, ketotifen, as well as GLP-1 have been proposed for IBS treatment as well.

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Intestinal microbiota in pathophysiology and management of irritable bowel syndrome

Kang Nyeong Lee, Oh Young Lee

Kang Nyeong Lee, Oh Young Lee, Department of Internal Medicine, Hanyang University College of Medicine, Seoul 133-791, South Korea
Author contributions: Lee KN performed the literature review and drafted the paper; Lee OY performed the critical revision of the manuscript.
Correspondence to: Oh Young Lee, MD, PhD, Department of Internal Medicine, Hanyang University College of Medicine, 222 Wangsimni-ro, Seongdong-gu, Seoul 133-791, South Korea. leeoy@hanyang.ac.kr
Telephone: +82-2-22908343 Fax: +82-2-22988314
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Abstract

Irritable bowel syndrome (IBS) is a functional bowel disorder without any structural or metabolic abnormalities that sufficiently explain the symptoms, which include abdominal pain and discomfort, and bowel habit changes such as diarrhea and constipation. Its pathogenesis is multifactorial: visceral hypersensitivity, dysmotility, psychosocial factors, genetic or environmental factors, dysregulation of the brain-gut axis, and altered intestinal microbiota have all been proposed as possible causes. The human intestinal microbiota are composed of more than 1000 different bacterial species and 10^{14} cells, and are essential for the development, function, and homeostasis of the intestine, and for individual health. The putative mechanisms that explain the role of microbiota in the development of IBS include altered composition or metabolic activity of the microbiota, mucosal immune activation and inflammation, increased intestinal permeability and impaired mucosal barrier function, sensory-motor disturbances provoked by the microbiota, and a disturbed gut-microbiota-brain axis. Therefore, modulation of the intestinal microbiota through dietary changes, and use of antibiotics, probiotics, and anti-inflammatory agents has been suggested as strategies for managing IBS symptoms. This review summarizes and discusses the accumulating evidence that intestinal microbiota play a role in the pathophysiology and management of IBS.

Key words: Immunity; Irritable bowel syndrome; Microbiota; Permeability; Probiotics

Core tip: Irritable bowel syndrome (IBS) is a functional bowel disorder with multiple pathophysiology, which is not fully understood. Intestinal microbiota has recently been postulated to be involved in the pathophysiology of IBS. Many studies of IBS focus on investigating the efficacy of modulating the microbiota by probiotics and antibiotics. However, the role of the intestinal microbiota in the pathophysiology and management of IBS is not clear. This review provides the accumulating evidence on it.

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INTRODUCTION

Irritable bowel syndrome (IBS) is a functional bowel disorder characterized by abdominal pain or discomfort relieved by defecation, and accompanied by changes in bowel habits such as diarrhea or constipation, which cannot be explained by structural, biochemical, or metabolic abnormalities[1]. The symptoms of IBS have been accounted for as resulting from visceral hypersensitivity, intestinal dysmotility, genetic or environmental factors,

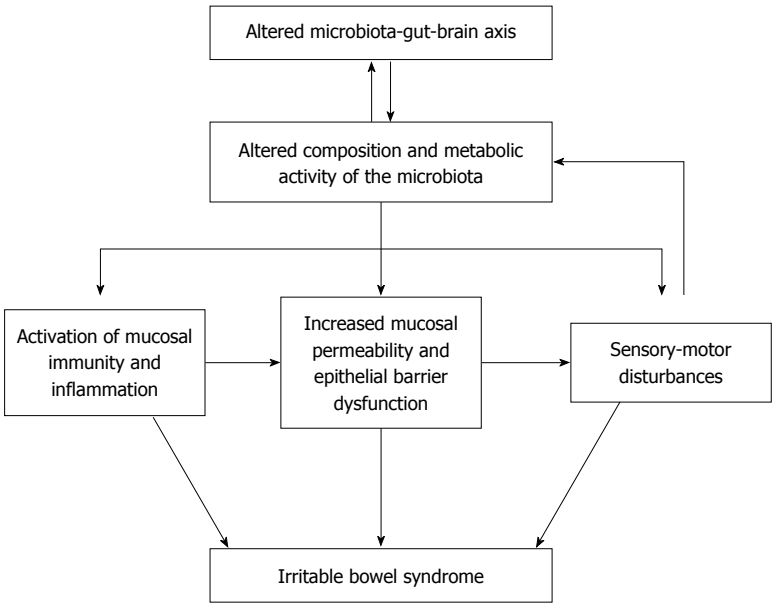

Figure 1 Putative pathophysiologic role of the microbiota in irritable bowel syndrome. Intestinal microbiota play a substantial role in irritable bowel syndrome (IBS). Although the microbiota may contribute directly to the symptoms of IBS, it is more likely that altered composition and metabolic activity of the microbiota caused by stress or other psychological disturbances indirectly activate mucosal immunity and inflammation, increase epithelial permeability, and reduce barrier function, thereby activating the sensory-motor dysfunction responsible for a variety of symptoms of patients with IBS.

psychological factors, or a dysregulated brain-gut axis[2]. In addition to these factors, bacterial infection, dysregulated intestinal immune function, and chronic low-grade mucosal inflammation have all been suggested as putative pathogenetic mechanisms, in which the intestinal microbiota might play an important role, but their role in IBS cannot be fully explained (Figure 1)[3,4].

Intestinal microbiota is a collective term for a complex ecosystem of microbes inhabiting the intestine[5]. In the human intestine, this ecosystem may include any one of over 1000 microbial species, and 10^{14} cells (*i.e.*, about 10 times more than the number of human cells in the body[6]), containing 150-fold more genes than the human genome[7]. The microbiota can be divided into mucosal and luminal subtypes[8], and it was previously thought to comprise three predominant enterotypes: *Bacteroides, Prevotella,* and *Ruminococcus*[9], although such a strict categorization is no longer widely accepted[10].

To evaluate the composition and metabolic activity of the intestinal microbiota, culture-dependent and -independent tests have been developed[11]. It has been shown that size and diversity of the microbiota increase distally from the upper to the lower gastrointestinal (GI) tract[12] and are modulated by gastric acid, intestinal motility, and the function of the ileocecal valve. Their distribution also varies according to the region of the GI tract with gram-positive facultative anaerobic bacteria in the proximal small intestine and gram-negative anaerobes in the distal small intestine. Although the composition and diversity of the microbiota are genetically controlled from birth and become stable after weaning and throughout life, qualitative and quantitative changes can occur over the longitudinal and cross-sectional axes of the intestine: changes in bacterial enzymes and metabolic activity, as well as in microbial populations. The composition and metabolic activity of the microbiota vary between, but also within, individuals due to many factors including mode of delivery at birth, diet, sanitation, antibiotics, and ageing[13]. At birth, contamination from the vaginal canal provides the intestine with the maternal microbiome, while during a delivery by cesarean-section, the gut comes into contact with commensals from the skin and the surgical environment[14]. The composition of the microbiota can also be altered by the feeding method: bifidobacteria increase in breast-fed babies (*i.e.*, babies receiving a high-carbohydrate and high-fiber diet), and *Bacteroides* spp. increase in formula-fed babies (babies receiving a high-fat diet)[15]. Lastly, it can vary across geographical regions, *e.g.*, between rural Africa and urban Europe[16].

The intestinal microbiota is essential for maintaining individual health, including normal GI function. In this context, its main functions are metabolic, protective, and trophic: it can help to digest and absorb nutrients, and produces a variety of beneficial compounds such as short-chain fatty acids (SCFA)[17], it can act as a barrier against pathogens by adhering to the mucosa, generating immune responses, and interacting with components of the epithelial layer, it can also influence the differentiation and proliferation of the intestinal epithelial cells and the development of the enteric immune system.

In parallel with the beneficial effects of microbial activity on the gut, bacterial fermentation may give rise to large amounts of gas and thus contribute to the symptoms of bloating, flatulence, and abdominal distension, which are commonly reported by patients with IBS[18]. An association between the microbiota and IBS has been supported by the evidence of modulation of mucosal immunity: IBS symptoms were found to be more frequent

after an episode of gastroenteritis, and some IBS symptoms were found to improve after antibiotic treatment targeting the intestinal microbiota[19]. This putative link was also demonstrated in studies of probiotics, which modulated the intestinal microbiota in IBS patients. Finally, mucosal immunity-gut microbiota-brain axis is being suggested as a possible pathway for the development of IBS due to altered intestinal microbiota. This review article explores the role of the microbiota in the pathophysiology and management of IBS, and provides a comprehensive summary of the evidence for the concept of IBS as a microbiota-related disorder.

Despite the large volume of studies of the intestinal microbiota, our understanding of its role in health and disease is still in its infancy. In studying the microbiota, culture-based methods are being replaced by advanced, culture-independent, molecular techniques. However, these two approaches are complementary: culture studies of fecal matter or colonic mucosa are valuable for identifying functional groups and for selective enumeration, whereas advanced molecular study are a powerful tool for monitoring changes in microbial composition. The molecular methodology includes sequencing of the small-subunit ribosomal RNA genes through amplification of nucleic acids extracted from fecal or mucosal samples, fingerprinting methods such as denaturing gradient gel electrophoresis, targeted methods such as fluorescence in situ hybridization and quantitative PCR, new high-throughput sequencing, and 16S rRNA-based microarraying[20].

PUTATIVE PATHOPHYSIOLOGIC ROLE OF INTESTINAL MICROBIOTA IN IBS

Alteration of the microbiota-gut-brain axis

The microbiota in the gut can be altered by brain function, and microbial alteration can, in turn, influence brain function. It is evidenced by the finding that patients with IBS frequently have accompanying psychological disorders, such as anxiety or depression, and those with psychological stress are more likely to develop post-infectious (PI)-IBS. This connection between the microbiota, the gut, and the brain in IBS postulates the existence of a bidirectional, homeostatic network, and it is an exciting area of ongoing research.

Animal studies have demonstrated the influence of the intestinal microbiota on brain development. Brain dysfunction in Germ-free (GF) mice was reported, including an exaggerated hypothalamic-pituitary response to mild stress[21], more exploratory and risk-taking behavior[22], and altered brain chemistry and memory, indicative of impaired hippocampal development[23]. Brain chemistry and behavior were also influenced by altered microbiota; a study showed that transient alteration of the microbial composition by diet provoked exploratory behavior, accompanied by changes of in the levels of brain-derived neurotrophic factor in the specific regions of the brain such as hippocampus and amygdala[24]. The gut microbiota and the brain may be communicated by neural, metabolic (bacterial and host), immunologic, or endocrine pathways[25]. The neural pathways was first suggested in animal models; anxiety-related behavior was reduced after probiotic treatment, provided vagus nerve integrity was maintained[26,27]. Metabolic pathways were revealed in a study that brain function and behavioral changes were closely associated with bacterial metabolites such as SCFAs (which comprise most of the circulating organic acids) and tryptophan metabolites[28,29]. A role of immunologic pathways was demonstrated in animal and human studies showing that certain psychological disorders were associated with pro-inflammatory cytokines, whose levels had been altered by manipulating the composition of the microbiota[30-32]. Endocrine pathways in microbiota-gut-brain axis were suggested in a study showing that the endocrine structure and function of the GI tract which secrets a variety of hormones such as cholecystokinin and serotonin [5-hydroxytryptamine (5-HT)] were reduced in GF rats[33].

Likewise, the intestinal microbiota can be affected by signals from the central nervous system produced in response to stress or psychological disturbances. Stress can change GI motility and secretions, which alter the microbial habitat. The microbial habitat may also be altered by changes in gene expression of some microbial species. Conversely, the intestinal microbiota can influence neurotransmitters like norepinephrine, dopamine, and serotonin in the brain, and activation of the hypothalamic-pituitary-adrenal axis is also thought to be involved in the microbiota-gut-brain axis.

As a result of alteration of the microbiota in this axis, mucosal immunity may be activate and thereby epithelial barrier function can be disrupted, which could contribute to the visceral hypersensitivity and dysmotility in IBS. Furthermore, the intestinal microbiota may not only release metabolites but also induce the formation of host-derived immune mediators, thereby affecting the enteric nervous system both directly and indirectly. However, much about the role of the microbiota-gut-brain axis in IBS remains poorly understood.

Dysbiosis: quantitative and qualitative changes in the microbiota

Altered composition of the intestinal microbiota: Intestinal microbiota can be grouped into luminal and mucosal microbiota. It is generally accepted that the composition of the luminal and mucosal microbiota differs between patients with IBS and healthy controls, and the composition may also vary according to the subtype of IBS[34], although studies of the intestinal microbiota have been as diverse and complex as the microbiota itself, with inconsistent and conflicting results[29,35-44] (Table 1). According to both the early culture-based and the more recent advanced molecular studies, it was found in IBS that the proportions of specific bacterial groups were altered, the diversity of microbial populations was reduced, and the degree of variability in the microbiota composition was different. The findings included decreased levels of

Table 1 Summary of studies of the intestinal microbiota in patients with irritable bowel syndrome

Ref.	Subject (*n*)	Method	Finding
Si *et al*[35]	IBS (25)	Culture	Decreased *Bifidobacterium*
	Control (25)		Increased *Enterobacteriaceae*
Malinen *et al*[36]	IBS (27)	qPCR	Decreased *Lactobacillus* in IBS-D
	Control (22)		Increased *Veillonella* in IBS-C
Mättö *et al*[37]	IBS (26)	Culture	Increased coliform and aerob to anaerob ratio
	Control (25)	PCR-DGGE	Temporal instability
Codling *et al*[38]	IBS (41)	PCR-DGGE	No difference in fecal/mucosal
	Control (33)		Temporal instability
Ponnusamy *et al*[39]	IBS (11)	DGGE	Increased diversity in *Bacteroidetes*, *Lactobacillus*
	Control (8)	qPCR-16sRNA	
Tana *et al*[29]	IBS (26)	Culture	Increased *Lactobacillus* and *Veillonella*
	Control (26)	q-PCR	
Lyra *et al*[40]	IBS (20)	qPCR	Increased *Ruminococcus* torques and decreased
	Control (15)		*Clostridium thermosuccinogenes* in IBS-D
Krogius-Kurikka *et al*[41]	IBS (10)	16S rRNA	Increased *Proteobacteria* and *Firmicutes*
	Control (23)	sequencing	Decreased *Actinobacteria* and *Bacteroidetes*
Kerckhoffs *et al*[42]	IBS (41)	FISH	Decreased *Bifidobacterium*
	Control (26)	qPCR	
Kassinen *et al*[43]	IBS (24)	16S rRNA	Decreased *Collinsella aerofaciens*, *Clostridium cocleatum*, and *Coprococcus*
	Control (23)	sequencing	*eutactus*
Maukonen *et al*[44]	IBS (24)	PCR-DGGE	Decreased *Clostridium coccoides*
	Control (16)		Temporal instability
Jeffery *et al*[46]	IBS (37)	16S rRNA	Increased ratio of *Firmicutes* to *Bacteroidetes*
	Control (20)	pyrosequencing	Clustering in IBS

DGGE: Denaturing gradient gel electrophoresis; FISH: Fluorescent in situ hybridization; IBS: Irritable bowel syndrome; qPCR: Quantitative polymerase chain reaction.

fecal lactobacilli and bifidobacteria, increased levels of facultative anaerobic bacteria dominated by streptococci and *Escherichia coli* (*E. coli*), increased ratios of *Firmicutes*: *Bacteroidetes* and higher counts of anaerobic organisms (such as clostridium)[44,45]. In addition, the microbiota of IBS patients reportedly belonged to entirely different enterotypes than those of healthy controls[34,46]. These inconsistent and sometimes conflicting results are thought to be due to the use of a single fecal sample irrespective of the fluctuating symptoms of IBS.

Altered metabolic activity of the intestinal microbiota: Intestinal microbiota may produce excessive amounts of gas by fermenting poorly absorbable carbohydrates (*e.g.*, the so-called FODMAPs, fermentable oligosaccharides, disaccharides, monosaccharides and polyols), which may cause abdominal pain, bloating, flatulence, and distension in IBS. Additionally, altered fermentation of poorly absorbable carbohydrates could increase the production of SCFAs, which would then lead to release of 5-HT from the intestinal mucosa[47]. In fact, increased numbers of acetic and propionic acid-producing bacteria (*Veionella* and *Lactobacillus* spp) were reported in patients with IBS[29]. It has been demonstrated that the release of 5-HT initiated high-amplitude, propagated colonic contractions, accelerated intestinal transit, and increased gut motility[47,48], all of which may contribute to IBS symptoms, suggesting that fermentation products play a potential role of in contributing IBS symptoms.

However, considering the large variability due to different methodologies of microbiota studies, and individual differences in relation to dietary, genetic and geographical factors, as well as heterogeneity of the disease, these results should be cautiously interpreted. Research on the luminal and mucosal microbiota is still in infancy, and further studies using advanced techniques such as 16s rRNA and DNA sequencing are needed to improve our understanding of the microbiota changes in IBS.

Activation of mucosal immunity and inflammation in IBS

The altered composition and metabolic activity of the intestinal microbiota found in IBS may be associated with activation of mucosal immunity and inflammation. Changes in the intestinal microbiota were observed after an episode of infective gastroenteritis with subsequent antibiotic use. In fact, some patients start to report IBS symptoms following such episodes[49], which suggests an association between IBS and activation of mucosal immunity and inflammation caused by altered microbiota. Chronic low-grade mucosal inflammation has been frequently observed in many studies of IBS patients and in animal models of IBS[50-56].

The intestinal microbiota plays an essential role in the development, functioning, and regulation of both intestinal and systemic immunities. By interacting with the microbiota, the intestinal (or enteric) immune system, composed of innate and adaptive immunity, helps to maintain normal GI function[57]. In IBS patients, however, the interactions between enteric immunity and commensal and/or pathogenic microbes were found to be dys-regulated. Under normal conditions, intestinal microbes are recognized via their ligands, identified by toll-

like receptors (TLRs) on intestinal immune cells. Expression of TLRs in the colonic mucosa of IBS patients was found to be increased[58], as was the level of circulating antibodies such as antiflagellin antibodies[59]. Together, these findings suggest that in IBS, bacterial components such as lipopolysaccharides (LPS) and flagellin are recognized more frequently due to the increased TLRs and circulating antibodies. In addition, one of the antibacterial proteins, β-defensin-2, was found to be elevated in IBS[60]. These increased interactions of immunologic components with the microbiota could eventually lead to the mucosal inflammation in IBS.

Mucosal inflammation provoked by dysregulated innate and adaptive enteric immunities has been observed in many studies of IBS[61,62]. The numbers of activated mast cells were shown to be increased in the colon of IBS patients, and also to be in close proximity to enteric nerves, which correlated well with IBS symptoms[63], although this increase was specific to diarrhea predominant IBS (IBS-D)[52], and varied according to the region of the intestine[64]. In addition to mast cells, lymphocytes ($CD4^+$ and $CD8^+$ T cells) were also found to be elevated, suggesting that they may play a role in IBS, although there are some inconsistencies[50,55,56,65]. Immune alterations associated with IBS were also found in IgA-producing B cells[66], IgG^+ B cells[67], and in the levels of pro- and anti-inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α) and interleukin (IL)-10, IL-6, and interferon-γ in the intestinal mucosa of IBS patients[68]. Similarly, in the peripheral blood, levels of pro-inflammatory cytokines such as IL-6, IL-1β, and TNF-α were higher in patients with IBS than in controls, but the levels varied according to IBS subtype[69]. It is thought that mucosal inflammation and activated immunity in IBS may lead to increased permeability of the intestinal mucosa, and may thus induce abnormal sensory and motor function, which could contribute to the symptoms of IBS. However, the association between activated immunity and the intestinal microbiota is not clearly established, and further studies in this area are warranted.

Altered mucosal permeability and the epithelial barrier in IBS

The activation of mucosal immunity and inflammation driven by the altered microbiota in IBS may increase mucosal permeability and impair epithelial barrier function. The intestinal epithelium functions not only as an exchanger, absorbing fluid and nutrients, but also as a protective barrier against pathogens. It is covered with a thick layer of mucus, composed of a complex mixture of glycoproteins, mucins, bactericidal enzymes, and secretory immunoglobulin A (IgA). Alterations to the epithelial barrier observed in IBS have included increased mucosal permeability, increased expression of specific proteins, *e.g.* MUC20 (gene involved in the production of mucin) and PARM1, and increased fecal excretion of the antibacterial protein β-defensin-2[60]. Increased mucosal permeability in the small intestine was observed in patients with IBS-D[70], and it was associated with the expression and distribution of tight junction proteins; lower levels of the protein zonula occludens (ZO)-1 were found in IBS patients than controls[71,72]. Elsewhere, increased permeability along with mast cell infiltration into the colon was found to be associated with the severity of IBS symptoms[73]. Both the increased permeability and symptoms of IBS were improved by lactic acid bacteria, suggesting that there may be an association between an altered epithelial barrier and IBS symptoms[74]. It is worth noting that one study of gut permeability found that the increase was limited to the colon[75], whereas another IBS study reported that the expression and distribution of ZO-1 was altered in the jejunum[76]. Generally, this increase in gut permeability was found to be associated with bacteria-related protease activity and its receptors in the intestinal epithelium[77]. It is also thought that a single-nucleotide polymorphism in the gene encoding the tight junction protein, E-cadherin, may increase the risk of developing PI-IBS[78]. On the other hand, some bacterial metabolites produced by the intestinal microbiota were found to improve epithelial barrier function[79]. It has also been suggested that the barrier dysfunction with increased mucosal permeability in IBS may also be associated with visceral hypersensitivity[80].

Sensory-motor disturbances caused by intestinal microbiota

In addition to the mucosal inflammation of the gut that may affect sensory-motor and secretory functions, neuronal structure, and neurotransmitter release in the gut[81], the intestinal microbiota can directly affect intestinal sensory-motor functions[82]. Alterations in the microbiota induced by antibiotic treatment were found to precipitate visceral hypersensitivity, which was restored by probiotic treatment[83]. Probiotic treatment was also found to reduce sensation of pain via the enteric nerve in a model of visceral pain induced by colorectal distension[84]. A similar level of pain modulation was also achieved by inducing the expression of opioid and cannabinoid receptors[85]. With respect to motor disturbances, it has been reported that colonic motor function was enhanced by supernatants from the *E. coli* strain Nissle 1917, and that this was mediated by stimulation of smooth muscle cells[86]. Also, probiotic treatment was found to increase small-intestinal motor function in rats[87]. Furthermore, transplantation of healthy human fecal microbiota into GF mice increased their colonic motility and shortened GI transit, which was closely associated with the type and amount of carbohydrates in the diet[88]. The beneficial effects of the microbiota on motility were shown to be region-specific with migrating motor complex velocity increased in the jejunum but decreased in the colon[89]. These interactions between intestinal microbiota and GI sensory-motor function may be related to IBS, although the exact mechanism of the interactions is not well understood.

It seems that normal GI motility relies on TLR4 sig-

naling stimulated by the microbiota. It was demonstrated that mice lacking TLR4, which is frequently stimulated by bacterial LPS, exhibited longer GI transit times and reduced abundance of colonic nitrergic neurons[90]. In addition to the microbiota itself, the metabolites from bacterial fermentation may also exert an effect on GI motility. One of the colonic metabolites, CH4, was shown to delay intestinal transit[91], H_2S was shown to inhibit the contraction of intestinal smooth muscle[92], SCFA, to stimulate colonic transit by triggering 5-HT release[47], and tryptamine from tryptophan, to increase intestinal contractions[93]. Other bacterial metabolites that may be related to GI motility include bile acid metabolites[94] and ligands of GABA receptors with a suppressive effect on GI motility[95]. While the microbiota may affect gut sensory-motor function, the reverse may also be true: the microbial ecosystem in the gut may be disturbed by accelerated or decelerated GI transit[88]. It is thought that the changes in GI transit may alter the flow rate of intestinal contents and thereby affect the environment for resident bacteria, which then impinges on both the organizational structure and the gene expressed in the microbiota.

MODULATION OF THE INTESTINAL MICROBIOTA FOR MANAGING IBS SYMPTOMS

Dietary modifications

An association between diet and symptom development in IBS is reported frequently but its mechanisms are not clearly defined. Some of the proposed causative factors include hypersensitivity and/or allergic reaction to specific foods, and alterations of the habitat and metabolic activity of the intestinal microbiota. Diet is thought to be a powerful factor influencing the composition and metabolic activity of the microbiota in an individual. The composition of the microbiota in babies change after weaning, and in adults it varies according to geographic regions due to differences in the food consumed, the type of meat consumed, and cooking methods (whether the food is fried, baked or boiled). Therefore, any dietary strategy aimed at modifying the microbiota should be matched to the individual because different microbial species are responsive to different kinds of dietary components.

However, whether a change in the diet can directly affect the microbiota in IBS is not clear. This is partly due to the lack of well-designed, controlled trials that investigate the effects of diet on IBS. Although specific diets, *e.g.*, the FODMAPs diet, have been shown to provoke IBS symptoms in some patients, not all studies regarding the effects of exclusion diets on the symptoms of IBS are completely reliable due to a variety of confounding factors, including a high placebo effect. Nevertheless, it can be speculated that in some IBS patients, intake of certain foods may provoke abnormal fermentation due to aspects of the composition of their intestinal microbiota and that the composition of the microbiota in those patients could be changed to normal by excluding the symptom-provoking foods.

Dietary fiber stimulates the production of SCFAs by mixing with microbes and enzymes. In a healthy gut, these by-products can improve the function and homeostasis of the GI tract. Although it has been suggested that some patients with IBS may benefit from dietary fiber, many patients report an increase in abdominal distension and bloating as a result of fermentation of the fiber. It may be that water holding properties of fiber and its ability to accelerate intestinal transit may alter the habitat for the microbiota and therefore indirectly affect its composition and metabolic activity.

It seems that individualized advice on dietary consumption of non-digestible carbohydrates in the management of IBS, as the inter-individual differences in the response of the microbiota lead to different responses to changes in diet[96].

Antibiotics

Antibiotic treatment in IBS assumes that small intestinal bacterial overgrowth (SIBO) plays an important role in the development of IBS. Despite the limited validity and lack of standardization of the methods used to evaluate SIBO, treatment with non-absorbable antibiotics such as rifaximin has yielded a therapeutic benefit. Double-blind, placebo-controlled trials of rifaximin in IBS yielded an improvement in IBS symptoms, which correlated well with the reduced excretion of hydrogen in the breath[97,98]. These findings together with the positive effects of other antibiotic treatments, suggest that a short course of poorly absorbable antibiotics may be of some use in the management of IBS symptoms in some patients. However, data on the long-term effects of antibiotics in IBS are limited. Furthermore, information on the optimal dose of antibiotics, and predictors of treatment success and failure are needed to confirm the benefit of this type of treatment[99].

Probiotics

Effects of probiotics: By adhering to intestinal epithelial cells and competing for nutrients and space, probiotics can protect against pathogens. This protective effect of probiotics has been demonstrated *in vitro* using intestinal cell lines with lactobacilli, bifidobacteria and *E. coli* subspecies[100-102]. In addition, probiotics can improve mucosal barrier function and thereby prevent pathogens from increasing intestinal permeability[103,104]. Intestinal permeability can also be increased by stress, which may facilitate the subsequent translocation of pathogenic bacteria. However, it was observed that the increase in intestinal permeability caused by stress was inhibited by lactobacilli[105-107]. In addition, lactobacilli increased levels of bacterial fermentation products such as SCFAs (acetic, propionic and butyric acids) and thereby acidifying the colon, which subsequently increased the numbers of *Bifidobacterium* and *Lactobacillus* species and decreased

clostridia[108]. In addition to these roles, probiotics were also shown to modulate immunity in animals with experimentally-induced colitis[109,110]. Furthermore, they were shown to reduce visceral hypersensitivity by increasing the expression of opioid and cannabinoid receptors in the intestinal mucosa[85].

However, regarding the effect of probiotics on IBS symptoms, the mechanism is not clearly defined. It is possible that probiotics may not only modulate gut dysmotility and hypersensitivity but also have anti-inflammatory properties. It was found that probiotic treatment attenuated intestinal dysmotility in a mouse model, induced intestinal cell mediators related to reduced hypersensitivity such as cannabinoid and opioid receptors, and normalized the ratio of cytokines IL-10/IL-12 in the systemic circulation.

Probiotic studies in IBS: A majority of studies of probiotics in IBS have been performed to evaluate their effect on either overall or specific IBS symptoms. Although most of them have used *Lactobacillus* or *Bifidobacterium* species, single strains or combinations of multiple strains have also been used with multiple doses (from 10^6/mL to 10^{10}/mL) and for variable durations. Similarly, primary and secondary outcomes in those studies were evaluated using variable factors such as abdominal pain, symptom severity, quality of life, and global IBS symptoms. On balance, these studies found a therapeutic benefit, *i.e.*, improvement in symptoms of bloating, flatulence, bowel frequency, and in global symptoms, although there are some inconsistencies between specific studies. In particular, beneficial effects of probiotics were reported in a well-designed study using bifidobacteria such as *Bifidobacterium infantis* 35624[30,111], *B. lactis*, *B. animalis* DN173010, and *B. bifidum* MIMBb75[112]. Symptom improvement was also reported in studies using probiotic mixtures such as *Escherichia coli* (DSM 17252) and *Enterococcus faecalis* (DSM 16440)[113], and *Lactobacillus rhamnosus* GG, *L. rhamnosus* LC705, *Bifidobacterium breve* Bb99 and *Propionibacterium freudenreichii* ssp. *shermanii* JS[114,115]. By contrast, negative results were reported in studies using other probiotic combinations[116], such as *Lactobacillus paracasei* spp. *paracasei* F19, *L. acidophilus* La5 and *Bifidobacterium lactis* Bb12[117,118], and *Lactobacillus plantarum* MF1298[119].

In recent studies, it was found that 4-wk treatment with probiotics improved IBS symptoms and altered composition of the microbiota as well[120], and that probiotic treatment in IBS patients reduced the genus Bacteroides to the levels of healthy controls and also improved global IBS symptoms[121]. However, as indicated in several meta-analyses, the previous studies of probiotics in IBS fail to report whether symptom improvement was accompanied by a change in the microbiota or not. Furthermore, many systematic reviews pointed out several study limitations including heterogeneity, inadequate statistical methods, and possible publication bias. Examples of heterogeneity include differences in types, doses, and delivery of probiotics[122-125], which may have produced different outcomes. Therefore, despite the reported benefits of probiotics in IBS, there are many aspects of potential treatment regimens that are yet to be established, such as adequate dosage, treatment duration, choice of species for each individual or symptom of IBS, target symptoms for probiotics, and probiotic formulation. Future studies should aim to identify which species, strains, and doses of probiotics provide the optimal therapeutic benefit to individual patients with IBS, and which specific symptoms of IBS should be the target of probiotic treatment.

CONCLUSION

Intestinal microbiota can play a substantial role in IBS. Although the microbiota may contribute directly to the symptoms of IBS, it is more likely that altered composition and metabolic activity of the microbiota caused by stress or other psychological disturbances indirectly activate mucosal immunity and inflammation, increase epithelial permeability, and reduce barrier function, thereby activating the sensory-motor dysfunction responsible for a variety of IBS symptoms. Therefore, our knowledge of the link between the microbiota and IBS may enable us to treat focusing on the possible mechanism of this disorder; Dysbiosis may be restored by probiotic or antibiotic treatment and also by diet modification. Activation of mucosal immunity and inflammation can be treated by immune-modulating agents. Increased intestinal permeability and barrier dysfunction can be a potential therapeutic target of probiotics. However, the microbial pathophysiology of IBS is not clearly understood, as microbiota alterations in IBS might be either a cause of IBS or a consequence of intestinal secretion and motility changed by IBS. Furthermore, due to the heterogeneity of IBS studies as well as IBS itself, there has been variability in the results of studies. Therefore, objective diagnostic modalities in IBS are warranted, and further studies using advanced molecular techniques are needed.

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WJG 20th Anniversary Special Issues (4): Irritable bowel syndrome

Constipation-predominant irritable bowel syndrome: A review of current and emerging drug therapies

Khaled A Jadallah, Susan M Kullab, David S Sanders

Khaled A Jadallah, Susan M Kullab, Department of Internal Medicine, King Abdullah University Hospital, Faculty of Medicine, Jordan University of Science and Technology, Irbid 22110, Jordan
David S Sanders, Department of Gastroenterology and Liver Unit, Royal Hallamshire Hospital, Sheffield, South Yorkshire S102JF, United Kingdom
Author contributions: Jadallah KA and Sanders DS conceived and designed the study; Jadallah KA and Kullab SM performed the literature search and wrote the manuscript; all authors revised and approved the final version.
Correspondence to: Khaled A Jadallah, MD, Assistant Professor of Medicine, Department of Internal Medicine, King Abdullah University Hospital, Faculty of Medicine, Jordan University of Science and Technology, Irbid 22110, Jordan. khaled-j@just.edu.jo
Telephone: +962-79-6673884 Fax: +962-2-7200624
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Abstract

Irritable bowel syndrome (IBS) is a highly prevalent medical condition that adversely affects patient quality of life and constitutes a significant economic burden on healthcare resources. A large proportion of patients suffer from the constipation subtype of IBS (IBS-C), most commonly afflicting older individuals and those with a lower socioeconomic status. Conventional pharmacologic and nonpharmacologic treatment options have limited efficacies and/or significant adverse events, which lead to increased long-term health care expenditures. Failure to effectively treat IBS-C patients over the past decades has largely been due to a poor understanding of disease pathophysiology, lack of a global view of the patient, and an inappropriate selection of patients and treatment endpoints in clinical trials. In recent years, however, more effective and safer drugs have been developed for the treatment of IBS-C. The advancement in the area of pharmacologic treatment is based on new knowledge of the pathophysiologic basis of IBS-C and the development of drugs with increased selectivity within pharmacologic classes with recognized efficacies. This narrative review covers the spectrum of available drugs and their mechanisms of action, as well as the efficacy and safety profiles of each as determined in relevant clinical trials that have investigated treatment options for IBS-C and chronic constipation. A brief summary of laxative-based treatment options is presented, followed by up-to-date assessments for three classes of drugs: prokinetics, prosecretory agents, and bile acid modulators.

Key words: Constipation; Irritable bowel syndrome; Drug therapy; Serotonergic agents; Prokinetics; 5-hydroxytryptamine type 4 agonists; Secretagogues; Prosecretory agents; Bile acid modulators

Core tip: Constipation-predominant irritable bowel syndrome (IBS-C) is one of the most common disorders seen by gastroenterologists worldwide, and is associated with a substantial burden on health care resources. Pharmacologic treatments for IBS-C have largely been unsatisfactory, mainly due to the multifaceted and poorly understood pathophysiology of this disorder. Recently approved drugs and novel investigational compounds are expected to streamline the management of IBS-C. This narrative review covers the mechanisms, clinical trial efficacies, and safety profiles of these pharmacologic agents, in order to help practicing physicians keep up with the rapidly developing field of IBS-C therapy.

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INTRODUCTION

Irritable bowel syndrome (IBS) is one of the most common gastrointestinal (GI) disorders across all ages and ethnicities, with a worldwide prevalence ranging between 5% and 20%[1-4]. The majority of individuals with IBS experience impairments to their performance of daily activities and decreased health-related quality of life, for which conventional treatments provide limited resolutions[5,6]. For some IBS sufferers, substantial psychologic and psychiatric disturbances develop over time, leading to polypharmacy accompanied by the inherent risk of drug interactions, further deterioration of health status, and increased health care expenditures[6,7].

The constipation-predominant subtype of IBS (IBS-C), defined by constipation associated with abdominal pain that is generally relieved by defecation[8], affects about 34% of the IBS population[9], of which a substantial fraction are of older age and lower socioeconomic status[3]. Recent evidence suggests that IBS-C is associated with higher rates of functional impairment, as compared to other subtypes of IBS[10-12]. Conventional laxative-based pharmacologic treatment of IBS-C, which is mostly symptom-based, is largely unsatisfactory[13,14]. Yet, despite the substantial burden of IBS-C-associated ailments and the well-recognized need for more efficacious and safer treatments, few novel treatment compounds have been approved for clinical use. The need for a drug therapy that effectively treats all of the symptoms of IBS-C (abdominal pain, constipation, and secondary symptoms of constipation), improves the patient's health-related quality of life, and can be used safely on a chronic basis remains unfulfilled.

Advancement in the treatment of IBS-C requires a greater focus on the pathophysiologic abnormalities underlying each of the symptoms of this complex disorder[15], which is the scientific basis for the development of new pharmaceutical compounds. The present article reviews the current pharmacologic agents for the treatment of IBS-C, in terms of their clinical trial efficacy, tolerability, and safety. A brief description of the broad spectrum of laxative-based treatment options is also presented. In general, this review focuses on the main classes of drugs that have been the subject of active research in recent years (prokinetics, prosecretory agents or secretagogues, and bile acid modulators). Furthermore, in addition to the well-established drugs (tegaserod and lubiprostone), newly-approved drugs (prucalopride, velusetrag, linaclotide, plecanatide, chenodeoxycholate (CDC) and elobixibat) as well as drugs currently in development for the treatment of IBS-C are discussed. As there is significant overlap between IBS-C and chronic constipation (CC)[16], drugs that are currently approved or being investigated for the treatment of CC are also included in this review, according to their potential for use in the management of IBS-C; for instance, lubiprostone, which was initially developed and approved for CC, has subsequently received approval for the treatment of IBS-C. Nonpharmacologic remedies, such as fiber supplements and probiotics, however, are not discussed.

Studies included in this review were collected from a PubMed search for English-language articles published between 1980 and December 2013 using the following keywords alone or in combination: irritable bowel syndrome, constipation, constipation-predominant irritable bowel syndrome, drug therapy, laxatives, prokinetics, serotonergic agents, 5-HT$_4$ agonists, secretagogues, prosecretory agents, bile acid modulators, randomized controlled trials (RCTs), meta-analysis. Governmental websites [www.clinicaltrials.gov (United States), www.clinicaltrialsregister.eu (European Union)] were searched for data concerning ongoing clinical trials. Only high quality studies were cited and discussed in the present review.

LAXATIVE-BASED PHRARMACOLOGIC AGENTS

Conventional laxatives and stool softeners have been used for decades for the treatment of CC, and have also been used by IBS-C patients to improve their bowel habits[13,14]. Clinical experience and, to a lesser extent, evidence from the literature indicate that about half of the patients treated with laxatives are disappointed by the lack of long-term efficacy[17-19]. Despite the high prevalence and the remarkable socioeconomic burden associated with IBS-C and CC, concrete evidence from high-quality RCTs on laxative efficacy and safety is very limited[20]. In fact, only recently have well-conducted studies provided evidence for the use of bisacodyl in CC and polyethylene glycol in IBS-C[21,22].

Although laxative-based treatments provide short-term relief of constipation in many CC and IBS-C patients, there is a lack of high quality evidence to support their regular use. However, laxatives remain a suitable therapeutic option for many patients because of their relative safety, low cost, and over-the-counter availability. Well-conducted RCTs comparing the most commonly used laxatives and newer pharmacologic agents will help to identify the safest and most effective therapy for regular use. The mechanisms and most common adverse events of different types of laxatives are summarized in Table 1.

PROKINETICS

Slow colonic transit is recognized as one of the most important mechanisms underlying constipation. Prokinetics have been designed to stimulate muscle activity to counter the underlying hypomotility that is linked with slow-transit constipation[23,24]. A crucial role for 5-hydroxytryptamine (5-HT, serotonin) in normal enteric nervous system function has been documented[25-27], and the ex-

Table 1 Main types of pharmacologic laxatives

Type	Agents	Mechanism of action	Most common adverse events
Bulking agents	Psyllium Methylcellulose Calcium polycarbophil	Increase in stool bulk and reduction in consistency by luminal water binding	Bloating Flatulence
Stool softeners (surfactants)	Docusate potassium Docusate sodium Docusate calcium	Softening and lubrication of stools by increasing water secretion	Nausea Vomiting Abdominal pain/cramps Rectal urgency
Osmotic laxatives	Milk of Magnesia (magnesium hydroxide) Magnesium citrate Magnesium sulphate Sodium picosulphate/magnesium citrate (Picoprep®) Lactulose/lactilol Sorbitol Polyethylene glycol (macrogol)	Osmotic water retention, decreased stool consistency, and increase fecal volume and peristalsis	Sweet taste Nausea Bloating Flatulence Abdominal pain/cramps Electrolyte disturbances (?)
Stimulant laxatives	Anthraquinones Senna Cascara Bisacodyl Phenolphthalein	Luminal water retention through activation of CAMP, and induction of colonic contractions by acting on enteric nerves	Abdominal pain/cramps Dehydration Electrolyte disturbances Muscle cramps Melanosis coli/colonic inertia (?)

CAMP: Cyclic adenosine monophosphate.

Table 2 Chemical and clinical characteristics of discontinued/failed prokinetics

	Cisapride	Renzapride	Tegaserod
Chemical structure	Piperidinyl benzamide	Benzamide derivative	Indole carboxaldehyde derivative
Target receptors	Nonselective 5-HT$_4$ agonist and 5-HT$_3$ antagonist	Full 5-HT$_4$ agonist and antagonist of 5-HT$_3$ and 5-HT$_{2b}$	5-HT$_4$ and 5-HT$_1$ partial agonist
Mechanism of action/ pharmacodynamic effects	Local acetylcholine release; Acceleration of GI transit	Local acetylcholine release; Acceleration of GI transit	Augmentation of the peristaltic reflex; Enhanced intestinal secretion; Reduced sensitivity to rectal distension
Most common adverse events	Diarrhea Abdominal pain	Diarrhea Abdominal pain Headache Flatulence	Diarrhea Abdominal pain Headache Flatulence
Safety	Prolongation of QTc interval and fatal arrhythmias	No prolongation of QTc interval	Increased risk of serious ischemic cardiac events
Approval status	Approved in 1993; Withdrawn in 2000	Phase 3 RCTs terminated due to insufficient efficacy	Approved in 2002 for IBS-C (not in EU) and in 2004 for CC; Withdrawn in 2007

CC: Chronic constipation; EU: European Union; GI: Gastrointestinal; IBS-C: Constipation predominant-irritable bowel syndrome; QTc: Corrected QT interval; RCT: Randomized controlled trial; 5-HT: 5-hydroxytryptamine.

pression of the 5-HT type 4 (5-HT$_4$) receptor in the GI tract has been associated with intestinal motility[23,28]. In the past two decades, several prokinetic agonists of the 5-HT$_4$ receptor have been introduced in clinical practice. Table 2 presents the chemical and clinical characteristics of the older prokinetics, whereas Table 3 summarizes the characteristics of the newer prokinetics.

Cisapride

Cisapride, a non-selective 5-HT$_4$ agonist, was originally developed for the treatment of functional upper GI disorders, and later found to be efficacious for treating constipation[29]. However, its interaction with human ether-a-go-go-related gene (hERG) potassium channels leads to cardiac arrhythmias, which caused the drug to be withdrawn from the global market[29]. This "rise and fall" of cisapride underscores the importance of longitudinal safety studies for newer drugs, as well as the need for post-market monitoring.

Tegaserod

Tegaserod, a partial 5-HT$_4$ agonist devoid of the arrhythmogenic effect elicited by cisapride, was demonstrated in RCTs to be an efficacious and well-tolerated promotility agent in IBS-C patients[30,31]. The drug received approval for the treatment of women with IBS-C in July 2002 in the United States and a few other countries, but not in the European Union. In August 2004, the United States's Food and Drug Administration (FDA) also approved tegaserod for the treatment of patients with CC, and a subsequent multinational high-quality randomized controlled trial demonstrated its efficacy and tolerability in these patients[32]. Nevertheless, due to ensuing reports of ischemic cardiac events, tegaserod was withdrawn from the

Table 3 Chemical and clinical characteristics of novel prokinetic agents

	Prucalopride	Naronapride	Velusetrag	ROSE-010
Chemical structure	Dihydrobenzofuran carboxamide	Benzamide	Dihydroxyquinoline-carboxamide	Glucagon-related peptide
Target receptor/affinity	High selectivity and affinity for 5-HT$_4$ (> 150-fold)	5-HT$_4$ full agonist in the GI tract; partial agonist in the heart	Potent selective agonist of 5-HT$_4$ with high affinity (500-fold)	GLP-1 analogue
Pharmacodynamic effects	Accelerated colonic transit in health and CC	Accelerated colonic transit in health	Dose-dependent acceleration of colonic transit in health	Acceleration of colonic transit; antinociceptive effect in IBS-C
Most common adverse events	Diarrhea Nausea Headache Abdominal pain	Diarrhea Headache	Diarrhea Nausea Headache Vomiting	Nausea Headache
Approval status/stage of development	Approved for CC in EU in 2009 and in Canada in 2011	Phase 2 RCTs in CC completed	Phase 2 RCTs in CC completed	Phase 2 RCTs in IBS-C completed

CC: Chronic constipation; EU: European Union; GI: Gastrointestinal; GLP-1: Glucagon like peptide-1; IBS-C: Constipation-predominant irritable bowel syndrome; RCT: Randomized controlled trial; 5HT: 5-hydroxytryptamine.

market in March 2007, and since 2009, its use has been limited to emergency situations[33]. Although tegaserod was eventually removed from the worldwide market, it is still considered to represent an important step in the development of novel serotonergic drugs for the management of IBS-C and CC.

Prucalopride

In recent years, three highly selective 5-HT$_4$ agonists, namely prucalopride, velusetrag, and naronapride, have been investigated mainly for the treatment of CC (Table 2). In contrast to nonselective 5-HT$_4$ agonists, these pharmacologic compounds have not been associated with adverse cardiovascular events[34]. Large, multicenter RCTs have shown that prucalopride, the most extensively investigated drug of this class, is efficacious and safe for treating patients with CC[34-36]. In October 2009 the European Medicines Agency (EMA) approved prucalopride (Resolor®, 2 mg once daily) for the treatment of CC in women for whom laxative-based approaches failed to grant adequate relief[36]. In November 2011 the drug received approval in Canada (Resotran®, 1 or 2 mg once daily) for the same indication; although, to date, the drug remains unapproved by the United States FDA.

Recently, a large phase 3 RCT conducted in 46 sites from five countries of the Asia-Pacific region evaluated the efficacy and safety of a 12-wk treatment with daily prucalopride (2 mg) in CC patients[37]. In that study, significantly more patients responded to prucalopride than placebo (33.3% *vs* 10.3%), with responding patients having a weekly average of $\geqslant$ 3 spontaneous complete bowel movements (SCBMs). The most frequently reported adverse events were diarrhea, nausea, abdominal pain, and headache, all of which mainly occurred during the first and second day of drug administration. Thus, the authors concluded that daily 2 mg prucalopride was effective and well tolerated, with a favorable safety profile. Although no studies have yet addressed the efficacy of prucalopride in IBS-C, it is expected that it will also be efficacious for the disease symptoms, even though worsening of abdominal pain would limit its use in clinical practice.

Velusetrag

The second highly selective 5-HT$_4$ agonist, velusetrag (TD5108), has demonstrated stimulatory effects on colonic motility and transit in a phase 1 RCT[38]. In that trial, 60 healthy volunteers received one of four doses of velusetrag (5, 15, 30 or 50 mg) as a single dose or once daily for six days. A significant increase in the colonic transit and bowel emptying time of the descending colon was observed in participants receiving the single dose, and accelerated gastric emptying occurred in participants receiving multiple doses, with no serious adverse events. A four-week phase 2 RCT in 401 patients evaluated the efficacy, safety and tolerability of different velusetrag doses (15, 30 or 50 mg/d) in CC patients[39]. Patients treated in that study showed significant improvement in SCBMs, stool consistency, and time to achieve the first bowel movement, with adverse events, such as diarrhea, headache, nausea and vomiting, mostly occurring in the first two days of treatment. The adverse events-related discontinuation rate was 5%, and no manifestations of cardiac toxicity were noted. The results of these RCTs indicate that velusetrag is a safe drug and efficacious for the treatment of CC, though larger and longer phase 3 trials are required before robust conclusions are drawn. Furthermore, treatment of IBS-C patients with velusetrag has yet to be evaluated.

Naronapride

A third drug, naronapride (ATI-7505), is a full agonist of 5-HT$_4$ receptors in the GI tract and partial agonist of these receptors in the heart. It is structurally similar to cisapride, but without affinity for 5-HT$_3$ receptors and negligible hERG potassium channel activity[40,41]. The drug is currently being investigated for the treatment of upper and lower GI functional disorders, but only limited

Table 4 Chemical and clinical characteristics of prosecretory agents

Drug	Lubiprostone	Linaclotide	Plecanatide
Chemical structure	A prostone, bicyclic fatty acid (metabolite of prostaglandin E1)	14-amino acid peptide, analogue of guanylin	Analogue of uroguanylin
Target receptor/ mechanism of action	Activation of ClC-2 by direct action on epithelial cells provoking intestinal fluid secretion, also mediated by CFTR	Binding to GC-C with stimulation of cGMP and CFTR-mediated secretion; desensitization of afferent pain fibers mediated by production of extracellular cGMP	GC-C receptor activation with CFTR-mediated secretion
Pharmacodynamic effects	Accelerated small bowel and colonic transit	Dose-related acceleration of colonic transit	Probable acceleration of colonic transit
Most common adverse events	Nausea Diarrhea Abdominal pain	Dose-dependent diarrhea	Dose-independent diarrhea Nausea
Potential other beneficial effects	Mucosal protection	Antineoplastic	-
Cost	AWP is $296 for one month supply	AWP is $255 for 30 capsules	-
Approval status/stage of development	United States FDA-approved for women with IBS-C and men and women with CC	United States FDA-approved for both IBS-C and CC EMA-approved for IBS-C only	Phase 2b RCT in CC completed; Phase 3 RCT in CC recruiting patients; Phase 2 RCT in IBS-C recruiting patients

AWP: Average wholesale price; CC: Chronic constipation; CFTR: Cystic fibrosis transmembrane conduction regulator; cGMP: Cyclic guanosine monophosphate; ClC-2: Chloride channel-2; EMA: European Medicines Agency; FDA: Food and Drug Administration; GC-C: Guanylate cyclase-C; IBS-C: Constipation-predominant irritable bowel syndrome; RCT: Randomized controlled trial.

data are available in the literature thus far.

Renzapride, clebopride, and mosapride

Renzapride, clebopride, and mosapride are nonselective 5-HT$_4$ agonists that are no longer considered for the treatment of patients with IBS-C or CC. Though they were shown to be safe from a cardiovascular standpoint[33], they did not show significant efficacy in IBS-C clinical trials and were therefore abandoned[42,43].

ROSE-010

ROSE-010 is an experimental glucagon-like peptide-1 (GLP-1) analogue that affects the motility of and nociception in the GI tract[44]. In one RCT investigating the effect on acute abdominal pain in IBS, ROSE-010 was favored over a placebo for patient-rated pain relief[45]. More recently, a phase 2 RCT investigating the effect of ROSE-010 on GI motor functions in women with IBS-C found that although gastric emptying was delayed, colonic transit was significantly accelerated after 48 h, providing relief of constipation in these patients[46]. Although these results are encouraging, phase 3 RCTs are needed to confirm the efficacy and safety of ROSE-010.

PROSECRETORY AGENTS (SECRETAGOGUES)

In the last decade, intestinal secretion has been the subject of active research for the development of treatments for CC and IBS-C. The chemical and clinical characteristics of prosecretory agents, drugs that augment intestinal secretion, thus acting as a stool lubricant and facilitating its evacuation, are summarized in Table 4.

Lubiprostone

Lubiprostone, a chloride channel activator, was the first secretagogue to be investigated and approved for treatment of CC and IBS-C. Chloride channels have been recognized as the major effectors of fluid transport and secretion in the intestinal lumen[47]. In particular, type-2 chloride channels (ClC-2) have been explored with regard to their role in CC and IBS-C[48,49]. Lubiprostone is a highly specific activator of ClC-2 channels that leads to increased intestinal secretion[50,51], an effect that requires the cystic fibrosis transmembrane conductance regulator (CFTR)[52]. A phase 2, 12-wk double-blind RCT demonstrated that lubiprostone [8, 16 and 24 μg, twice daily (BID)] reduced abdominal pain in IBS-C patients, though higher doses were associated with more adverse events, namely nausea and diarrhea[53]. Schey and Rao demonstrated that 8 μg lubiprostone BID offered the best risk-benefit ratio for IBS-C patients[54].

The positive results from the phase 2 studies led to two phase 3, multicenter RCTs involving 1171 IBS-C patients treated for three months with 8 μg lubiprostone BID[55]. The primary efficacy endpoint was the percentage of overall responders that were at least moderately relieved for all four weeks of the month or significantly relieved for at least two weeks of the month. Patient-rated symptoms were significantly improved with lubiprostone treatment, with no increase in adverse events compared to the placebo. As the lubiprostone regimen was effective, well tolerated and safe, the long-term (up to 52 wk) efficacy, safety, and tolerability was evaluated in an extension study including 522 of these same IBS-C patients[56]. The results of this extended trial confirmed the efficacy of lubiprostone, with a favorable safety and tolerability profile for up to 13 mo. However, the absence

of a placebo arm raises some questions about the statistical validity of the data gathered.

Lubiprostone was approved by the United States FDA in April 2006 for the treatment of CC in men and women, and in April 2008 for the treatment of IBS-C in women. The recommended dose is 24 μg BID for CC and 8 μg BID for IBS-C. A four-week phase 3 RCT evaluated the efficacy and safety of 24 μg lubiprostone BID in 237 patients with CC and demonstrated significant improvement in the number of SCBMs, stool consistency, straining effort, and global bowel satisfaction[57]. Thus, lubiprostone was considered to be the "ideal" drug for IBS-C, as it was shown to be effective on all symptoms of IBS-C, including abdominal pain. However, recent data has suggested that lubiprostone may not have an anti-nociceptive effect in IBS-C. In fact, Whitehead *et al*[58] demonstrated that lubiprostone has no effect on visceral sensory thresholds in 62 IBS-C patients who completed a barostat test of pain and urge sensory thresholds. The authors concluded that lubiprostone did not relieve abdominal pain directly, but that the reduction in clinical pain in patients appeared to be secondary to changes in stool consistency.

Linaclotide

Linaclotide, a minimally absorbed first-in-class peptide agonist of guanylate cyclase C (GC-C), was recently approved for the treatment of IBS-C and CC. GC-C mediates intestinal secretion in response to heat-stable enterotoxins, the major cause of *Escherichia coli*-induced secretory diarrhea[59]. Linaclotide binds to GC-C, which is richly present on the luminal surface of the intestinal enterocytes[60], and ultimately activates CFTR, resulting in the secretion of chloride and bicarbonate into the intestinal lumen. Consequently, intestinal fluid secretion is increased, stools are softened, and colonic transit may be accelerated. The effect of linaclotide on ascending colonic transit has been demonstrated in a phase 2 RCT involving 36 women with IBS-C[61]. Additionally, unlike lubiprostone, linaclotide has been also shown to reduce visceral nociception in laboratory rodents[62]. More recently, this visceral antihyperalgesic effect has been replicated in healthy mice and those with chronic visceral hypersensitivity[63]. The dual action of linaclotide on both constipation and abdominal pain in IBS-C is likely related to its approval by both the United States FDA and the EMA.

The efficacy and safety of linaclotide for the treatment of IBS-C patients have been demonstrated in four well-conducted RCTs[61,64-66]. In a 12-wk RCT study of 420 IBS-C patients, Johnston *et al*[64] found that various doses of linaclotide (75, 150, 300 and 600 μg, once daily) were effective in improving all symptoms of IBS-C. The only observed adverse event in that trial was a dose-dependent diarrhea, whereas other adverse events were comparable between the treatment and placebo groups. A phase 3, 26-wk RCT[65] was recently conducted with linaclotide (290 μg daily) in 804 IBS-C patients according to the recommended United States FDA primary endpoints (responder: a patient who reported (1) ⩾ 30% improvement in an average daily worst abdominal pain score; and (2) an increase of ⩾ 1 average weekly SCBMs for at least half of the trial duration)[67]. The results of that trial showed that 33.7% of treated patients were United States FDA endpoint responders, compared to only 13.9% of those receiving a placebo. Specifically, 48.9% of treated patients met the criterion for pain responder, and 47.6% met the SCBM responder criterion, compared to 34.5% and 22.6% respectively of placebo-treated patients. In terms of safety and tolerability, diarrhea was the most common adverse event, occurring most often within the first four weeks of therapy, while the discontinuation rates were 10.2% and 2.5% for linaclotide and placebo, respectively. Another phase 3 RCT included a 12-wk treatment period followed by a four-week randomized withdrawal period[66]. The outcome measures of that study were the United States FDA endpoints for IBS-C and three other endpoints based on improvement in abdominal pain and SCBMs. The results of this trial also indicated that linaclotide was safe and effective in relieving IBS-C symptoms, with diarrhea being the most common adverse event and no worsening of symptoms in the withdrawal period.

Linaclotide (145 μg, once daily) was also shown by four well-conducted RCTs to be safe and effective for the treatment of CC[68-70]. Moreover, the safety and efficacy of linaclotide for the treatment of patients with IBS-C and CC has been confirmed by a recent meta-analysis study[71]. In August 2012, linaclotide (Linzess®; Ironwood Pharmaceuticals, Inc., Cambridge, MA, United States) was approved by the United States FDA for the treatment of IBS-C at a dose of 290 μg once daily and CC at a dose of 145 μg once daily[72]. In the European Union, the drug received approval for IBS-C patients but not for CC patients. The approval of linaclotide represented an important development in the treatment of IBS-C and CC, especially for those patients with poor tolerance or response to lubiprostone.

In summary, there is evidence showing that linaclotide is an effective, well tolerated, and safe therapeutic option for patients with IBS-C and CC, though the long-term safety and efficacy of linaclotide as well as a direct comparison with lubiprostone need to be investigated. Importantly, this drug has the advantage of improving both bowel symptoms and abdominal pain. However, the high cost of linaclotide and lubiprostone may limit their use in clinical practice, especially because a large proportion of IBS-C and CC patients belong to lower socioeconomic groups.

Plecanatide

Similar to linaclotide, plecanatide is a minimally absorbed GC-C agonist believed to act on both intestinal secretion and nociception. A phase 1 RCT was conduced in 72 healthy volunteers to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of various doses

Table 5 Chemical and clinical characteristics of bile acid modulators

	Chenodeoxycholate	Elobixibat
Chemical structure	Sodium chenodeoxycholic acid (primary bile acid)	Enantiomer of 1,5-benzothiazepine
Mechanism of action	Deconjugation to secondary bile acids, thus inducing colonic secretion and propulsive contractions	IBAT inhibition resulting in delivery of endogenous bile acids to the colon, thus inducing colonic secretion and propulsive contractions
Pharmacodynamic effects	Accelerated colonic transit	Dose-dependent acceleration of colonic transit
Most common adverse events	Diarrhea Abdominal cramping/pain Nausea	Diarrhea Abdominal cramping/pain
Potential other beneficial effects	Probable lowering of LDL	Lowering of LDL and cholesterol
Stage of development	Phase 3 RCT in IBS-C completed	Phase 3 RCTs in CC, completed; extended safety and tolerability RCTs enrolling

CC: Chronic constipation; IBAT: Ileal bile acid transporter; IBS-C: Constipation-predominant irritable bowel syndrome; LDL: Low-density lipoprotein; RCT: Randomized controlled trial.

(ranging from 0.1 to 48.6 mg) of oral plecanatide[73]. The study found no measurable systemic absorption of plecanatide, with adverse events similar to the placebo; thus, it was concluded that the drug acts locally in the intestine and is well tolerated and safe. However, low statistical power prevented the authors from making any conclusions with respect to the pharmacodynamic parameters. Preliminary results from a phase 2a RCT that is underway in patients with CC have suggested that plecanatide is effective, well tolerated, and safe at doses up to 9 mg[74]. Moreover, plecanatide-treated patients showed significant improvement in bowel symptoms without any observed serious adverse events. Other phase 2 RCTs using plecanatide in CC and IBS-C patients are still recruiting patients, and no results have been reported thus far.

BILE ACID MODULATORS

Bile acid modulators have been used to treat constipation disorders based on the observation of increased incidence of diarrhea in patients taking bile acids for gallstones or cholestatic liver diseases[75], and in patients with terminal ileum disease or resection[76]. The enhancement of colonic secretion and motility is caused mainly by the deconjugation of bile acids in the colon to secondary bile acids[77,78]. Thus far, two drugs, CDC and elobixibat, have been investigated for the treatment of IBS-C and CC. Their chemical and clinical characteristics are shown in Table 5.

CDC

CDC is a primary biliary acid that has been in use for many years for the dissolution of gallstones. In clinical studies, the main adverse event of CDC (Chenodal®; Manchester Pharmaceuticals, Fort Collins, CO, United States) was a dose-dependent diarrhea[77] that is of the secretory type, due mainly to intracellular activation of adenylate cyclase and increased intestinal permeability[77,79,80]. In a four-week placebo-controlled RCT of 20 gallstone patients with CC, Bazzoli *et al*[81] found that CDC significant improved bowel frequency and stool consistency. In a recent four-day double-blind RCT of 36 women with IBS-C, CDC (500 or 1000 mg, once daily) increased stool frequency, softened stools and improved straining, with lower abdominal cramping as the most commonly reported adverse event[82]. The authors concluded that the effect in these female patients was dependent on specific genetic variations in the negative feedback inhibition of bile acid synthesis. Therefore, CDC has the potential to be used as a "physiologic laxative" for the treatment of both IBS-C and CC; although, its use in IBS-C may be limited by the concern for worsening of abdominal pain.

Elobixibat

Elobixibat (formerly A3309) is a first-in-class ileal bile acid transporter inhibitor that is currently being investigated for the treatment of CC. Elobixibat has some potential advantages over currently approved drugs (prucalopride, lubiprostone, linaclotide). First, given its negligible systemic absorption, it is unlikely to induce cardiovascular toxicity, a theoretical effect of prucalopride. Second, it has a positive effect on both secretion and motility of the colon, while lubiprostone and linaclotide are only secretagogues, without any direct effect on colonic motility[77,78].

In the first human study of the pharmacokinetic and pharmacodynamic actions of elobixibat, Simrén *et al*[83] assessed the safety and tolerability of the drug in 30 patients with CC. The efficacy and metabolic parameters of patients receiving one of five elobixibat doses (from 0.1 to 10 mg, once daily) were favorable, with no significant adverse events. Two phase 2 RCTs focusing on the efficacy of elobixibat in CC patients with doses ranging from 5 to 20 mg once daily demonstrated significant improvement of all constipation parameters[84,85]. Furthermore, safety and tolerability analyses showed no serious adverse events, with lower abdominal cramping being the most common. Based on the results of these studies, elobixibat appears to be a promising pharmacologic option for patients with CC. The efficacy of elobixibat for the treatment of IBS-C has not yet been investigated, though the abdominal pain that is commonly observed might limit

Table 6 Chemical and clinical characteristics of drugs approved for other gastrointestinal indications and currently investigated for constipation-predominant irritable bowel syndrome

	Itopride	Neomycin/Rifaximin
Brand name	Ganaton®	Neomycin: Neo-Fradin® Rifaximin: Xifaxan®
Chemical structure	Benzamide derivative	Neomycin: aminoglycoside Rifaximin: semisynthetic antibiotic based on rifampicin
Mechanism of action	Dopamine D2 antagonist and acetylcholinesterase inhibitor	Neomycin: inhibition of protein synthesis Rifaximin: inhibition of bacterial RNA synthesis
Pharmacodynamic effects	Gastrokinetic; Acceleration of intestinal transit (?)	Eradication of methane; accelerated intestinal transit (?)
Most common adverse events	Diarrhea Headache Hyperprolactinemia	Neomycin: Neurotoxicity Ototoxicity Nephrotoxicity Rifaximin: Headache Nausea Dizziness Fatigue
Approval status/stage of development	Approved in Japan for functional dyspepsia; Phase 2 RCT in IBS-C completed in the United States	FDA-approved for hepatic encephalopathy and traveler's diarrhea; Phase 2 efficacy RCT in methane + IBS-C patients, comparing neomycin *vs* combination rifaximin and neomycin (completed)

FDA: Food and Drug Administration; IBS-C: Constipation-predominant irritable bowel syndrome; RCT: Randomized controlled trial.

Table 7 Quality of evidence supporting different pharmacologic agents for constipation-predominant irritable bowel syndrome and chronic constipation

Pharmacologic agent	Quality of evidence for IBS-C	Quality of evidence for CC
Laxatives		
Psyllium	No RCTs	Moderate
Docusate sodium	No RCTs	Low
Lactulose	No RCTs	Moderate
PEG	Moderate	High
Senna	No RCTs	Low
Bisacodyl	No RCTs	Moderate
Prokinetics		
Prucalopride	No RCTs	High
Naronapride	No RCTs	Low
Velusetrag	Low	Low
Rose-010	Moderate	No RCTs
Secretagogues		
Lubiprostone	High	High
Linaclotide	High	High
Plecanatide	Low	Low
Bile acid modulators		
CDC	Low	Low
Elobixibat	No RCTs	Moderate

The quality of evidence was assessed according to the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) system[86], which defines study quality as high (further research is very unlikely to change confidence in the estimated effect); moderate (further research is likely to have an important impact on confidence in the estimated effect and may change the estimate); low (further research is very likely to have an important impact on confidence in the estimated effect and is likely to change the estimate); or very low (any estimate of effect is very uncertain). CC: Chronic constipation; CDC: Chenodeoxycholate; IBS-C: Constipation-predominant irritable bowel syndrome; PEG: Polyethylene glycol; RCT: Randomized controlled trials.

its use in clinical practice.

OTHER INVESTIGATIONAL AGENTS

The search for safer and more effective drugs for the treatment of IBS-C is ongoing, with phase 1 and phase 2 clinical trials underway to evaluate various pharmacologic options, including drugs already approved for other gastrointestinal indications [Ganaton® (Abbott India Ltd., Mumbai, India), Neo-Fradin® (X-Gen Pharmaceuticals Inc., Horseheads, NY, United States), Xifaxan® (Salix Pharmaceuticals Inc., Raleigh, NC, United States)] (Table 6), as well as novel molecules (DA6886, AZD1722, RDX5791, TC6499). Thus far, no results from completed studies are available, and other studies are still recruiting patients.

PERSPECTIVES AND CONCLUSION

IBS-C has been, and probably will remain for some time, a troubling disease for many sufferers and an enormous challenge for the treating physician. The multifactorial pathogenesis of the disease and the ill-defined drug targets make the goal of manufacturing a "universal drug" for IBS-C a hard one to attain. In recent years, new drug therapies have been added to the armamentarium for the treatment of IBS-C. The current available evidence indicates that linaclotide is the "ideal" treatment option for IBS-C patients at this time, but other investigational agents are showing promise as well. However, large scale, high quality longitudinal studies of such agents and post-

market monitoring of approved drugs are needed to confirm the efficacy, tolerability and safety of these treatments. The quality of current evidence in support of different drug classes is summarized in Table 7. However, drug choice is dictated not only by the supporting evidence, but also by the patients' and societal perspectives.

Patient-relevant symptoms in conjunction with a better understanding of the pathophysiologic mechanisms underlying IBS-C should drive the development of novel pharmacologic agents for this complex disorder. Novel drug therapies are expected to streamline the management of IBS-C, thus increasing patient satisfaction and ultimately reducing the use of healthcare resources. This could indeed compensate for the high cost of these drugs, which is one of the major concerns for many patients and insurers. Finally, since IBS-C is a spectrum disorder resulting in a broad range of responses to different drug regimens, the treatment of most IBS-C patients should be individualized. It is anticipated that in the near future, a multitude of pharmacologic agents with divergent mechanisms of action will be effective for diverse subsets of IBS-C patients, and the reconciliation of past pharmacologic treatment successes and failures will ultimately improve future management of IBS-C.

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Irritable bowel syndrome: A clinical review

Rosa LS Soares

Rosa LS Soares, Internal Medicine Department, Faculty of Medicine, Federal Fluminense University, Rio de Janeiro 24030-210, Brazil
Author contributions: Soares RLS contributed to the manuscript.
Correspondence to: Rosa LS Soares, MD, PhD, Department of Clinical Medicine, Federal Fluminense University, Marques do Parana 303, 24030-210 Niterói, Rio de Janeiro, Brazil. rosaleonora@gmail.com.br
Telephone: +51-21-26299016 Fax: +51-21-26299017
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Abstract

Irritable bowel syndrome (IBS) remains a clinical challenge in the 21st century. It's the most commonly diagnosed gastrointestinal condition and also the most common reason for referral to gastroenterology clinics. Its can affect up to one in five people at some point in their lives, and has a significantly impact of life quality and health care utilization. The prevalence varies according to country and criteria used to define IBS. Various mechanisms and theories have been proposed about its etiology, but the biopsychosocial model is the most currently accepted for IBS. The complex of symptoms would be the result of the interaction between psychological, behavioral, psychosocial and environmental factors. The diagnosis of IBS is not confirmed by a specific test or structural abnormality. It is made using criteria based on clinical symptoms such as Rome criteria, unless the symptoms are thought to be atypical. Today the Rome Criteria Ⅲ is the current gold-standard for the diagnoses of IBS. Secure positive evidence of IBS by means of specific disease marker is currently not possible and cannot be currently recommended for routine diagnosis. There is still no clinical evidence to recommend the use of biomarkers in blood to diagnose IBS. However, a number of different changes in IBS patients were demonstrated in recent years, some of which can be used in the future as a diagnostic support. IBS has no definitive treatment but could be controlled by non-pharmacologic management eliminating of some exacerbating factors such certain drugs, stressor conditions and changes in dietary habits.The traditional pharmacologic management of IBS has been symptom based and several drugs have been used. However, the cornerstone of its therapy is a solid patient physician relationship. This review will provide a summary of pathophysiology, diagnostic criteria and current and emerging therapies for IBS.

Key words: Irritable bowel syndrome; Clinical review; Pathogenesis; Diagnostic; Treatment; Biopsychosocial model

Core tip: Irritable bowel syndrome (IBS) remains a clinical challenge in the 21st century. Various mechanisms and theories have been proposed about its etiology, but the biopsychosocial model is the most currently accepted. Today the Rome Criteria are the current gold-standard for the diagnoses of IBS. Traditional management of IBS has been symptom based and several drugs have been used. However, the cornerstone of its therapy is a solid patient physician relationship. This review will provide a summary of pathophysiology, diagnostic criteria and current and emerging therapies for IBS.

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INTRODUCTION

The functional gastrointestinal disorders (FGIDs) are a heterogeneous group of chronic conditions that are considered important to public health because they are remarkably common, can be disabling, and induce a major

social and economic burden. Irritable bowel syndrome (IBS) is the most prevalent FGID noted in the general population worldwide and also the most common reason for referral to gastroenterology clinics[1-4]. Even though it was described to 150 years ago, IBS remains a clinical challenge in the 21st century[5,6]. Its can affect up to one in five people at some point in their lives, and has a significantly impact of life quality and health care utilization[7,8]. The prevalence varies according to country and criteria used to define IBS[9-21]. Various mechanisms and theories have been proposed about its etiology, but the biopsychosocial model is the most currently accepted for IBS[22]. The complex of symptoms would be the result of the interaction between psychological, behavioral, psychosocial and environmental factors[23-27]. The diagnosis of IBS is not confirmed by a specific test or structural abnormality. It is made using criteria based on clinical symptoms such as Rome criteria, unless the symptoms are thought to be atypical. There is still no clinical evidence to recommend the use of biomarkers in blood to diagnose IBS. Today the Rome Criteria are the current gold-standard for the diagnoses of IBS[26,28,29]. There is no definitive treatment for IBS and the traditional management has been symptom based but recent developments in the understanding of complex interaction between the gut, immune system and nerve system have led to an expanded arsenal of therapeutic options for relief of both bowel movement-related symptoms and pain[30-34]. However, a strong doctor-patient relationship is the key for effective treatment of patients and realistic expectations.

This review will provide a summary of pathophysiology, diagnostic criteria and current and emerging therapies for IBS[35-39].

PATHOGENESIS

In despite its high prevalence, the pathophysiology of IBS is not yet completely understood and seems to be multifactorial[22-25]. Various mechanisms [gastrointestinal (GI) dismotility[40,41] visceral hypersensitivity[42,43] intestinal mucosa activation[44-48], Increased intestinal permeability[49-54], have been proposed about the IBS pathophysiology. Studies suggest interplay between luminal factors (*e.g.*, foods and bacteria residing in the intestine], the epithelial barrier, and the mucosal immune system[48]. However, the biopsychosocial model[22,25,26,36] is the most currently theory accepted for IBS. The complex of symptoms would be the result of the interaction between psychological, behavioral, psychosocial and environmental factors Since fifty years ago, several theories have proposed regarding the etiology of IBS of which the most important are as follows.

Evidences and not evidences of GI motility disorders in IBS

IBS is a complex disorder that is associated with altered GI motility, secretion and sensation[55,56]. In some patients with IBS motor abnormalities of the GI are detectable, *e.g.*, increased frequency and irregularity of luminal contractions, prolonged transit time in constipation-predominant IBS and an exaggerated motor response to cholecystokinin and meal ingestion in diarrhea-predominant IBS. Despite this no predominant pattern of motor activity has emerged as a marker for IBS[57-60] and the relevance of these motor function alterations to symptoms has yet to be established. However, pharmacological stimulation of gut motility in IBS patients appears to reduce gas retention and improve symptoms. These data suggest that a motility disorder could be associated with this complaint in some patients[61,62]. Role of serotonin in the pathophysiology of IBS. Serotonin (5-HT) plays a critical role in the regulation of GI motility, secretion and sensation. It is an important signaling molecule in the gut targeting enterocytes, smooth muscles and enteric neurons. Most of the body serotonin is present in enterochromaffin cells. Serotonin activates both intrinsic and extrinsic primary afferent neurons to, respectively initiate peristaltic and secretory reflexes and to transmit information to the central nervous system. Serotonin activates both intrinsic and extrinsic primary afferent neurons to, respectively initiate peristaltic and secretory reflexes and to transmit information to the central nervous system. It is inactivated by the serotonin reuptake transporter (SERT) in the enterocytes or neurons[22,24,30,32]. There are lines of evidence that FGIDs, as IBS, are associated with defective enteric serotonergic signaling. Altered serotonin signaling could lead to intestinal and extra intestinal symptoms in IBS. These results support the concept that diarrhea-predominant (IBS-D) IBS is characterized by reduced 5-HT reuptake, whereas impaired release may be a feature of constipation predominant IBS (IBS-C). However, exogenous serotonin application evokes so many responses that it is difficult to determine which is physiologically relevant. Therapeutic agents targeting altered serotonin signaling may provide new effective treatment for patients with IBS[62-65].

Evidences and not evidences of visceral hypersensitivity in IBS

Visceral hypersensitivity is considered to be one of the main factors that cause symptoms in IBS patients and increased sensation in response to stimuli is a frequent finding in IBS patients[42,43,65-68]. This selective hypersensitization results from stimulation of various receptors in the gut wall of visceral afferent nerves in the gut[69-71], triggered by bowel distention or bloating, as a possible explanation for IBS symptoms[72-77]. Rectal distension in patients with IBS also increased cerebral cortical activity more than in controls. The increased sensitivity of the colon could be influenced by a psychological tendency to report pain and urgency, rather than increased neurosensory sensitivity[78]. About half of patients with IBS (mainly those with constipation) have a measurable increase in abdominal girth associated with bloating (sensation of abdominal fullness), although this may not be related to the volume of intestinal gas[72,74,79]. In addition, other

factors may contribute to visceral hyperalgesia, such as specific GI mediators (serotonin, linins), or increases in spinal cord excitability due to activation of an N-methyl-D-aspartate (NMDA) receptor[80]. In addition, IBS patients show an increased secretion in the duodenum and jejunum. Larsson *et al*[81] proposed that the enhanced secretion may reflect disturbed enteric network behavior in some patients with IBS.

Evidences and not evidences of intestinal barrier disorders in IBS

Some authors reported an increase in permeability of the intestinal mucosa and disruption of tight junctions in sub-groups of patients with IBS often triggered by some factors[45-50]. The possible mechanisms underlying these changes could be associated with the interaction between mucosa permeability, visceral hypersensitivity and inflammation mucous[48-54,82,83]. Studies suggest that an interaction between luminal factors (for example, food and bacteria that reside in the gut), the epithelial barrier and mucosal immune system could result in pain through the inflammatory stimulation of afferent nerves[45-48,51,82,83]. Some factors were described as triggers for intestinal permeability alterations. They are stress, food, bile, infection and dysbioses[48,49].

Role of stress: The association between IBS and psychological factors, especially anxiety and stress, has been described for many years[22-25,36]. In rats chronically stressed the consequently increased corticosterone release leads to intestinal inflammation with consequent mucosal barrier dysfunction[84-88]. However, the direct association between intestinal barrier dysfunction and stress in patients with IBS still needs confirmation.

Role of food and bile: Some patients with IBS report worsening of symptoms after eating and perceive food intolerance to certain foods[89-93]. Multiple factors have been considered to contribute to food sensitivity in patients with IBS. Investigations have centered on food specific antibodies, carbohydrate malabsorption, and gluten sensitivity. Although some IBS patients related relief of symptoms on a gluten-free diet the specific relationship between gluten and increased intestinal permeability in IBS have not yet confirmed[93-96]. We reported that IBS patients have difficulties with food in general and specific foods may not be involved in IBS pathogenesis.

It is reasonable to assume that IBS causes food sensitivity, rather than vice versa[97,98]. Certain bile acids could increase intestinal permeability through the phosphorylation of epidermoid growth factor receptor, which induces occludin desphosphorylation[99] or *via* dysfunction of the enteric neurons[100].

Role of infection-IBS post-infectious: Gastroenteritis is a common trigger for IBS. The IBS symptoms can be triggered by an enteric infection and can persist for weeks, months and years[52,101-104]. Two meta-analyses demonstrated an increased risk of IBS in patients who experienced an episode of acute gastroenteritis. Risk factors for post infectious IBS included young age, prolonged fever, anxiety, and depression. A longer duration of the initial infection has also been associated with increased risk for IBS. One of the largest prospective studies included a total of 2069 individuals who had been exposed to contaminated drinking water after heavy rainfall[105,106]. The cause of the intestinal symptoms after PI-IBS is not yet defined. The likely increase in intestinal permeability during the episode of acute gastroenterite could cause inflammation and intestinal microbiota change, leading to intestinal barrier dysfunction and infection-induced dysbiosis[107,108]. Development of idiopathic malabsorption bile acids and increase in serotonin-containing enteroendocrine cells and T lymphocytes[108-111]. The use of antibiotics for GI or other infections was observed to be a risk factor for developing functional bowel symptoms[112].

Evidences of small intestinal bacterial overgrowth in IBS

Small intestinal bacterial overgrowth (SIBO) is associated with an increased number and/or type of bacteria in the upper GI tract[113]. However, data reporting an association between IBS and SIBO have been conflicting. In support of an association between SIBO and IBS are studies demonstrating abnormal breath hydrogen levels in IBS patients after receiving a test dose of a carbohydrate, as well as improvement in symptoms after eradication of the overgrowth[114,115]. In addition, increased methane production, a gas by product of intestinal bacteria, has been associated with constipation predominant IBS[116,117]. Other studies have failed to support an association between SIBO and IBS The improvement of symptoms with antibiotics described in some patients with IBS may be due to improved intestinal motility or a change in the flora of the colon, rather than SIBO[118-121].

Evidences and not evidences of abnormalities of intestinal flora in IBS

The relationship between stress and microbiota goes back many decades, when Tannack and Savage reported that stressed mice showed dramatic reductions in these populations of lactobacilli[122]. Recent studies demonstrated that the intestinal microbiota can influence the gut-brain communication in health and disease, and consequently altering brain chemistry and behavior. However, it's perhaps premature to extrapolate the current preclinical work to the clinic. The complex ecology of the fecal microflora has led to speculation that changes in its composition could be associated with diseases including IBS. Emerging data suggest that the fecal microbiota in individuals with IBS differ from healthy controls and varies with the predominant symptom[122-124]. However, not all studies have found disturbances in the microbiota composition of IBS patients and his currently unclear whether the alteration that have been reported are primary or secondary in nature. The contribution of altered intestinal composition or function in IBS remains contro-

versial and additional studies are needed to validate these observations[125-129].

Evidences and not evidences of low grade mucosal inflammation and IBS

Increased numbers of lymphocytes have been reported in the colon and small intestine in a subset of patients with IBS[130,131]. These cells release mediators (nitric oxide, histamine and proteases) capable of stimulating the enteric nervous system, leading to abnormal motor and visceral responses within the intestine[132-134]. Studies have demonstrated a correlation between abdominal pain in IBS and the presence of activated mast cells in proximity to colonic nerves[135]. In addition, peripheral blood mononuclear cells of IBS patients produce higher amounts of tumor necrosis factor than healthy controls[136]. Changes in mucosal barrier function and a consequent increase in intestinal permeability could be the basis for the increased inflammation in IBS[45-49]. The interaction between the increased intestinal permeability, low levels of inflammation and hypersensitivity could be the key to the pathophysiology of IBS[48,136-138].

Evidences and not evidences of genetic contribution in IBS

The pathogenesis of IBS has traditionally been based on the biopsychosocial model that emphasizes that the symptom manifestations of IBS and consulting behavior are influenced at least in part by psychological processes. However, there has been increasing interest in trying to identify potential molecular mechanisms in IBS, and this endeavor has been driven by some evidence that there is a true genetic contribution to IBS[138-140]. IBS does aggregate in families, and the concordance of IBS is twice as great in monozygotic compared with dizygotic twins in most, but not all studies[141-144]. A number of genetic polymorphisms have been associated with IBS but most remain to be independently confirmed, and unknown gene-environment interactions probably remain essential for the disorder to manifest[144-146]. A future direction of investigation includes genome-wide approaches and further delineation of the role of epigenetic factors in IBS. By studying the genetic associations between candidate genes and intermediate phenotypes that are associated with manifestations of the clinical phenotype, one can also evaluate the role of the candidate mechanism in IBS. The intermediate phenotypes most commonly used in IBS are colonic transit, colonic motility and compliance, and sensation thresholds and ratings[147,148].

Evidences and not evidences of brain-gut axis and psychosocial dysfunction in IBS

Psychosocial factors may influence the expression of IBS[149-153]. Though the role of the BGA is not fully understood, there is strong evidence of a crucial involvement of the BGA in the development of IBS and IBS like symptoms[22-24,36]. In patients with IBS the dysregulation of the BGA, a bidirectional and integrated system modified by psychosocial processes and environmental influences, could induce dysmotility or visceral sensitivity. The importance of the knowledge of concepts related brain-gut interactions improves patient physician relationship and identifies what level pharmacological treatment can be beneficial for patients with IBS[154-159].

DIAGNOSIS

Although it is among the most common disorders in gastroenterology and primary care practices IBS continues as a substantial diagnostic challenge[1,3,4,20,22,24,27,160]. Frequently, the IBS diagnosis is missed or delayed. There are several medical conceptions concerning the SII. While a large number of doctors consider that IBS would be a mixture of different organic diseases and others believe IBS does not exist and in your point of view these symptoms are normal and these patients are not medical priority, only a few doctors consider IBS as a functional bowel disease well defined by the byopsychosocial model[22,25,26,36]. This fact is an important obstacle to making IBS diagnosis. However millions of IBS patients around the world are still looking for responses and relief of symptoms. For these reasons is so important to make a diagnosis of IBS. The key issues is to diagnose IBS safely through minimal risks and reasonable costs[9,26,27,29,36,161-164]. To standardize clinical research protocols, was published a definition of consensus for the diagnosis of IBS in 1992 called Rome criteria, which was recently revised in 2005 and named as Rome Ⅲ criteria[28]. The diagnosis must be based on clinical data, using symptoms based on criteria of Rome, unless the symptoms are atypical[165,166]. When the criteria are filled in IBS diagnosis and alarm features are absent, the number of diagnostic tests should be minimal.Reports and guidelines emphasize that IBS is not a diagnosis of exclusion and encourage clinicians to make a positive diagnostic using the Rome Criteria alone[9,26-29,36,167-171].

Definition, clinical manifestations and diagnostic criteria

IBS is the most commonly diagnosed GI condition and also the most common reason for referral to gastroenterology clinics (up to 50% of all offices visits to gastroenterologists). Its can affect up to one in five people at some point in their lives, and has a significantly impact of life quality and health care utilization. The prevalence varies according to country and criteria used to define IBS. IBS is more frequent in women than in men, and its prevalence is less for individuals over 50 years, when compared with those of less than 50 years. Are considered typical clinical manifestations in IBS discomfort or abdominal pain relieved by defecation, associated with a change in stool form[172-176].

Pain

Patients with IBS can present with a variety of symptoms which include both GI and extraintestinal complaints. However, the symptom complex of chronic abdominal pain and altered bowel habits remains the nonspecific pri-

mary characteristic of IBS. It's chronic nature, signs and symptoms which vary periodically from mild to severe have many negative effects on the quality of life for the suffers. Many factors, for example. emotional stress and eating may exacerbate the pain. In contrast defecation usually provides some relief[28,164,168,177-180].

Altered bowel habits

Patients with IBS complain of altered bowel habit, ranging from diarrhea (IBS-D), constipation (IBS-C), or alternating diarrhea and constipation (IBS-M). One half of patients with IBS-D complain of mucus discharge. Large volume diarrhea, bloody stools, nocturnal diarrhea, and greasy stools are not associated with IBS and suggest organic disease. Another sub-group of patients with IBS-D describe an acute viral or bacterial GI before the onset of symptoms compatible with IBS. This clinical entity is called post-infectious IBS. It's important to remember that the most bowel movements are preceded by lower abdominal cramps. 8-Patients with IBS-C may experience a sensation of incomplete evacuation and periods of constipation can last from days to months alternating with diarrhea or normal bowel function[28,164,168,180].

Other GI symptoms

Bloating or feeling of abdominal distension are very frequent complaints in IBS and may be included in the diagnostic criteria for IBS in the future. Other digestive symptoms as dysphagia, early satiety, intermittent dyspepsia, nausea and non-cardiac chest pain patients with are also often associated with IBS. Comorbidity with other FGIDs is high and can be caused by shared as visceral hypersensitivity pathophysiological mechanisms. Comorbidity with other FGIDs is high and may be caused by shared pathophysiological mechanisms such as visceral hipersensivity[180-185].

Extra-intestinal symptoms

Psychiatric disorders, especially major depression, anxiety, and somatoform disorders occur frequently[158,186,187]. The nonGI nonpschiatric disorders with the best documented association are fibromyalgia, chronic fatigue syndrome, temporal mandibular joint disorder and chronic pelvic pain[188-190]. In addition, IBS is often accompanied by other extra-intestinal symptoms as asthma and cerebral pain symptoms as primary headache[191-193]. The high prevalence of co morbidities in IBS patients has led investigators to develop hypothesis regarding underlying pathophysiologic mechanisms linking these disorders[194,195]. The comorbidities are correlated with enhanced medical help seeking, worse prognosis, and higher rates of anxiety and depression all resulting in a reduced quality of life. The identification of this clinical problem could improve the therapeutic options and the prevention strategies[196-198].

Diagnostic criteria

The concept of utilization of the clinical criteria for IBS diagnosis was formulated at first time for Manning in 1978[199]. Other criteria have also been proposed[200-202]. Today the Rome Criteria Ⅲ are the current gold-standard for the diagnoses of IBS[203]. IBS was defined as recurrent abdominal pain or discomfort associated with altered defecation and IBS patients are grouped into different subtypes based on the predominant stool consistency. Formally, the Rome Ⅲ Criteria require recurrent abdominal pain or discomfort ⩾ 3 d/mo in the last 3 mo associated with ⩾ 2 of the following: 1- improvement with defecation; 2- onset associated with a change in form (appearance) of the stool[203]. Supportive symptoms that are not part of the Rome Ⅲ Criteria include: abnormal stool frequency, abnormal stool form, defecation straining, urgency or a feeling of incomplete bowel movement, passing mucus and bloating[204].

Diagnostic approach

The basic diagnosis should include a careful and thorough medical history. This complaint data should be quantified as precisely as possible (*e.g.*, by symptom diaries). The aim is the most accurate detection of symptom constellation and dynamics, as well as the active queries alarm symptoms. There is evidence that the (patient and doctor alike convincing) exclusion of relevant other causes can contribute for the mutual improved trust and due to also to the success of the treatment[205,206]. The substantial human and economic costs associated with IBS needs to development of efficient diagnostic and management strategies[6,27]. Patients are first identified as having a symptom complex compatible with IBS based upon Rome Ⅲ Criteria. If the patient who have IBS suggestive symptoms, and no alarm symptoms or no family history of IBD or colorectal cancer are present, a limited number of diagnostic studies is required to exclude organic disease in most patients and a considerable number do not require any tests at all. This limited diagnostic approach excludes organic disease in more than 95 percent of patients[201,205,206]. Routine laboratory studies (complete blood count, chemistries) are normal in IBS[22,26,201,207-210]. The rates of prevalence of IBD, colorectal cancer and thyroid disease are different in patients with IBS when compared with the general population. Lactose intolerance seems to be more prevalent in patients with IBS symptoms when compared with controls other carbohydrates such fructose and sucrose can also cause or exacerbate IBS symptoms. However, there is no evidence of cause and effect between lactose intolerance and IBS[211-213]. Stool examination for ova and parasites would be indicated only in patients who live in developing countries or were there recently[214]. There is insufficient evidence to recommend routinely test for SIBO in patients with IBS[26,168,215-222]. The utility of abdominal imaging tests in patients with suspected IBS and no alarm features is scarce. In absence of alarm signals characteristic IBS patients aged less than 50 years need not be submitted to colonoscopy. The image of the colon would not be useful in obtaining colonic imaging that could explain the symptoms of patients with IBS[223].

Alarm features or red flags

In the presence of alarm features or atypical symptoms which are not compatible with IBS, it's important to exclude other causes. The alarm symptoms (*e.g.*, anemia and weight loss) have a high specificity for the presence of inflammatory or malignant diseases. Rectal bleeding, nocturnal or progressive abdominal pain, weight loss, anemia and another laboratory abnormalities such as elevated inflammatory markers, or electrolyte disturbances, a family history of colorectal cancer, IBD or celiac disease are often associated with IBS-like symptoms[216-218]. Faced with a patient with IBS symptoms and alarm signals the colonoscopy should be performed to exclude organic disease[22,26,168,219,224]. We suggest performing screening tests based upon the patient's clinical history in patients with IBS-M, and in IBS with refractory symptoms (change of progression of symptoms or absence in response to general therapeutic measures)[26,225,226]. further evaluation depends upon the predominate symptoms. In IBS C the evaluation is similar to other patients with chronic constipation and in patients with predominant diarrhea is similar to other with chronic diarrhea[26,168,226].

Biomarkers in IBS - the future newer innovative tests for IBS

Secure positive evidence of IBS by means of specific disease marker is currently not possible and cannot be currently recommended for routine diagnosis. However, a number of different changes in IBS patients were demonstrated in recent years, some of which can be used in the future as a diagnostic support. Several non-invasive approaches were investigated for their ability to discriminate IBS from non-IBS disorders. Although a larger number of data are necessary these tests show potential as adjuncts to traditional diagnosis methods in IBS and may reduce unnecessary testing in clinical practice[227-231]. They are examination of stools forms, fecal markers, and serological markers. A blood screening test approved in the United States for the IBS ("Prometheus® IBS diagnostics") tests a constellation from a total of 10 "IBS blood biomarkers" and can thus supposedly secure diagnosis "IBS" in combination with the other clinical parameters[224]. The practical value of this test currently (still) cannot clearly be evaluated, because the published evidence is insufficient. Secure positive evidence of IBS by means of specific "disease marker" is currently not possible and cannot be currently recommended for routine diagnosis.

TREATMENT

General principles in the treatment of IBS

Over the past 2 decades very few agents have achieved regulatory approval for the treatment of IBS. IBS has no definitive treatment but could be controlled by eliminating of some exacerbating factors such certain drugs, stressor conditions and changes in dietary habits. Traditional management of IBS has been symptom based. Because of the abnormalities in bowel states associated with each IBS subtype, it is not likely that one agent would successfully treat all three subtypes. As a result, clinical trials have focused, for the most part, on one IBS subtype[2,25,22,29,32,36,168,229].

NON-PHARMACOLOGIC MANAGEMENT

Fundamental aspects of the doctor patients-interaction as the basis of IBS therapy

Many patients with IBS have bounced around the field of medicine for many years with different diagnoses, due to lack of interest or deep frustration of the doctor in the treatment of IBS. The absence of biological markers for the diagnosis of IBS or even its characterisation as a mental illness. The absence of biological markers for the diagnosis of IBS or even its characterisation as a mental illness could lead to inadequate interpretation. Patients should be informed that the nature of the disease is chronic, benign, and educated on how to deal with and control symptoms of the disease, which vary periodically from mild to severe and have many negative effects on quality of life. Patients should be also informed that their diagnosis is not like being altered, but that it is possible to have a normal life. A detailed medical history and physical examination physician should pay particular attention to their patient's concerns. The treatment goal in patients suffering with IBS is to try eliminating or decreasing the patient's primary symptoms which should be addressed on first encounter with the patient[35].

DIET RECOMMENDATIONS ABOUT DIETARY HABITS

It should be noted that the intake of foods does not cause IBS. However, many IBS patients have non-specific intolerance to foods. The dietary restriction of fermentable carbohydrates popularly termed the low FODMAP (fermentable oligosaccharides, disaccharides, monosaccharides, and polyols) diet has received considerable attention is now accepted as an effective strategy for managing symptoms of IBS. However, limitations still exist with this approach in part due to the fundamental difficulty of placebo control in dietary trials[230]. In essence, IBS patients should avoid foods that trigger an onset of their symptoms, consume a minimum of high fat foods and take part in regular physical activity[168,229,231].

RESPONSES TO PSYCHOLOGICAL FACTORS

In patients with IBS, psychological factors (such as stress factors in career, family, *etc.*) such anxiety and depression, as well as the tendency towards summarization should be evaluated in interdisciplinary collaboration. Trauma and abuse should be considered and carefully be explored. As a result, the treatment success can be positively influ-

enced. The evidence suggests that IBS patients who alternate the intestinal habits more often, are more affected by its symptoms, exhibit a greater tendency towards somatization and have a higher prevalence of psychiatric comorbidities[31,37,232-234].

COMPLEMENTARY OR ALTERNATIVE FORMS OF TREATMENT

The treatment of IBS, with forms of alternative therapy cannot be recommended on the basis of insufficient evidence. However, in IBS patients who did not respond to conventional treatment, complementary therapy could be effective. More recent studies are related to hypnotherapy[168,235-242].

MEDICATIONS\PHARMACOTHERAPY

Due to the heterogeneity of IBS, there is no standard treatment. The chronic use of drugs should be minimized as much as possible or even avoided. Different time periods may apply for not pharmacotherapy treatment approaches. However a medical therapy attempt without response should be terminated at the latest 3 months and the duration of therapy should be discussed with the patient[30-34,168].

MANAGEMENT OF IBS PAIN

Visceral hypersensitivity is felt to be a major contributing factor in abdominal pain experienced by IBS patients. Managing abdominal pain in IBS has changed very little over the past few decades. The antispasmodics remain a cornerstone of therapy. The antispasmodic are the most prescribed pharmacological agents for IBS, and their effect in reducing abdominal pain would be related to direct action in the contractility of muscle wall. As its use may lead to constipation should be used with caution in patients with IBS. Anxiolitc agents such benzodiazepines are of limited use in IBS. However, they can reduce acute anxiety that may contribute to the symptoms. Their use may be indicated for short-term (less than two weeks)[168,243-245].

ANTIDEPRESSANTS

Antidepressants have analgesic properties. The postulated mechanisms of pain modulation with tricyclic antidepressants (TCAs) and possibly selective serotonin reuptake inhibitors (SSRIs) in IBS are facilitation of endogenous endorphin release, blockade of norepinephrine reuptake leading to enhancement of descending inhibitory pain pathways, and blockade of the pain neuromodulator serotonin[32,168]. Beside imipramine, nortriptilina and desipramine, amitryptline are of the tricycle antidepressant drugs commonly used in the treatment of IBS patients at low doses. TCAs and SSRIS appear to be more effective than placebo in the overall reduction of symptoms associated with IBS. However, the degree of tolerability and safety of use of these patients is not well defined[246-250].

MANAGEMENT OF IBS-C

Dietary modifications and lifestyle should be the initial tools of the treatment of patients with constipation predominant IBS who have mild to moderate symptoms. The consumption of fiber-enriched foods and the increased fluid intake to prevent stool dehydration should be stimulated by the physician in this sub-group of IBS patients. Some improvement has been demonstrated in primary complains. However some patients may experience increased bloating. There is no evidence for the use of laxatives in patients with IBS[168]. In refractory cases polyethylene glycol can be used to improve only the frequency of bowel movements and gaseousness due to colonic metabolism of non-digestible[251,252].

Lubiprostone is a locally acting chloride channel activator that enhances chloride chloride-rich intestinal fluid secretion[32,253]. In a first step it was approved by the Food and Administration (FDA) for use in chronic idiopathic constipation and for women with IBS-C. However, its use is currently only suitable for women with IBS and severe constipation that has been refractory to other forms of treatment. Serious adverse events were similar to placebo. However, the long-term security remains to be established. been refractory to other treatments[168,254-256].

Tegaserode, a first of the agonists of the 5-hydroxityptamine (5-HT4) receptor class of drugs that stimulate the release of neurotransmitters and increase colonic motility, was approved for IBS and constipation but removed from the market in 2007 because of cardiovascular side effects[32,255-258]. It's a 5-HT4 receptor agonist that in clinical trials has been reported to reduce the general symptoms of IBS patients in comparison to attested placebo[259]. The Linaclotide, a guanylate cyclase agonist stimulates intestinal fluid secretion and transit, has been approved by the United States FDA for treatment of IBS with constipation in 2012. Their approval was two randomized controlled trials in phase Ⅲ. The patients initially randomized to placebo had significant improvement in abdominal pain and complete and spontaneous bowel movements. Diarrhea was the most common side effect. However, the long term risks of linaclotide are unknown and therefore its role on the treatment of IBS with constipation remains to be determined[260,261].

MANAGEMENT OF IBS-D

In this group of patients the anti-diarrheal agents are generally effective. There is evidence which suggests that the use of regular low doses of anti-diarrheal agents could be effective in such patients[168]. Among the most commonly used anti of diarrhea agents loperamide is one that has been more studied in patients with diarrhea predominant IBS. Constipation is the major side effect of Loperamide[262,263]. It should not be used in patients with constipation and in patients with IBS diarrhea con-

stipation alternating with diarrhea should be used with caution[168]. In patients with diarrhea predominant IBS reports of stressors (*e.g.*, eating, stressful encounters, travel) that lead to symptoms are frequent. When the predictors of crises are known the physician may start a first line of treatment, using antidiarrhea agents[263]. The use of cholestyramine could be beneficial in patients with IBS-D. However, there are still no definitive evidence for its use in IBS treatment[168].

Alosetron (such as cilansetron, ondasetron and granisetron) is a 5-hydroxitriptamine (serotonin) 3-receptor antagonist. Its modulates visceral afferent activity from GI tract and in IBS patients could act favorably on colonic motility and secretion and afferent neural systems Constipation was reported in approximately one third of patients using alosetron. It was recertified by the FDA (after the withdrawal from the market) with restrictive guidelines and is prescribed under a specific Protocol. Its benefits are more favorable in women with severe IBS and diarrhea that are refractory to conventional therapies[168,264-268].

MANAGEMENT OF IBS WITH CONCOMITANT BLOATING AND THE USE OF ANTIBIOTICS IN IBS

Abdominal bloating, a symptom commonly witnessed in IBS patients, is unfortunately very subjective and often observed in constipation predominant IBS patients. Probable mechanisms of bloating include: psychosocial, weak abdominal muscles, paradoxal relaxation of abdominal muscles and changes in visceral sensitivity. The role of prokinetic agents, simethicone and activated charcoal need to be better assessed with further well-designed studies. Dietary fiber supplementation and no absorbable sugars like lactulose can worse bloating and gaseous food, beans, carbonated beverages can lead to aerophagia symptoms. Some patients with IBS have shown improvement in symptoms of bloating, abdominal pain, or altered bowel habits. when treated with antibiotics[266-268]. The mechanism responsible for the improvement of the symptoms of these patients could be the suppression of gas produced by colonic bacteria or by alteration of colonic flora or by decreasing of the small bacterial overgrowth. It's a question to be answered[269-271]. However, the benefit from the treatment appears to be transient. Currently is not recommended breath testing for intestinal bacterial overgrowth neither. It is not recommended to use antibiotics routinely for all patients with IBS and there are no data available to justify the prolonged use of of nonabsorbable antibiotics in these patients[168]. In patients with moderate to severe IBS without constipation (particularly those with bloating) who failed to respond to all other therapies it's reasonable to try a 2 wk trial (not long term) of a nonabsorbable antibiotic such rifamixin.

PROBIOTICS USE IN IBS PATIENTS-A SHORT-REVIEW

The rationale for the use of probiotics in IBS is its association with infectious diarrhea. It's accepted that IBS-like symptoms are highly prevalent in the months after cure from infectious enteritis. About 7%-30% of patients with infectious diarrhea can develop IBS, in particular associated after travel to tropical countries. Among the possible mechanisms of probiotic therapy is the promotion of the endogenous defense barrier of the gut. These include the normalization of intestinal permeability and increase intestinal microecology changed, as well as improvement of gut immune barrier through the downregulation of a proinflammatory State[272]. The *Bifidobacteria*, *Saccharomyces boulardii* and other combinations of probiotics demonstrate some efficacy in IBS. The *Bifidobacteria* (especially *Bifidobacterium infantis* 35624), *Saccharomyces boulardii* and other combinations of probiotics demonstrate some efficacy in IBS Trials to date remain conflicting and no clear benefit has yet to be established for lactobacilli[273]. However, due to the number of clinical studies available, the role of probiotics in the relief of symptoms of IBS remains uncertain.

CONCLUSION

IBS affects up to one in five people at some point in their lives. However its remains a clinical challenge in the 21st century. The pathogenesis of IBS is likely multifactorial, including disorders the intestinal barrier, motility, secretion, visceral sensitivity and interactions between psycho logic and psychosocial factors. The biopsychosocial model is the most currently accepted for IBS. It's not confirmed by a specific biomarker. Guidelines emphasize that IBS is not a diagnosis of exclusion and encourage clinicians to make a positive diagnostic using the Rome Criteria alone Today the Rome Criteria Ⅲ are the current gold-standard for the diagnoses of IBS.

IBS has no definitive treatment but could be controlled by eliminating of some exacerbating factors such certain drugs, stressor conditions and changes in dietary habits. Traditional management of IBS has been symptom based. Because of the abnormalities in bowel states associated with each IBS subtype, it is not likely that one agent would successfully treat all three subtypes. As a result, clinical trials have focused, for the most part, on one IBS subtype. The modulation of the brain-gut axis is being seen as an attractive target for the development of novel treatments for a wide variety of disorder. However, the cornerstone of its therapy is a solid patient physician relationship. There are no recommendations for preven-

tion for IBS.

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Irritable bowel syndrome: A microbiome-gut-brain axis disorder?

Paul J Kennedy, John F Cryan, Timothy G Dinan, Gerard Clarke

Paul J Kennedy, Timothy G Dinan, Gerard Clarke, 1.15 Biosciences Institute, Department of Psychiatry, Alimentary Pharmabiotic Centre, University College Cork, Cork, Ireland
John F Cryan, Department of Anatomy and Neuroscience, Alimentary Pharmabiotic Centre, University College Cork, Cork, Ireland
Author contributions: Clarke G, Dinan TG and Cryan JF devised the study; Clarke G, Dinan TG, Cryan JF and Kennedy PJ reviewed and evaluated the literature for inclusion in the review; Clarke G and Kennedy PJ prepared the initial draft of the manuscript; Clarke G, Dinan TG, Cryan JF and Kennedy PJ reviewed, edited and approved the final version of the manuscript.
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Correspondence to: Gerard Clarke, PhD, 1.15 Biosciences Institute, Department of Psychiatry, Alimentary Pharmabiotic Centre, University College Cork, Cork, Ireland. g.clarke@ucc.ie
Telephone: +353-21-4901408 Fax: +353-21-4901722
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Abstract

Irritable bowel syndrome (IBS) is an extremely prevalent but poorly understood gastrointestinal disorder. Consequently, there are no clear diagnostic markers to help diagnose the disorder and treatment options are limited to management of the symptoms. The concept of a dysregulated gut-brain axis has been adopted as a suitable model for the disorder. The gut microbiome may play an important role in the onset and exacerbation of symptoms in the disorder and has been extensively studied in this context. Although a causal role cannot yet be inferred from the clinical studies which have attempted to characterise the gut microbiota in IBS, they do confirm alterations in both community stability and diversity. Moreover, it has been reliably demonstrated that manipulation of the microbiota can influence the key symptoms, including abdominal pain and bowel habit, and other prominent features of IBS. A variety of strategies have been taken to study these interactions, including probiotics, antibiotics, faecal transplantations and the use of germ-free animals. There are clear mechanisms through which the microbiota can produce these effects, both humoral and neural. Taken together, these findings firmly establish the microbiota as a critical node in the gut-brain axis and one which is amenable to therapeutic interventions.

Key words: Irritable bowel syndrome; Microbiome; Anxiety; Tryptophan; Abdominal pain; Gastrointestinal motility; Cognition

Core tip: A dysregulated gut-brain axis may be responsible for the main features of irritable bowel syndrome (IBS). However, the role of the gut microbiota is an underappreciated but critical node in this construct. Numerous clinical studies have documented various alterations in the composition of the gut microbiota in IBS, indicating defects in stability and diversity of this virtual organ. Manipulation of the gut microbiome influences the symptom profile in IBS and clear mechanisms have been elucidated to explain these interactions. This has important clinical implications and may offer hope for future treatment options to alleviate the suffering caused by this debilitating disorder.

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INTRODUCTION

Irritable bowel syndrome (IBS) is the most common functional gastrointestinal (GI) disorder accounting for up to 50% of visits to general practitioners for GI complaints[1]. Despite considerable research efforts, adequate treatment of GI symptoms in IBS has proved a considerable challenge and remains a venture undermined by a poorly understood pathophysiology[2]. That such a rudimentary grasp of this debilitating condition persists despite a high worldwide community prevalence, between 10%-25% in developed countries, offers some perspective on the complex character of the disorder[3-5]. Impairments in the quality of life of afflicted individuals are associated with a chronic symptom profile incorporating abdominal pain, bloating and abnormal defecation[6]. Patients with IBS were painfully aware of the kind of signals the gut can send to the brain long before the concept of a dysregulated gut-brain axis emerged as the favoured explanation for their travails[7]. This bidirectional communication system provided the basis for incremental and much needed improvements in our understanding of IBS[8]. In parallel, it has become increasingly apparent that the gut microbiome constitutes a critical node within this axis in both health and disease[9-11].

In this review, we briefly detail the key components of the microbiota-gut-brain axis and critically evaluate the evidence, both direct and indirect, supporting a role for microbiome perturbations in IBS. The ability of this virtual organ to influence the gut-brain axis and relevant behaviours is explored and putative mechanisms outlined. Finally, we discuss the diagnostic and therapeutic implications arising from this corpus of knowledge.

MICROBIOME-GUT-BRAIN AXIS

The microbiome-gut-brain axis comprises a number of fundamental elements including the central nervous system (CNS), the neuroendocrine and neuroimmune systems, both the sympathetic and parasympathetic limbs of the autonomic nervous system, the enteric nervous system (ENS) and, of course, the gut microbiome[9,12]. Signalling along the axis is facilitated by a complex reflex network of afferent fibers projecting to integrative cortical CNS structures and efferent projections to the smooth muscle in the intestinal wall[13]. Thus, a triad of neural, hormonal and immunological lines of communication combine to allow the brain to influence the motor, sensory, autonomic and secretory functions of the gastrointestinal tract (Figure 1). These same connections allow the gastrointestinal tract to modulate brain function[7,10]. Although reciprocal communication between the ENS and the CNS is well described, the proposed role of the gut microbiota within this construct remains to be fully defined. The commitment to building a more complete picture of our legion of gastrointestinal inhabitants in both health and disease and their myriad of functions is clear from large-scale projects such as the NIH funded Human Microbiome Project[3]. Thus, it is becoming increasingly certain that our gut microbiome has a hand in virtually all aspects of normal physiological processes including those immunological features which buttress the gut-brain axis[14,15]. Interestingly, in the context of IBS as a stress-related disorder, the composition of the gut microbiota can be influenced by stressors[16,17] and the gut microbiome can itself regulate the host endocrine repertoire[18,19].

IBS AND MICROBIOME: DIRECT EVIDENCE

The true nature of gut microbiota disturbances in IBS and the functional consequences remains elusive and although direct evidence for alterations does exist, it is perhaps not as conclusive or consistent as one might expect for consideration as a prototypical *microbiome*-gut-brain axis disorder (Table 1). Much of the evidence predates the metagenomic approaches which now dominate this terrain and these early studies indicated subtle qualitative and quantitative alterations as well as a temporal instability in the composition of the microbiota in IBS compared to healthy controls[20-24]. Since a stable but diverse microbiota is generally considered beneficial to health, these studies provided a plausible basis to further consider shifts in microbiota composition as a pathogenic factor in IBS.

Although no consensus has emerged regarding the precise differences which are present, the application of modern high-throughput culture-independent techniques of superior resolution has largely supported the general thrust of the earlier findings[25]. At the phylum level, one of the more consistent findings across techniques appears to be the enrichment of Firmicutes and a reduced abundance of Bacteroidetes[26-28]. Such alterations may contribute to the reported lower diversity in the gut microbiota of IBS subjects compared to healthy controls[29-31]. More work remains to determine whether the Rome Ⅲ defined subtypes of IBS[32] are reflected in distinct microbiota conformations but it has been reported that there is a lower abundance of mucosa-associated *Bifidobacteria* in diarrhea predominant IBS (IBS-D) compared to constipation predominant IBS (IBS-C) patients[33]. There are also reports of subtype specific faecal microbiome compositions in children with IBS[34]. Interestingly, it has also been reported that children diagnosed with IBS-D also have a lower abundance of some members of the Bifidobacterium genera compared to healthy controls[35]. This suggests that alterations in the gut microbiota occur early in life and could be a chronic feature of IBS across the lifespan but this possibility requires further investigation and verification. The application of pyrosequencing technology to faecal samples has yielded a number of interesting findings including cohorts within the overall IBS group with both an altered and similar microbiota compared to healthy controls suggesting that microbiota differences might only be a feature in a subset of IBS patients[27]. Of further note

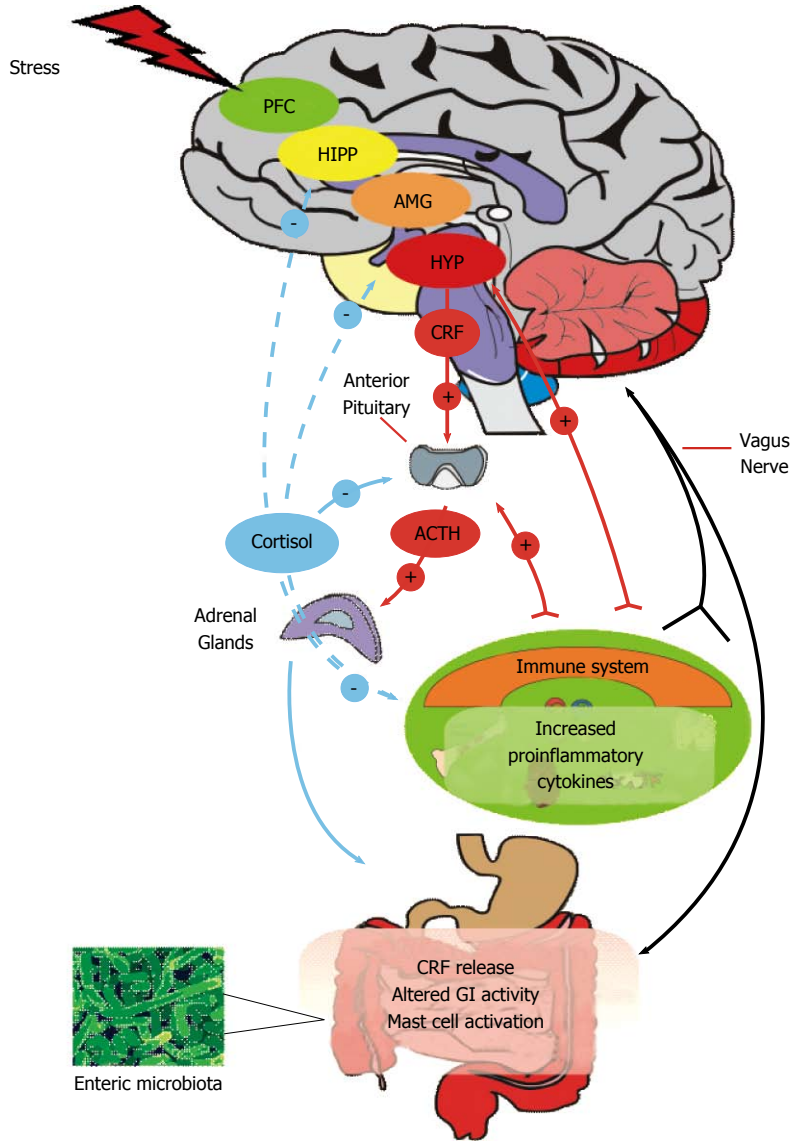

Figure 1 Microbiome-gut-brain Axis. The central nervous system (CNS) and enteric nervous system (ENS) communicate along vagal and autonomic pathways to modulate many gastrointestinal (GI) functions. The enteric microbiota influence the development and function of the ENS and immune system which affects CNS function. The hypothalamic pituitary adrenal (HPA) axis forms a key component of brain-gut signalling, responding to stress or heightened immune activity. Mood and various cognitive processes can mediate top-down bottom / bottom-up signalling. The HPA axis can be activated in response to environmental stress or by elevated systemic proinflammatory cytokines. Cortisol released from the adrenal glands feeds back to the pituitary, hypothalamus (HYP), amygdala (AMG), hippocampus (HIPP) and prefrontal cortex (PFC) to shut off the HPA axis. Cortisol released from the adrenals has a predominantly anti-inflammatory role on the systemic and GI immune system. In response to stress, GI activity can be altered and corticotropin releasing factor (CRF) increased. Stress can increase systemic proinflammatory cytokines which can act at the pituitary to activate the HPA axis and can signal to the central nervous system via the vagus nerve, which also transmits changes due to mast cell activation in the GI tract.

in this study was the presence of distinct microbiota defined subtypes of IBS among those cohorts with an altered microbiota which were unrelated to the Rome Ⅲ defined categories.

Differential microbiota compositions might not necessarily have functional consequences but there are some indications that the reported alterations have relevance for symptom expression in IBS. Of note is the report in healthy adults that subjects who experienced pain, assessed by questionnaire, over the 7 wk duration of the study had over five-fold less *Bifidobacteria* compared to those without pain[36]. However, in general, the association between specific symptoms and microbiota alterations remains under-investigated in IBS. Studies which have examined this topic have reported associations between stool frequency and musoca-associated *Bifidobacteria* and *Lactobacilli*[33], a correlation between *Firmicutes* and *Proteobacteria* and symptom scores[28] as well as a correlation between symptom scores and a *Ruminococcus-torques*-like phylotype[37].

Although the findings discussed above affirm the likelihood of a perturbed microbiome in IBS, some caution is advisable and a number of caveats should be considered before reaching this conclusion. All studies, not just those concerned with characterising the microbiota, must contend with the considerable heterogeneity within

Table 1 Microbiota alterations in irritable bowel syndrome

Sample type/method	Subjects recruited	Key finding	Ref.
Faecal microbiota (at 3 mo intervals)/Q-PCR (covering about 300 bacterial species)	IBS (27, Rome Ⅱ Criteria; IBS-D = 12; IBS-C = 9; IBS-A = 6); Healthy Controls (22)	Decreased *Lactobacillus spp* in IBS-D; Increased Veillonella spp in IBS-C; Differences in the *Clostridium coccoides* subgroup and *Bifidobacterium catenulatum* group between IBS patients and controls	[22]
Faecal microbiota/Q-PCR (10 bacterial groups), Culture, HPLC	IBS (26, Rome Ⅱ/Ⅲ; IBS-D = 8; IBS-C = 11, IBS-A = 7); Healthy Controls (26)	Higher counts of *Veillonella* and *Lactobacillus* in IBS *vs* controls; Higher levels of acetic acid, propionic acid and total organic acids in IBS *vs* controls	[52]
Faecal microbiota(0, 3, 6 mo)/Culture-based techniques, PCR-DGGE analysis	IBS (26, Rome Ⅱ; IBS-D = 12; IBS-C = 9; IBS-A = 5); Healthy Controls (25)	More temporal instability in IBS group; No difference in the *bacteroides*, *bifidobacteria*, spore-forming bacteria, *lactobacilli*, *enterococci* or yeasts, Slightly higher numbers of coliforms as well as an increased aerobe:anaerobe ratio in IBS group	[23]
Faecal microbiota/DNA-based PCR-DGGE, RNA-based RT-PCR-DGGE	IBS (16, Rome Ⅱ; IBS-D = 7; IBS-C = 6; IBS-A = 3); Healthy Controls (16)	Higher instability of the bacterial population in IBS compared to controls; Decreased proportion of *C. coccoides-Eubacterium rectale* in IBS-C	[24]
Faecal Microbiota/GC Fractionation, 16S ribosomal RNA gene cloning and clone sequencing, qRT-PCR	IBS (24, Rome Ⅱ; IBS-D = 10; IBS-C = 8; IBS-A = 6); Healthy Controls (23)	Significant differences in phylotypes belonging to the genera Coprococcus, Collinsella and Coprobacillus	[20]
Faecal Microbiota/GC Fractionation, 16S ribosomal RNA gene cloning and clone sequencing, qRT-PCR	IBS (12, Rome Ⅱ, All IBS-D); Healthy Controls (22)	Significant differences between clone libraries of IBS-D patients and controls; Microbial communities of IBS-D patients enriched in *Proteobacteria* and *Firmicutes*, reduced *Actinobacteria* and *Bacteroidetes* compared to control; Greater abundance of the family *Lachnospiraceae* in IBS-D	[26]
Faecal Microbiota/qRT-PCR	IBS (20, Rome Ⅱ; IBS-D = 8; IBS-C = 8; IBS-M = 4); Healthy Controls (15)	Intestinal microbiota of the IBS-D patients differed from other sample groups; A phylotype with 85% similarity to *C. thermosuccinogenes* significantly different between IBS-D and controls/IBS-M; A phylotype with 94% similarity to R. torques more prevalent in IBS-D than controls; A phylotype with 93% similarity to R. torques was altered in IBS-M compared to controls; *R. bromii*-like phylotype altered in IBS-C comparison to controls	[244]
Faecal Microbiota/DGGE 16s rRNA	IBS (11, Rome Ⅱ); Healthy Controls (22)	Biodiversity of the bacterial species was significantly lower in IBS than controls; presence of *B. vulgatus*, *B. ovatus*, *B. uniformis* and *Parabacteroides* sp. in healthy volunteers distinguished them from IBS	[31]
Faecal Microbiota/DGGE 16s rRNA, qRT-PCR, GC-MS	IBS (11, Rome Ⅱ; Non-IBS patients (8)	IBS subjects had a significantly higher diversity *Bacteroidetes* and *Lactobacillus* groups; Less diversity for *Bifidobacteria* and *C. coccoides*; Elevated levels of amino acids and phenolic compounds in IBS which correlated with the abundance of *Lactobacilli* and *Clostridium*	[51]
Faecal Microbiota and sigmoid colon biopsies/DGGE 16s rRNA	IBS (47, Rome Ⅱ); Healthy Controls (33)	Significant difference in mean similarity index between IBS and healthy controls; Significantly more variation in the gut microbiota of healthy volunteers than that of IBS patients	[29]
Faecal Microbiota and brush duodenal samples/FISH + qRT-PCR	IBS (41, Rome Ⅱ; IBS-D = 14, IBS-C = 11; IBS-A = 16); Healthy Controls (26)	2-fold decrease in the level of bifidobacteria in IBS patients compared to healthy subjects; no major differences in other bacterial groups. At the species level, *B. catenulatum* significantly lower in IBS patients in both faecal and duodenal brush samples than in healthy subjects	[21]
Faecal Microbiota and brush duodenal samples/DGGE 16s rRNA, q-RT-PCR	IBS (37, Rome Ⅱ; IBS-D = 13, IBS-C = 11; IBS-A = 13); Healthy Controls (20)	Higher levels *P. aeruginosa* in the small intestine and faeces of IBS than healthy subjects	[47]
Faecal Microbiota and colonic mucosal samples/Culture, qRT-PCR	IBS (10, Rome Ⅲ, all IBS-D); Healthy Controls (10)	Significant reduction in the concentration of aerobic bacteria in faecal samples from D-IBS patients when compared to healthy controls 3.6 fold increase in concentrations of faecal *Lactobacillus* species between D-IBS and healthy controls; No significant differences were observed in the levels of aerobic or anaerobic bacteria in colonic mucosal samples between D-IBS patients healthy controls; No significant differences in mucosal samples between groups for *Clostridium*, *Bacteroides*, *Bifidobacterium* and *Lactobacillus* species and *E. coli*	[46]
Faecal Microbiota and colonic mucosal samples/T-RFLP) fingerprinting of the bacterial 16S rRNA gene	IBS (16, Rome Ⅲ, All IBS-D); Healthy Controls (21)	1.2-fold lower biodiversity of microbes within faecal samples from D-IBS compared to healthy controls; No difference in biodiversity of mucosal samples between D-IBS and healthy controls	[30]

Faecal Microbiota/Phylogenetic microarray, qRT-PCR	IBS (62, Rome Ⅱ; IBS-D = 25; IBS-C = 18; IBS-A = 19); Healthy Controls (46)	2-fold increased ratio of the *Firmicutes* to *Bacteroidetes* in IBS; 1.5-fold increase in numbers of *Dorea*, *Ruminococcus* and *Clostridium* spp; 2-fold decrease in the number of *Bacteroidetes*; 1.5-fold decrease in *Bifidobacterium* and *Faecalibacterium* spp; 4-fold lower average number of methanogens	[28]
Rectal biopsies/FISH	IBS (47, Rome Ⅲ; IBS-D = 27, IBS-C = 20); Healthy Controls (26)	Greater numbers of total mucosa-associated bacteria per mm of rectal epithelium in IBS than controls, comprised of bacteroides and *Eubacterium rectale-C. coccoides*; *Bifidobacteria* lower in the IBS-D group than in the IBS-C group and controls; Maximum number of stools per day negatively correlated with the number of mucosa-associated *Bifidobacteria* and *Lactobacilli* only in IBS	[33]
Faecal Microbiota/16s rRNA amplicon pyro-sequencing	IBS (37, Rome Ⅱ; IBS-D = 15, IBS-C = 10, IBS-A = 12); Healthy Controls (20)	IBS subgroup (n = 22) defined by large microbiota-wide changes with an increase of *Firmicutes*-associated taxa and a depletion of *Bacteroidetes*-related taxa	[27]
Faecal Microbiota/Phylogenetic microarray, qRT-PCR	IBS (23, Rome Ⅱ; IBS-D = 12, PI-IBS = 11); 11 Healthy Controls (11); Subjects who 6 mo after gastroenteritis experienced no bowel dysfunction (PI-nonBD, n = 12) or had recurrent bowel dysfunction (PI-BD, n = 11)	Bacterial profile of 27 genus-like groups separated patient groups and controls; Faecal microbiota of patients with PI-IBS differs from that of healthy controls and resembles that of patients with IBS-D; Members of Bacteroidetes phylum were increased 12-fold in patients, while healthy controls had 35-fold more uncultured *Clostridia*; Correlation between index of microbial dysbiosis and amino acid synthesis, cell junction integrity and inflammatory response	[50]
Faecal Microbiota/Phylogenetic Microbiota Array, high-throughput DNA sequencing, r-RT- PCR, FISH	IBS (22, pediatric Rome Ⅲ, All IBS-D); Healthy Controls (22)	At the higher taxonomical level gut microbiota was similar between healthy and IBS-D children. Levels of *Veillonella*, *Prevotella*, *Lactobacillus* and Parasporo bacterium increased in IBS, *Bifidobacterium* and *Verrucomicrobium* less abundant in IBS	[35]
Faecal Microbiota/16s rRNA pyrosequencing, DNA microarray (Phylochip)	IBS (22, Pediatric Rome Ⅲ; IBS-D = 1, IBS-C = 13; IBS-U = 7, other = 1); Healthy Controls (22)	Greater percentage of the class gamma-proteobacteria in IBS compared to controls; Novel *Ruminococcus*-like microbe associated with IBS; Greater frequency of pain in IBS correlated with an increased abundance of several bacterial taxa from the genus Alistipes	[34]

IBS (D/C/A/U): Irritable bowel syndrome (diarrhoea/constipation/alternating/unsub typed); PI: Post-infectious; Q: Quantitative; DGGE: Denaturing gradient gel electrophoresis; qRT: Quantitative reverse transcriptase; PCR: Polymerase chain reaction; HPLC: High performance liquid chromatography; GC: Gas chromatograph; DNA: Deoxyribonucleic acid; RNA: Ribonucleic acid; rRNA: Ribosomal ribonucleic acid; FISH: Fluorescence in situ hybridization; T-RFLP: Terminal restriction fragment length polymorphism.

this patient group. There is no doubt that this lack of uniformity contributes to some of the inconsistencies in the reported data and larger studies are required which factor in not just IBS subtypes but also the influence of gender, genetics, presence of comorbidities, whether the patients recruited are in the active or quiescent phase at the time of sampling and increased standardisation in healthy control cohorts[38]. This feature is then superimposed on our rapidly evolving impressions of what constitutes a healthy microbiome which is highly individual specific but still lacks full definition[39-41]. Diet plays a major role in shaping the gut microbiota[42-44] and given the often self-imposed dietary restriction practises among the IBS population[45], it is difficult to rule out the possibility that the observed alterations are a consequence of these changes. Indeed, in isolation, the studies outlined do not clearly establish a causal role for the microbiome in IBS and the alterations described could be a consequence not just of dietary alterations but also the main GI symptoms, which wax and wane, as well as the altered stress reactivity.

Considerable debate also exists surrounding the sample type used across the various studies. Practical logistical reasons favour faecal sampling protocols but this strategy fails to capture the complexity of the gut microbiota and the clear distinction between the mucosa-associated and lumen residing microbiota. There is also a microbiota gradient along the gastrointestinal tract which is not captured by a faecal microbiota analysis. Although some studies have logically attempted to link alterations in the faecal microbiota with disturbances in the musosa-associated microbial complement[21,29,30,33,46,47], the precise relationship between altered composition, diversity and/or stability in the faecal compartment and microbe-mucosa interactions remains to be fully defined. Indeed, the subtleties of any equilibrium between these different microbial niches is the subject of on-going investigation in both and health and disease and we cannot yet confidently predict how one affects the other, either positively or negatively. Other methodological considerations relating to the merits and limitations of the variety of techniques which have been used to characterise the microbiome in IBS are likely to have contributed to some of the inconsistencies reported[48,49]. The consequences of any altered composition are less frequently reported although a number of interesting studies have taken this approach[50-52] which is likely to feature more prominently as the field undertakes to establish not just who is or isn't there but also what they are or are not doing.

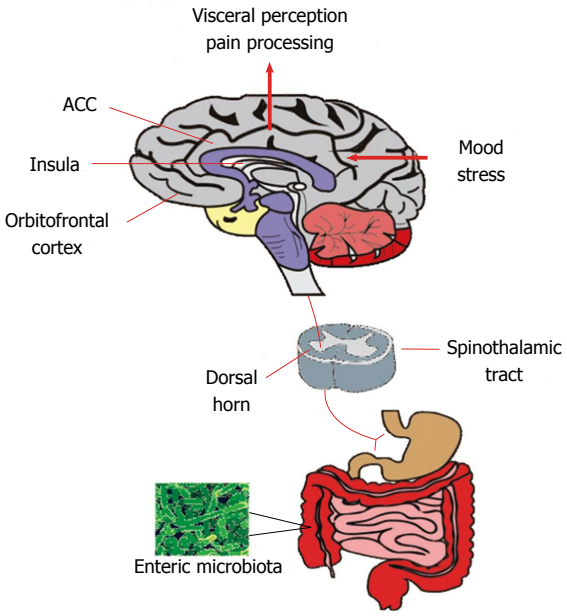

Figure 2 Visceral pain perception. The microbiota can influence the spinothalamic projections from the gastrointestinal tract which reach higher cortical areas including the insula, anterior cingulate cortex and orbitofrontal cortex where visceral sensory and pain signals reach the conscious awareness. These regions mediate the cognitive processing of visceral signals and integrate mood and stress-related information and initiate autonomic and behavioural responses. ACC: Anterior cingulate cortex.

IBS AND MICROBIOME: INDIRECT EVIDENCE

The lack of consensus in studies which have sought to directly quantify microbiota alterations in IBS has prompted the consideration of alternative but more indirect lines of support. These approaches are in line with the recognised requirement for a better knowledge of the mechanisms through which changes in microbiota composition can promote disease to help the transition for correlation to causation[53,54].

An endorsement of the importance of the gut microbiome is taken from the emergence of IBS following an enteric infection, post-infectious IBS (PI-IBS), which bears most similarity to IBS-D[55]. One of the highest incidences of this phenomenon, 36%, was reported following a gastroenteritis outbreak in Walkerton due to contamination of the town water supply[56]. The ability of certain probiotic strains to ameliorate some symptoms of IBS also indicts dysbiosis of the microbiota as an important factor in the disorder[57]. Interestingly, antibiotic usage has been linked with both an increased risk for IBS[58,59] as well having some beneficial effects as in the case of rifaximin[60,61]. Small intestinal bacterial overgrowth (SIBO) has also been proposed as a factor in IBS and while it can be responsible for IBS-like symptoms, it remains a controversial topic and inadequately substantiated[62]. The presence of low grade inflammation could potentially be driven by an altered microbiota composition and in turn support a proinflammatory microbial community and offers a further strand of support[8,25,63]. Taken together, this direct and indirect evidence makes a plausible case to include the microbiome as a critical conceptual node in a framework for understanding the disorder.

IBS SYMPTOMS AND MICROBIOTA

If gut microbiome disturbances are pertinent to IBS, then this virtual organ should demonstrate an ability to influence the canonical symptoms of the disorder as well as other prominent behavioural alterations. In addition, it should be possible to therapeutically target the microbiome to ameliorate the symptoms which are purported to be under its influence. This certainly seems to be the case for the abdominal pain component of the disorder which is underpinned by visceral hypersensitivity (Figure 2) in a large proportion of individuals with IBS[64-66]. It appears, for example, that the visceral hypersensitivity phenotype characteristic of IBS can be transferred *via* the microbiota of IBS patients to previously germ-free rats[67]. In other preclinical approaches, visceral hypersensitivity is also induced following manipulation of the intestinal microbiome with antibiotics[68] and following deliberate infection[69,70] or endotoxin administration[71]. Moreover, maternal separation, an early-life stress based animal model of IBS, produces an adult phenotype with both an altered microbiota and visceral hypersensitivity[13,17].

From a therapeutic perspective, certain probiotic strains, such as *B. infantis* 35624 and *Lactobacillus acidophilus*, can ameliorate colonic hypersensitivity in animal models[72-74] and this and other probiotic strains are also of some benefit in clinical populations[57,75]. Interestingly, visceral hypersensitivity due to chronic psychological stress in mice can be prevented by pre-treatment with oral rifaximin[76]. Also of note is that mast cells have been implicated as a downstream mediator of microbiota-driven immune alterations in the pain component of IBS[77-80] and a mast cell stabiliser, disodium cromoglycate, can reverse colonic visceral hypersensitivity in a stress-sensitive rat strain used to model IBS[81].

Although not simply a bowel habit disorder of disrupted gastrointestinal motility and transit[82], it does appear likely that these features might at least partially explain the altered defecatory patterns that are typical of IBS[83]. Clearly, it has long been known that both enteric infections and antibiotics can induce diarrhoea[84,85]. Certain strains of probiotic have demonstrated efficacy for the treatment of diarrhoea[86]. Thus, a role for the microbiota in the regulation of colonic motility has been proposed[87] and the interaction between the intestinal microbiota and the gastrointestinal tract also regulates absorption, secretion and intestinal permeability[88]. The olfactory bulbectomy mouse model of depression has recently been shown to have both an altered microbiota and aberrant colonic motility[89]. However, the effect of the gut microbiota on gastrointestinal transit is complex and studies in humanized mice indicate that while GI transit can be regulated by the microbiota, this is a diet-dependent feature[90]. Of course, gut motor patterns can

also influence the microbiota, highlighting further the bi-directional, intricate nature of the relationship[91]. Studies in mice indicate a role for gut microbial products in the regulation of gastrointestinal motility *via* toll-like receptor 4 (TLR4)[92]. Given the recent association between this receptor and the control of stress-induced visceral pain in mice[93], it may represent an interesting target for modulation of two cardinal features of IBS.

Psychiatric comorbidity in IBS

It is well established that psychiatric comorbidities, particularly anxiety and depression, are common among patients with IBS[94,95]. Although concerns about the screening instruments such as the Hospital Anxiety and Depression Scale (HADS) used in research studies are noted[96-98], psychiatric co-morbidity is readily identifiable in IBS when well validated instruments such as the structured clinical interview for DSM-IV-TR are employed[99]. Following acute gastroenteritis, prior anxiety and depression has been identified as a risk factor for the subsequent development of PI-IBS[100,101]. Higher anxiety and depression scores have also been reported in this population following the initial infection[102]. Prenatal infection can also result in a depressive phenotype in adult mice[103]. Following endotoxin challenge in rodents, depressive-like behaviours can emerge once the initial inflammation-induced sickness behaviours subside[104]. This complexity indicates that a reciprocal relationship is likely, an important consideration when discussing the association between changes in the gut microbiota in IBS and central disturbances. Such alterations may then be secondary to changes in the composition of the gut microbiota, or indeed, perturbations of the gut microbiota, *via* pathways of the brain-gut axis, may arise as a result of changes in central function.

While as yet neither correlative or causative clinical studies exist that directly interrogate the qualitative and quantitative structure of the gut microbiome in psychiatric illnesses for abnormalities, there is now strong evidence from the preclinical literature that changes in the microbiome can influence these aspects of brain and behaviour[12,14]. This is most convincing for anxiety-like behaviours and multiple independent teams of researchers have confirmed in proof of principle studies that germ-free mice are less anxious than their conventionally colonised counterparts[105-107] while reintroduction of the microbiota prior to critical time windows can normalise these behaviours[105]. Ablation of the microbiota in mice using a non-absorbable antimicrobial cocktail reproduces this behavioural feature while it has also been established that this is a trait which is transmissible *via* the microbiota[108]. Interesting, in germ-free rats, absence of the microbiota seems to confer elevated levels of anxiety-like behaviours[109] but regardless of the direction of the alterations, these studies confirm that this is a behaviour under the influence of the microbiota. Deliberate infection of the GI tract in mice also consistently produces an anxious phenotype[110-112] while certain probiotic strains may have anxiolytic potential[113].

Although there are now a number of examples of animal models of depression which have an altered microbiota[17,89,114], the preclinical evidence linking the microbiota to depressive-like behaviours is mostly derived from probiotic studies where certain strains such as *L. rhamnosus*[115], *B. infantis*[116] and a formulation of *L. helveticus* and *B. longum* displayed antidepressant like properties[117]. Interestingly, the latter study also demonstrated that at least in healthy volunteers, targeting the microbiota in this manner could alleviate psychological distress including an index of depression.

Evidence from the clinical domain comes indirectly from the utility of a variety of antibacterial agents in the modulation of depression. This includes, in addition to support from preclinical studies[118,119], preliminary clinical confirmation that minocycline (a broad-spectrum tetracycline antibiotic) possesses antidepressant properties[120,121]. Whether this effect generalises to all tetracycline antibiotics is not known but another member of this class, doxycycline, seems to have similar beneficial effects, at least in preclinical studies[122]. The mechanism of action of minocycline has been considered in the context of neuroprotection, suppression of microglial activation or anti-inflammatory actions and it does reach clinically relevant concentrations in the CNS[123]. Even if its anti-inflammatory action is distinct from its antimicrobial action as when used in preclinical stroke models[124], the action of minocycline against bacteria in the gut now need to be considered in its putative antidepressant effects. Indeed, a number of other antimicrobial agents have shown some potential as antidepressants but all have other relevant mechanisms of action which have been preferentially adopted to explain their efficacy. This includes D-cycloserine[125] [antibiotic effective against tuberculosis which is also a partial agonist of the N-methyl-D-asparate (NMDA) receptor] and ceftriaxone[126] (a beta-lactam antibiotic that also stimulates uptake of glutamate). Moreover, in aged populations fluoroquinolone antibiotics can potentially induced depressive symptoms[127]. Similarly, norfloxacin (a quinoline antibiotic with antibacterial activity against gram-positive and gram-negative bacteria) has been linked with depressive side effects in the clinic[128]. It is also interesting to note that iproniazid, a drug which in many ways sparked the monoamine hypothesis of depression and heralded the psychopharmacological era in the management of depression, is primarily an antimicrobial agent whose antidepressant effects were presumed to be mediated *via* inhibition of monoamine oxidase[129]. It would not be without irony if future treatment options for depression, as has been suggested, focus instead on targeting the microbiota[114,130].

Cognition function in IBS

Extensive cognitive testing in germ-free animals has not been carried out, likely as it is logistically challenging and the difficulty in conducting the lengthy testing protocols

Table 2 Cognitive performance in irritable bowel syndrome

Cognitive domain	Sample size: IBS/Control/Other	IBS subtype	Sex Male:Female	Mean age IBS/Control/Other	Key finding	Ref.
Visuospatial memory	39/40	IBS-D = 7; IBS-C = 4; IBS-A = 28	6:33 (IBS) 11:29 (Control)	28/28	Impaired performance which correlated with salivary cortisol levels	[140]
	40/41	N.S.	13:27 (IBS) 16:25 (Control)	37/43	No group differences	[245]
Working memory	39/40	IBS-D = 7; IBS-C = 4; IBS-A = 28	6:33 (IBS) 11:29 (Control)	28/28	No group differences	[140]
	40/41	N.S.	13:27 (IBS) 16:25 (Control)	37/43	No group differences	[245]
Cognitive flexibility	30/30	IBS-D = 13; IBS-C = 13; IBS-A = 4	15:15 (IBS) 15:15 (Control)	21/21	Impaired cognitive flexibility and altered frontal brain activity in IBS	[141]
	39/40	IBS-D = 7; IBS-C = 4; IBS-A = 28	6:33 (IBS) 11:29 (Control)	28/28	No group differences	[140]
	40/41	N.S.	13:27 (IBS) 16:25 (Control)	37/43	No group differences	[245]
Selective attention	39/40	IBS-D = 7; IBS-C = 4; IBS-A = 28	6:33 (IBS) 11:29 (Control)	28/28	No group differences	[140]
	40/41	N.S.	13:27 (IBS) 16:25 (Control)	37/43	No group differences	[245]
	27/27	N.S.	3:24 (IBS) 3:24 (Control)	45/42	No group differences	[246]
Reaction time	40/41	N.S.	13:27 (IBS) 16:25 (Control)	37/43	No group differences	[245]
Affective attention	15/15	IBS-D = 6; IBS-C = 3; IBS-A = 3; Other = 3	4:11 (IBS) 5:10 (IBS)	30/30	Enhanced attention to GI symptom-related words	[247]
	20 (Rome Ⅱ Criteria)/33	N.S.	2:18 (IBS) 12:21 (Control)	31/27	Enhanced attention to pain-related words	[248]
	36 (Rome Ⅱ Criteria)/40 (mixed organic GI disease)	N.S.	12:24 (IBS) 16:24 (mixed organic GI disease)	35/36	Enhanced recognition of GI-related words	[249]
Affective memory	30 (Manning criteria)/30/28 (depressed patients)/28 (organic GI disease)	N.S.	N.S.	36/35/38/27 (median age)	Enhanced recall of negative words compared to control and organic GI disease - no difference in comparison to depression group	[250]

GI: Gastrointestinal; IBS (D/C/A): Irritable bowel syndrome (diarrhoea/constipation/alternating); N.S.; Not specified.

required while simultaneously maintaining the animal in a germ-free state should not be underestimated. Nevertheless, studies which have used the most feasible paradigms such as novel object recognition and the T-maze have demonstrated non-spatial, hippocampal mediated, and working memory deficits[131]. In addition, germ-free animals also exhibit pronounced social-cognitive deficits relevant to neurodevelopmental disorders which can be partially ameliorated by bacterial colonisation of the gut[132]. Studies in conventional mice have shown that infection with *C. rodentium* combined with acute stress, leads to memory dysfunction which could be prevented by daily administration of a probiotic prior to infection[131], thus highlighting a complex interaction between stress and the gut microbiota on brain function. In addition, modulating the composition of the gut microbiota using a specific diet has been shown to affect cognition in conventional mice[133,134].

Clinically, the influence of microbial disturbances on cognitive performance has long been recognised in hepatic encephalopathy where cognitive impairment, which in some cases may present as dementia, can be reversed with oral antibiotic treatment[135,136]. Although data linking changes in the gut microbiota with cognitive function in IBS is currently lacking, there is nevertheless a growing body of evidence that cognitive alterations may be a key feature of IBS and other brain-gut axis disorders[7,137]. Initial studies focusing on cognitive function within the cognitive-behavioural model of IBS[138,139] identified that patients exhibit greater attention to GI symptom and pain related stimuli (Table 2 for details). This enhanced attention to, and inability to re-direct attention from, GI symptoms, purportedly maintains a continual cycle of symptom exacerbation which can be ameliorated in some patients using cognitive-behavioural psychotherapeutic techniques (for extensive review of the cognitive-behavioural model of IBS[137]).

An advanced understanding of cognitive alterations in IBS has been provided by recent studies utilising well validated and sensitive neuropsychological measures with patients. For example, patients with IBS have been found to exhibit a hippocampal mediated visuospatial memory deficit which was related to hypothalamic-pituitary-adrenal (HPA) axis activity[140]. In addition, a study employing functional brain imaging reported that patients were impaired on a test of cognitive flexibility, whilst also displaying abnormal brain activity in frontal brain regions during the task[141]. However, there is a disparity in find-

ings between studies (Table 2) which likely reflects the noted heterogeneity of IBS and different approaches to subject matching on the basis of demographic and other important variables. Regardless of these methodological drawbacks, such studies have added to our understanding of the complex behavioural phenotype of IBS. When considering the gut microbiota mediated alterations in brain function and cognition that have been shown preclinically[115,131,132,134], it is likely that an altered gut microbiota may leverage a significant influence on cognitive dysfunction in IBS. Of note then, is a recent study in a healthy human population which has provided preliminary evidence that intake of a fermented milk product with probiotic can modulate brain activity in regions involved in mediating cognitive performance[142]. As such, interventions targeting the gut microbiota in IBS may prove beneficial in alleviating impaired cognition and associated central alterations.

STRESS, IBS AND THE GUT MICROBIOTA

Stress impacts greatly on virtually all aspects of gut physiology relevant to IBS including motility, visceral perception, gastrointestinal secretion and intestinal permeability while also having negative effects on the intestinal microbiota[17,143,144]. A maladaptive stress response may thus be fundamental to the initiation, persistence and severity of symptoms in IBS as well as the stress-related psychiatric comorbidities[145]. Although the findings pertaining to HPA axis irregularities in IBS are far from consistent[8,137], the well validated Trier Social Stress Test (TSST)[146] has recently been used to demonstrate a sustained HPA axis response to an acute stress in IBS, possibly indicating an inability to appropriately shutdown the stress response[147].

Accumulating evidence suggests aberrant stress responses could be mediated *via* the gut microbiota. A landmark study by Sudo *et al*[148] neatly validated this possibility by demonstrating the absence of a gut microbiota impaired control of the stress response, at least in terms of the exaggerated corticosterone production following acute stress in germ-free mice[148]. Subsequently independently replicated[105], the ability of the microbiota to modulate the stress response is also evident following probiotic administration[115], *C. rodentium* infection[131] and indeed following colonisation of germ-free mice[148]. Many now view the gut microbiota as an endocrine organ and as a key regulator of the stress response[18,19]. It must also be acknowledged that whilst the microbiota can modulate the stress response, stress can also affect the composition of the gut microbiota[17]. Thus, stress induced changes in the microbiota may precede any subsequent GI and central disturbances in IBS.

Mechanisms

When considering the preclinical evidence reviewed above, and preliminary evidence from healthy humans[142] it appears that the perturbations in composition of the gut microbiota may be considered as a primary factor in driving changes in central function in IBS. However, as IBS is a stress related disorder, the preclinical evidence indicating that chronic stress can alter the gut microbiota must also be borne in mind. As noted, stress and the gut microbiota have been shown to interact in a complex manner to influence brain function, at least in rodents[131] and it will be important to delineate this interaction in IBS. Nevertheless, when considering the gut microbiota as the primary factor in driving changes in central functioning, a number of potential mechanisms have been considered, with varying degrees of evidence supporting both humoral and neural lines of communication to the level of the CNS as well as more localised effects from compositional alterations.

Tryptophan, an essential amino acid and precursor for the neurotransmitter serotonin (5-HT), in particular has received much attention (Figure 3). 5-HT is a key signalling molecule in the brain-gut axis, both in the enteric nervous system[149] and the CNS[150]. The information gleaned from studies in germ-free animals suggests that the peripheral availability of tryptophan, which is critical for CNS 5-HT synthesis, is coordinated by the gut microbiota[105]. Plasma tryptophan concentrations can be normalised following colonisation of germ-free animals[105] and can also be augmented following administration of the probiotic *B. infantis*[116]. How the bacteria in our gut regulate circulating tryptophan concentrations is unclear but may involve controlling the degradation of tryptophan along an alternative and physiologically dominant metabolic route, the kynurenine pathway[151,152]. The enzymes responsible for the initial metabolic step in this pathway, indoleamine-2,3-dioxygenase (IDO) and tryptophan-2,3-dioxygenase (TDO), are immune and glucocorticoid responsive respectively and the decreased ratio of kynurenine to tryptophan (an index of IDO/TDO activity) in germ-free animals implicates this pathway in the reported alterations (Figure 4)[105]. Moreover, an increased ratio is observed following infection with *Trichuris Muris*, likely due to increased IDO activity following the associated chronic gastrointestinal inflammation[153].

The relevance of these preclinical findings to IBS is well reflected in the clinical literature which has demonstrated increased IDO activity in both male and female IBS populations[154-156]. Interestingly, TLR receptors, which have altered expression and activity in both clinical IBS populations[157,158] and animal models of the disorder[159], might drive the low grade inflammation in IBS and mediate the immune consequences of the misfiring engagement between the microbiota and the host in IBS. In this context, it is interesting to note that once TLR receptors are engaged by their cognate ligands, degradation of tryptophan can ensue in general[155,160,161] and there appears to be a differential TLR-specific pattern of kynurenine production in IBS[155].

There are also other potential explanations for the alterations in tryptophan supply due to microbiota alterations and in addition to the growth requirements

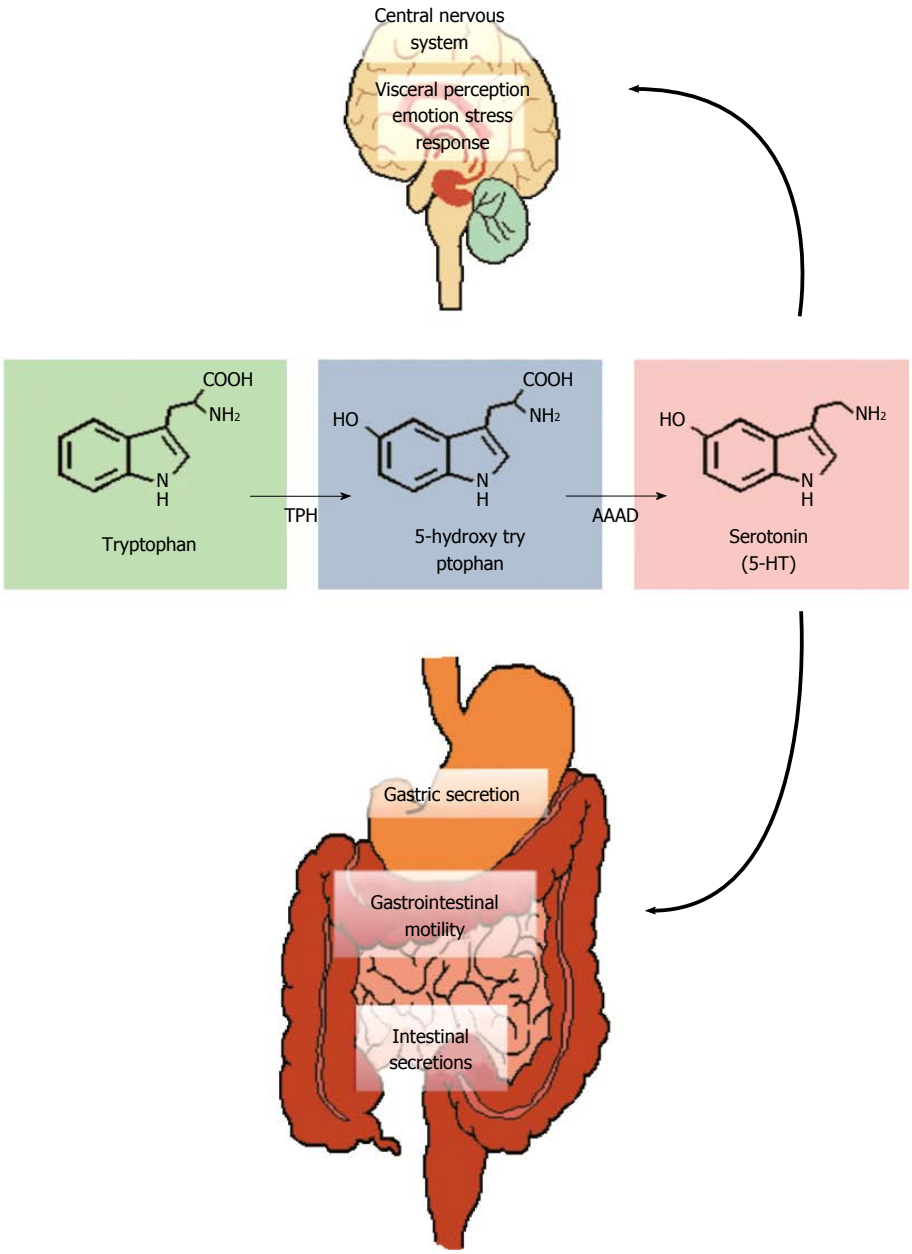

Figure 3 Tryptophan metabolism. Tryptophan is converted to 5-hydroxytryptophan by tryptophan hydroxylase (TPH) and this is the rate limiting step in the pathway. Aromatic amino acid decarboxylase (AAAD) subsequently converts 5-HTP into serotonin (5-HT). These reactions occur both in the central nervous system (CNS) (where 5-HT regulates a myriad of functions including emotion, cognition, stress and visceral perception) and in the enteric nervous system (gastrointestinal motility and secretion).

for bacteria[162], a bacteria-specific tryptophanase enzyme also recruits tryptophan for indole production[163,164]. One such bacteria, *Bacteroides fragilis*, harbours this enzyme and has recently been linked to gastrointestinal abnormalities in autism spectrum disorders[165]. Of further interest and adding to the complexity of the narrative is that, in contrast to eukaryotes, bacteria retain a capacity for tryptophan biosynthesis *via* enzymes such as tryptophan synthase[166,167]. It seems a curious quirk of the evolutionary process that we have lost the capacity for endogenous tryptophan synthesis, given the pivotal nature of this amino acid not alone as a precursor to serotonin, which itself has an expansive physiological repertoire[168], but also the other metabolic pathways it serves[150,151].

The production of serotonin from tryptophan, at least *in-vitro*, is also possible in some bacterial strains[169-171]. Harnessing this knowledge to specifically target the 5-HT receptors and receptor subtypes expressed in the gut of most relevance to IBS[172-175] or indeed alternative receptors activated by kynurenine pathway metabolites that interact with gastrointestinal functions[176] presents an interesting challenge. Similarly, whether we can accurately "titer" the gut microbiota to deliver precise circulating or regional tryptophan concentrations is an intriguing possibility but one beyond our current capabilities.

Of course, immune system mediators and glucocorticoids can impact both locally in the gut and at the level of the CNS independently of their effects on trypto-

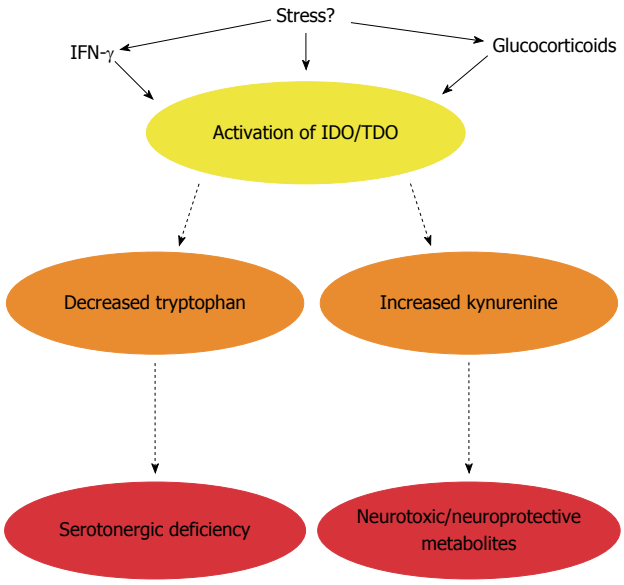

Figure 4 Impact of altered tryptophan metabolism in irritable bowel syndrome. In addition to serotonin, tryptophan can also be metabolised along the kynurenine pathway to generate neurotoxic and neuroprotective metabolites. The enzymes responsible for degradation along this pathway are immune (indoleamine-2,3-dioxygenase, IDO) and stress (tryptophan-2,3-dioxygenase, TDO) responsive. In IBS, this pathway is activated leading to a potential serotonergic deficiency and/or altered enteric nervous system (ENS) and central nervous system (CNS) availability of kynurenine and its metabolites. The microbiota appears to directly or indirectly regulate enzyme activity.

phan metabolism and represent viable alternative routes through which the gut microbiota can modulate gut-brain axis signalling and influence IBS symptoms[14,19,104,177,178]. In addition, the more general concept of a "leaky gut" has been proposed to explain the common feature of a low-grade circulating inflammation in both IBS itself and depression, which, as outlined above, is a prominent psychiatric comorbidity in IBS[179-182]. This model relies on the presence of increased intestinal permeability in IBS which allows the gut microbiota to drive the reported proinflammatory state and influence the CNS *via* the ensuing elevations in circulating cytokines[104] as well as visceral hypersensitivity *via* local gut mechanisms[183]. There is certainly accumulating evidence to support the hypothesis of altered intestinal permeability, a compromised integrity of the intestinal epithelial barrier and related tight junction disturbances in IBS, if not in depression[183-187].

Defects of the intestinal epithelial barrier may also play a significant role in cognitive dysfunction in IBS. The maternal immune activation (MIA) mouse model produces epithelial barrier defects, changes in the gut microbiota, and associated cognitive and behavioural features of neurodevelopmental disorders in rodents[188]. A recent study has provided strong evidence that maternal infection in the MIA model drives changes in the gut microbiota in the offspring, which subsequently leads to the cognitive and behavioural alterations in this model. Treatment with *B. fragillis* in MIA offspring restored gut barrier integrity and alleviated some of the cognitive and behavioural defects displayed by these animals[189]. Importantly, restoration of gut barrier integrity in MIA offspring appeared to stop a number of neuroactive metabolites being released systemically to reach the CNS and affect behavioural and cognitive function[189]. Thus, when extrapolated to IBS, epithelial barrier dysfunction may lead to the release of numerous metabolites that could impact centrally and impair cognitive performance. Of note, some probiotic strains have shown efficacy in repairing epithelial barrier function[190] in preclinical models which may also explain the efficacy in treating some GI symptoms in IBS[57]. If probiotics also prove beneficial in alleviating central disturbances in IBS, this may potentially be *via* restoration of epithelial barrier integrity leading to the reduction of harmful neuroactive metabolites being released from the gut and impacting centrally.

The gut microbiome can also be considered a metabolic organ[191,192] and the array of microbial metabolites produced can impact greatly on GI health and the gut-brain axis scaffolding. Interestingly, dietary restriction of fermentable carbohydrates (fermentable oligosaccharides, disaccharides, monosaccharides and polyols: the low FODMAP diet) has received much attention for the management of symptoms in IBS[193,194]. Although microbial metabolism of carbohydrates, proteins and amino acids by human gut bacteria generates a variety of compounds[195], short chain fatty acids (SCFAs) may be of particular importance in the context of microbiome-gut-brain axis signalling. For example, these organic acids are altered in IBS and may be related to symptoms[52,196]. Preclinically, administration of sodium butyrate increases visceral sensitivity in rats[197]. Interestingly, it has recently been demonstrated that butyrate can regulate intestinal macrophage function *via* histone deacetylase inhibition[198] which is in line with the proposed epigenetic mechanism of gut-brain axis dysfunction[199,200]. Butyrate can also mediate its immunomodulatory effects *via* G-protein coupled receptors[201] or indirectly *via* TLRs[202].

Receptors and transporters for SCFAs are expressed in the gastrointestinal tract and appear to be of relevance to gastrointestinal function[203-208]. For example, SCFAs may modulate both 5-HT secretion[18] and peptide YY release, an important neuropeptide at multiple levels of the gut-brain axis[209]. Thus, there is patently a role for these microbial metabolites beyond the regulation of energy homeostasis[210]. Interestingly, intraventricular administration of propionic acid in rats induces a variety of behavioural alterations although it is unclear if this occurs *via* similar mechanisms to the periphery[211]. It is worth noting that G protein-coupled receptor (GPR) 41, a receptor activated by propionic acid, is highly expressed in rat brain tissue[212]. Although we know that fibre metabolized by the gut microbiota can increase the concentration of circulating SCFAs[213], it remains to be established if this is reflected at physiologically relevant concentrations in the CNS.

The gut microbiota can also engage neural mechanisms to influence brain-gut axis signalling. In particular,

many of the behavioural effects of specific probiotic strains are abolished in vagotomized animals[113,115]. Germ-free studies have confirmed that the presence of intestinal bacteria is also essential for normal postnatal development of the ENS[214] and for normal gut intrinsic primary afferent neuron excitability in the mouse[215]. Thus, there is direct evidence of bacterial communication to the enteric nervous system while as indicated above, the microbiota is also a potential source of relevant ENS neurotransmitters including serotonin and GABA[216-218]. Interestingly, colorectal distension induces specific of patterns of prefrontal cortex activation in the viscerally hypersensitive maternal separation model of IBS, in which microbiota alterations are also manifested[219]. Taken together, it seems likely that the gut microbiota can modulate both the physiological information flow to the CNS *via* vagal afferents and the noxious information that is encoded by spinal afferents[10,220].

Implications and perspectives

Human microbiome science has become a focal point across multiple research domains and is now a mainstream endeavour. The benefits of the associated theoretical, practical and technological advances can be accrued to advance research in IBS. From a diagnostic perspective, it is difficult on the basis of the present clinical data to pinpoint with accuracy a microbiota-derived signature of IBS. Conceptually, the notion of the microbial community as a pathological entity is challenging for traditional biomarker approaches. Moreover, it is unclear if the current subtyping of IBS according to the dominant bowel habit aligns with specific alterations in the microbiota. In fact, research points to subtypes defined by the microbiota which are bowel-habit independent[27,50,221]. The constant stream of improvements in the technology used to qualitatively and quantitatively describe the gut microbiome make it likely that if a microbiota-based biosignature is present, it will be uncovered[49]. However, the challenges associated with analysis of these datasets should not be underestimated and it will be interesting to see if a format can be devised which would facilitate more routine and affordable screening.

The fact that the composition of the gut microbiota is malleable make it an interesting therapeutic target. Of the options available, certain probiotic strains have already shown some potential[57] while antibiotics also seem beneficial in some cases[222]. Probiotics are probably the more appealing option given their long record of safety although as for their efficacy, this does need to be evaluated on a strain-by-strain basis[223]. Prebiotics should also be considered on the basis of some studies indicating efficacy in the treatment of GI symptoms in IBS[224-226], and preclinical data indicating that prebiotic administration can modulate levels of important cognitive and behavioural related neurotrophins such as brain derived neurotrophic factor (BDNF) and glutamatergic receptor expression[227]. Diet offers an alternative mechanism to sculpt the gut microbiome[44] although it is difficult to grapple with the subtleties of using the approach to engender a switch from a "diseased" to a "healthy" microbiota. It is also worth noting the capacity of the gut microbiota to metabolise dietary components and associated health consequences, as in the case of L-carnitine which is associated with cardiovascular risk[228].

There is much current interest in the therapeutic potential of faecal microbiota transplantation[229]. This has largely stemmed from the demonstrated efficacy of donor faecal infusions in the treatment of recurrent *C. difficile*[230-232]. However, there are legitimate safety concerns regarding, for example, the provenance of the donor sample. The Food and Drug Administration (FDA) has taken a two track approach to its regulation strategy, opting not to enforce an investigational new drug (IND) requirement for use *in C. difficile* infections but adopting a stricter policy for other indications[229]. The IND requirement is an onerous and time consuming process which may impede or delay the emergence of FMT as a potential treatment option for IBS, if indeed it does prove effective. However, it is interesting to note the emergence of stool banks like OpenBiome (http://www.openbiome.org/) that provide screened, filtered, and frozen material ready for clinical use in the treatment of *C. difficile*. It is thus likely an extensive infrastructure will already be in place by the time FMT is more fully evaluated in IBS.

The contribution of the gut microbiome to drug metabolism, with potential implications for efficacy and toxicity, is also an emerging area of interest[233]. Recently, for example, it has been demonstrated that digoxin, a cardiac drug, can be inactivated by the gut Actinobacterium *Eggerthella lenta*[234]. Whether specific enzyme targets expressed by the microbiota can be selectively targeted to achieve desirable clinical outcomes is an interesting question[235] and may be of relevance to IBS. Clearly, achieving a superior mechanistic understanding of how the gut microbiota directly and indirectly affects drug metabolism could be of great benefit[236]. This is likely a bidirectional relationship with host-targeted drugs also modulating the composition and activity of the gut microbiome[237]. In this regard, it is interesting to note that the adverse impact of olanzapine (an antipsychotic) on metabolic function, possibly mediated by alterations in microbiota composition, can be attenuated by concurrent antibiotic administration in rats[238,239]. Some members of the selective serotonin reuptake inhibitors (SSRIs) may also possess antimicrobial activity[240]. This will need to be considered in the context of antidepressant agents used to treat IBS[241] or in any renewed attempts to more successfully target specific serotonergic receptors in the future[174,242]. The therapeutic potential in targeting microbial metabolites or their receptors (*e.g.* SCFAs) also warrants consideration[243].

CONCLUSION

There are biologically plausible mechanisms through

which the gut microbiome can influence both the cardinal symptoms and other prominent features of IBS. Moreover, the outcomes of a variety of experimental strategies offer convincing evidence that this is indeed the case. Although no consensus exists on the precise compositional alteration of the gut microbiota, the clinical data converges to support the concept of a less diverse and unstable community of bacteria in the disorder. While a causal role is yet to be verified clinically, it seems likely that this will be addressed once the necessary longitudinal studies are embraced. Moving forward the concept of IBS as a microbiome-gut-brain axis disorder offers a solid framework to further advance our understanding of the disorder. This approach promises much needed diagnostic and therapeutic innovations, but requires a continued concerted effort from researchers and clinicians across multiple disciplines.

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Impact of psychological stress on irritable bowel syndrome

Hong-Yan Qin, Chung-Wah Cheng, Xu-Dong Tang, Zhao-Xiang Bian

Hong-Yan Qin, Department of Pharmacy, First Hospital of Lanzhou University, Lanzhou 730000, China
Hong-Yan Qin, Chung-Wah Cheng, Zhao-Xiang Bian, Lab of Brain and Gut Research, School of Chinese Medicine, Hong Kong Baptist University, Kowloon Tong, Hong Kong
Xu-Dong Tang, China Academy of Chinese Medical Sciences, Beijing 100700, China
Author contributions: Qin HY wrote the manuscript about experimental evidence; Cheng CW contributed to the manuscript writing on clinical evidence; Tang XD contributed to the structure design of the manuscript; Bian ZX designed the aim of the editorial and finalized the manuscript.
Correspondence to: Zhao-Xiang Bian, MD, PhD, Professor, Lab of Brain and Gut Research, School of Chinese Medicine, Hong Kong Baptist University, No 7 of Baptist Road, Kowloon Tong, Hong Kong. bzxiang@hkbu.edu.hk
Telephone: +852-34112905 Fax: +852-34112929
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Abstract

Psychological stress is an important factor for the development of irritable bowel syndrome (IBS). More and more clinical and experimental evidence showed that IBS is a combination of irritable bowel and irritable brain. In the present review we discuss the potential role of psychological stress in the pathogenesis of IBS and provide comprehensive approaches in clinical treatment. Evidence from clinical and experimental studies showed that psychological stresses have marked impact on intestinal sensitivity, motility, secretion and permeability, and the underlying mechanism has a close correlation with mucosal immune activation, alterations in central nervous system, peripheral neurons and gastrointestinal microbiota. Stress-induced alterations in neuro-endocrine-immune pathways acts on the gut-brain axis and microbiota-gut-brain axis, and cause symptom flare-ups or exaggeration in IBS. IBS is a stress-sensitive disorder, therefore, the treatment of IBS should focus on managing stress and stress-induced responses. Now, non-pharmacological approaches and pharmacological strategies that target on stress-related alterations, such as antidepressants, antipsychotics, miscellaneous agents, 5-HT synthesis inhibitors, selective 5-HT reuptake inhibitors, and specific 5-HT receptor antagonists or agonists have shown a critical role in IBS management. A integrative approach for IBS management is a necessary.

Key words: Psychological stress; Irritable bowel syndrome; Microbiota-gut-brain axis; Immune activation

Core tip: Evidence from both clinical and experimental studies showed that psychological stress, acute or chronic, occurring in early life or adulthood, has marked impact on intestinal sensitivity, motility, secretion and permeability, and the underlying mechanism has a close correlation with mucosal immune activation, alteration in central nervous system, peripheral neurons and gastrointestinal microbiota. This review provides an overview about how psychological stress contributes to the development of irritable bowel syndrome (IBS) and aggravation of IBS symptoms, and informs a more comprehensive approach to the management of IBS.

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INTRODUCTION

Irritable bowel syndrome (IBS) is a chronic continuous or remittent functional gastrointestinal (GI) disorder affecting, statistically, 11.2% of the global population[1]. It is characterized by abdominal pain or discomfort associ-

ated with a change in consistency or frequency of stools but without gross abnormalities[2]. The pathophysiology of IBS is still inadequately understood, but it is most likely due to complex interactions between the immune, hormonal and nervous systems[3]. Diverse factors, including psychological stress, food intolerance or allergy, intestinal infection, injury (*e.g.*, abdominal or pelvic surgery), intestinal immune disruption and/or inflammation, changes in the intestinal microbiota or bacterial overgrowth, and genetic transmission, abuse and early life learning, have been found to contribute to the development of IBS syndrome according to the research in the last decade[4,5].

Recently, disturbance of the bidirectional brain-gut axis is increasingly recognized as a conceptual model of IBS pathophysiology, involving abnormal function in the enteric, autonomic and/or central nervous systems[6]. As stress can result in overactivity or underactivity along the hypothalamic-pituitary-adrenal (HPA) axis and of the autonomic nervous (ANS), metabolic, and immune systems, it can alter brain-gut interactions, ultimately affecting different physiological functions of the gastrointestinal tract[7]. The relationship between psychological stress and visceral hypersensitivity has been studied and well described by Musial *et al*[8] and Larauche *et al*[9], so this review will not cover that aspect of this topic. The purposes of this review are (1) to provide an overview of how psychological stress contributes to the development of IBS and aggravation of IBS symptoms; and (2) to inform a more comprehensive approach to the management of IBS.

PSYCHOLOGICAL STRESS AND STRESS-ACTIVATED PATHWAYS

Stress as a specific medical term was first defined by the endocrinologist Hans Selye in 1936[9] as the physiological adaptive responses to perceived (psychological) or real (physical) threats ("stressors") to an organism[7,9]. An acute (sudden or short-term) stressor can evoke a "fight or flight" response that prepares to defend the stability of the internal environment in order to ensure the survival of the organism. When the stress passes, a negative feedback is triggered to terminate the stress response and bring the body back to a state of homeostasis or eustasis[10]. However, if the stressor becomes chronic and/or exceeds the organism's ability to maintain the stress response, it becomes harmful because basal homeostasis cannot be reached[9]. For most humans in modern societies, psychological stress is more frequent than physical stress and it may be induced by various social and emotional triggers, some of which can be unique for an individual.

In the stress-activated pathways, the corticotrophin releasing factor (CRF) signaling system is a key element in the biochemical mechanism by which the brain translates a stimulus into an integrated physical response[10]. This system is composed of the 41 amino acid peptide, three related peptides, namely urocortin 1, urocortin 2 and urocortin 3, as well as the CRF receptors CRF1 and CRF2 and their variants[9]. When the body experiences stress, the CRF signaling system plays a primary neuroendocrine role in stimulating the HPA axis, acting as a neurotransmitter/neuromodulator to coordinate the immune and visceral efferent limbs, and activating the locus coeruleus and its noradrenergic projections. The CRF system can also modulate the forebrain, hindbrain and spinal sites for regulating the autonomic nervous system activity, leading to the stimulation of the sympathetic nervous system, release of catecholamines and induction of sacral parasympathetic activity[10]. In addition, stress affects directly or indirectly the composition and the growth of microbiota, which helps to maintain bidirectional communication between the components of the brain and the gut axis[7]. The impact of stress on the brain-gut axis has been reviewed by Grenham *et al*[11] and O'Malley *et al*[12].

Cellular effectors are also considered to play an important role in stress-induced alterations of the gut. These factors include mast cells, enterochromaffin (EC) cells, and lymphocytes, as well as the neurotransmitters, *e.g.*, proteases, 5-HT, and pro-inflammatory cytokines. It is well known that mast cells, EC cells and lymphocytes located in the lamina propria and mucosa constitute the major subpopulation of mucosal leukocytes which are involved in mucosal innate immunity against alimentary allergens and infections. Immune activation has been observed more frequently in IBS patients than in healthy controls. A wide array of mediators released by immune cells in IBS patients have been found to evoke peripheral sensitization of mucosal neuronal afferents and recruitment of "silent" nociceptors[13,14]. Moreover, at the cellular level, immune cells are known to express receptors for several different stress-related peptides including CRF, and the CRF family of peptides has potent immunomodulatory actions, suggesting that there may be crosstalk between stressors and immune factors in IBS[12].

The role of intestinal microbiota in the pathogenesis of IBS has drawn much attention in recent years. As a natural reservoir of microbiota, the GI tract plays a physiological role in metabolic, protective and structural functions in the body, while dysbiosis may contribute to several diseases, including IBS[15]. Chronic stress can induce dysbiosis and enhanced bacterial wall adherence, while the interaction between host and microbiota can modulate the neuro-immune-endocrine systems[16], suggesting that stress-induced microbiota alteration of the gut also plays a critical role in the pathogenesis of IBS. It has been reported that the abnormal GI microbiota interacts with the immune system and nervous system, which may cause the GI tract dysfunction by disturbing the brain-gut axis[17]. Now, the emerging concept of a microbiota-gut-brain axis suggests that targeting the gut microbiota may be a viable approach to treating complex disorders of the central nervous system[18].

Stress stimulates the HPA axis and then triggers the

release of CRF, ACTH, and cortisol, which directly or indirectly affect gut function, influences the composition and the growth of microbiota, and also stimulates the sympathetic nervous system. Stress alters the quantity of mast cells, EC cells, lymphocytes as well as their produced neurotransmitters, which are all involved in mucosal immune activation and further interact with gut microbiota and gut function. Stress-related changes in gut microbiota help maintain contact between the brain and gut.

PSYCHOLOGICAL STRESS IN IBS DEVELOPMENT

Evidence from clinical research

The co-morbidity of IBS and psychological distress is common, and the prevalence of at least one psychiatric disorder typically ranges from 40% to 60% and has been reported as high as 80%[19,20]. A strong correlation can also be observed between the severity of IBS and its co-morbid psychiatric disorders, especially depression and anxiety[12,19]. One review about the psychosocial determinants of IBS published in 2013[21], reports a significant increase in stressor score just before progression from IBS non-patient to IBS patient. And also major life traumas (*e.g.*, disruption of a close relationship, a marital separation, a family member leaving home, or break-up of a serious girl/boyfriend relationship) were frequently reported 38 wk prior to onset of IBS symptoms. In addition, other previous studies have demonstrated that early adverse life events (EALs) are associated with the prevalence of IBS[22,23]. EALs refer to traumatic experiences during childhood (*e.g.*, maladjusted relationships, severe illness or death of a parent, and physical, sexual or emotion abuse). In patients, the occurrence of IBS is typically associated with a higher total early life trauma score and impacted on health related quality of life (HRQOL)[22]. These studies strongly and clearly suggest that psychological or psychosocial stressors determine the development of IBS.

At the same time, there is some conflicting evidence about the relationship between stress and severity of IBS. In his review, Surdea-Blaga *et al*[21] showed that stressful life events can exacerbate abdominal pain and abdominal distension in up to one-third of IBS patients. In contrast, Blanchard *et al*[24] showed that the relation between stress and IBS symptoms was in a reciprocal, not causal, relationship after studying 254 treatment-seeking IBS patients for 4 wk. In yet another cross-sectional study of 153 consecutive patients diagnosed with different IBS subtypes (*i.e.*, constipation-predominant, diarrhea-predominant and mixed), Farzaneh *et al*[25] found no significant difference in the psychological profiles.

Evidence from animal studies

Based on the stress-related modulation in IBS patients, different experimental animal stress models have been developed to assess the vulnerability, the triggering and perpetuating factors determining stress. These include: an acute/chronic mild stress model with exposure to water avoidance stress; neonatal maternal separation stress model; restraint stress, genetic models of chronic stress, post-traumatic stress disorder model, neonatal inflammation/neonatal pain models, post-infectious IBS model and post-inflammatory IBS model. They provide a variety of approaches to explore hypotheses regarding the pathophysiological mechanisms underlying stress-related modulation of pain, visceral sensation and motility[9,10].

Experimental studies have shown that mucosal mast cells are activated after acute stress, and that they are increased or located closer to enteric nerves after chronic stress[26-28]. These mast cells release neuropeptides, *i.e.*, 5-HT, proteases and pro-inflammatory cytokines, known to be the mediators responsible for the altered intestinal sensation, motility, secretion and permeability characteristic of IBS[29]. As the majority of enteroendocrine cells, intestinal EC number and its product 5-HT content are elevated after early life stress (neonatal maternal separation)[30-32], and the increased 5-HT has been confirmed to have close correlation with the symptom generation of IBS[33,34]. In addition to mast cells and EC cells, the increased numbers of immune cells, such as T cells, with production of various cytokines are observed in the intestinal mucosa, which may be responsible for the immune activation in IBS. Dysfunction of the intestinal barrier, such as increased intestinal permeability and reduced intestinal blood flow, can be caused by different types of stress and the underlying mechanism was found to have a correlation with release of acetylcholine, glucocorticoids and corticotrophin-releasing hormone, activation of intestinal mast cells, and even splanchnic vasoconstriction driven by activation of the parasympathetic nervous system[35]. Intestinal barrier dysfunction may cause local or systemic inflammatory reactions and immune activation, which further affect the neuro-endocrine-immune pathways and lead to abnormal GI function. It is becoming well recognized that low-grade inflammation and the activated innate and adaptive immune responses play a vital role in the pathogenesis of IBS[36,37]. Now, psychological stress was found to mediate the immune activation and alter the body's responses to stress, which may facilitate the immune activation and/or exacerbate the dysregulation of stress response in IBS, and thus cause symptom flare-ups or exaggeration[12].

Besides the gut, early life stress also has an impact on the central nervous system and peripheral neurons. For example, stress up-regulates the tyrosine kinase receptor A nociceptive fibers, *c-fos* expression and CRF expression in the spinal cord and brain[38-40]; while stress down-regulates voltage-gated potassium channels and up-regulates sodium channels in colonic DRG neurons[41,42]. All of these factors contribute to the altered visceral hypersensitivity in IBS. It is found that both chronic water avoidance stress and acute restraint stress can increase colonic motility and induce sustained visceral hyperalgesia in rats, and CRF has been reported to be the key

factor responsible for stress-induced intestinal dysfunction[43,44]. In recent years, the fecal microbiota was also found altered in the rats exposed to early life stress, and gastrointestinal microbiota has been considered to play an important role in the pathogenesis of IBS[45,46].

The above experimental evidence from animals shows that stress, acute or chronic, applied in early life or adulthood, has marked impact on intestinal functions, and that the underlying mechanism has close correlation with alterations in mucosal immune cells, the central nervous system, peripheral neurons and gastrointestinal microbiota. The strong linkage of psychological stress and IBS originates from the brain-gut axis. Under normal conditions, the brain (central nervous system) communicates with the gut (enteric nervous system). The enteric nervous system (also named as "little brain") plays an essential role in the regulation of gut physiology, including secretion, motility and release of various neuropeptides and hormones[7]. Stress can induce alternations in central stress and arousal circuits (emotional motor system), result in increased CRF and noradrenergic release and activation of behavioral and autonomic responses. These may disrupt the sympathetic and parasympathetic nervous systems, HPA axis, endogenous pain modulation systems, and ascending aminergic pathway[22].

In fact, the communication between the central nervous system and enteric nervous system can be two-directional; whereas the brain can influence the function of the enteric nervous system. Therefore, stress can be the etiology for the development of IBS or aggravation of IBS symptoms (top-down model). IBS symptoms (*e.g.*, chronic continuous or remittent abdominal pain or discomfort associated with a change in consistency or frequency of stools) can elucidate or aggravate psychological disorders (*e.g.*, depression and anxiety) and lower the health related quality of life (bottom-up model). Combination of the both top-down and bottom-up models is also available for this bi-directional mechanism[47].

MANAGEMENT OF IBS

There is strong evidence that IBS is a stress-sensitive disorder. Therefore, the treatment of IBS should pay much attention to managing stress and stress-induced responses. Due to the failure of traditional pharmaceuticals, *e.g.*, laxatives and secretagogues, to give permanent relief, non-pharmacological approaches are now getting more and more attention. They include physician-patient relationship and placebo, patient education, utility of hypnotherapy, cognitive behavioral therapy, dietary modification including probiotics, exercise, and biofeedback[48]. Furthermore, a growing body of experimental data and clinical observations indicate the existence of a three-limbed microbiota-gut-brain axis. This is worthy of much further investigation for the development of any microbiota-based and microbiota-specific therapeutic strategies for IBS in the future. Moreover, pharmacological elements targeting GI symptoms and psychological symptoms should be developed further to treat the irritable bowel and irritable brain.

It is well known that 5-HT plays a critical role in stress-related alteration of gut motility, visceral sensitivity, and intestinal secretion, and also in the pathophysiology of many extra-intestinal stress-related disorders, such as anxiety, depression or chronic pain syndrome[9]. Therefore, therapeutic strategies that target 5-HT availability has been studied extensively, such as the inhibitor of 5-HT synthesis enzyme (tryptophan hydroxylase, TPH), selective 5-HT reuptake inhibitors, 5-HT-norepinephrine reuptake inhibitors, 5-HT3 receptor antagonists, and 5-HT4 receptor agonists have been investigated and some of them have been applied clinically to relieve symptoms of IBS. LX1031, an locally acting, small molecule inhibitor of TPH, has been confirmed to relieve symptoms and increase stool consistency in non-constipating IBS patients in a phase 2 study[49]. Alosetron, a representative antagonist of 5-HT3 receptors, is an effective agent in the symptom improvement of diarrhea-predominant IBS, but the serious adverse effects (*i.e.*, constipation and ischemic colitis) made it only being used under restrictive guideline. Tegaserod, a selective partial agonist of the 5-HT4 receptor, improved bowel habit but not abdominal pain in IBS patients, but the possible cardiovascular adverse effects made it being withdrawn. Nowadays, strategies targeting serotonergic systems remain active, 5-HT4 agonists, *i.e.* prucalopride, ATI-7505 and TD-5108, have been reported to have selectivity for 5-HT4 and will be advanced to human trial[50]. Ramosetron, a 5-HT3 receptor antagonist, was found effective in the treatment of visceral pain in IBS patients[51].

The pharmacological approaches for stress-related disorders also include tricyclic antidepressants, atypical antipsychotics and some miscellaneous agents[20]. Thorough development and assessment from the brain-gut aspect will provide more solid evidence about their usage in IBS patients.

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Genetic polymorphism in pathogenesis of irritable bowel syndrome

Cynthia KY Cheung, Justin CY Wu

Cynthia KY Cheung, Justin CY Wu, Institute of Digestive Disease, The Chinese University of Hong Kong, Hong Kong, China
Author contributions: Cheung CKY and Wu JCY equally contributed to this paper.
Correspondence to: Justin CY Wu, Professor, Institute of Digestive Disease, The Chinese University of Hong Kong, 9/F, Clinical Science Building, Prince of Wales Hospital, Shatin, Hong Kong, China. justinwu@cuhk.edu.hk
Telephone: +852-26323593 Fax: +852-26373852
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Abstract

Irritable bowel syndrome (IBS) is a complex symptom-based disorder without established biomarkers or putative pathophysiology. IBS is a common functional gastrointestinal disorder which is defined as recurrent abdominal pain or discomfort that has at least two of the following symptoms for 3 d per month in the past 3 mo according to ROME Ⅲ: relief by defecation, onset associated with a change in stool frequency or onset with change in appearance or form of stool. Recent discoveries revealed genetic polymorphisms in specific cytokines and neuropeptides may possibly influence the frequencies and severity of symptoms, as well as the therapeutic responses in treating IBS patients. This review gives new insights on how genetic determinations influence in clinical manifestations, treatment responses and potential biomarkers of IBS.

Key words: Irritable bowel syndrome; Genetic polymorphism; Cytokines; Serotonin; Psychiatric distress; Endocannabinoids

Core tip: Irritable bowel syndrome (IBS) is a complex symptom-based disorder without established biomarkers or putative pathophysiology. This review gives new insights on how genetic determinations influence in clinical manifestations, treatment responses and potential biomarkers of IBS. Although a number of IBS- related genes have been identified, the majority of the identified genes required further validation as each of them may only contribute to the pathophysiology in 1%-5% in patients with functional gastrointestinal disorders.

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INTRODUCTION

Irritable bowel syndrome (IBS) is defined according to ROME Ⅲ criteria as recurrent abdominal pain or discomfort for at least 3 d per month during the previous months with two or more of the following characteristics: relief by defecation, onset associated with a change in the frequency of stools, onset associated with change in form or appearance of stools[1,2]. IBS is often subcategorized according to the predominant stool pattern reported by the patients. These subcategories include constipation-predominant IBS (IBS-C), diarrhea-predominant IBS (IBS-D), and so-called mixed stool pattern IBS (IBS-M) which involves both constipation and diarrhea.

The prevalence of IBS ranges from 4.7% to 19.1% in western countries, however the prevalence in eastern countries ranges from 3.7% to 15.7% according to ROME Ⅱ criteria[3]. Although the pathophysiology of IBS still remains unknown, there is growing evidence that genetic contributions, inflammatory activation, psychosocial factors may play important roles to the development of IBS.

Recent studies in genetic polymorphisms reported that cytokines and neuropeptides may be involved in etiology and clinical manifestations of IBS. This review summarizes the recent discoveries in association of genetic polymorphisms and their impacts on the symptom development and severity, pathogenesis as well as treatment responses to IBS.

GENE POLYMORPHISMS

As IBS is a complex symptom based disorder without single identified pathophysiology or biomarker, multiple mechanisms such as motility dysregulation, visceral hypersensitivity, immune activation, psychosocial factors and altered brain-gut axis has been proposed. Although a number of IBS- related genes have been identified, the majority of the identified genes required further validation. Besides, each of them may only contribute to the pathophysiology in 1%-5% in patients with functional gastrointestinal disorders[4]. This review will summarize how genetic determinations may possibly regulate the putative mechanisms mentioned.

Serotonergic system

Serotonin (5-HT) is one of the most abundant neurotransmitter molecules in the gastrointestinal tract. It is stored in the secretory granules of enterochromaffin (EC) cells in the enteric nervous system, and its release is believed to be responsible for eliciting appetite regulation[5], gut motility[6] and visceral sensitivity[7]. Abnormal levels and activities of 5-HT had been reported in functional gastrointestinal (GI) disorders such as functional dyspepsia (FD)[8] and IBS. Increased plasma 5-HT levels were found in female IBS-D patients[9-11] while decreased postprandial plasma serotonin levels have been reported in IBS-C patients[10]. Excessive 5-HT release in the bowel may lead to diarrhea, nausea and vomiting. Studies reported that single nucleotide polymorphisms (SNPs) in serotonin modulators showed significant associations with IBS. First, tryptophan hydroxylase (TPH) is the rate limiting enzyme in the biosynthesis of serotonin[12]. The two isoforms TPH1 and TPH2 showed associations to clinical manifestations in patients with IBS. TPH1 is located on chromosome 11p15.3-p14 and composed of 11 exons[13] and mainly expressed in gut and peripheral organs. TPH2 is located at chromosome 12q21.1 and composed of 11 exons[14] and mainly expression in CNS and peripheral neurons[15]. Homozygous for minor allele (GG) of rs4537731 in promoter region of TPH1 reported more severe diarrhea, bloating, and a trend of more watery stool compared to two genotype groups(AA and AG genotypes) in IBS patients[16]. Genotypes reported with a minor allele (GT and GG genotypes) of rs211105 in intron 3 of TPH1 also reported more severe diarrhea symptoms and trend of more watery stool. Homozygous for the minor allele (T) of TPH2 rs4570625 reported more days with both very hard and watery stools compared to other genotype groups (GG and GT genotypes) in the promoter region of TPH2[16].

Serotonin reuptake transporter (SERT) is a protein that removes serotonin from the sites of action and recycles serotonin back into presynaptic neurons. SERT is located on the chromosome 17q11.2-q1. Wang *et al*[17] showed that the homozygous genotype (L/L) in the promoter region of SERT (L variant bp-1440 to +22) can increase the mRNA and protein level expression of SERT promoter activity in the colonic mucosa. Yeo *et al*[18] found that a strong genotypic association was established between SERT promoter deletion/deletion genotype and female IBS-D patients. Fukudo *et al*[19] further reported SERT linked promoter region polymorphism with long (L, 528bp) and short (S, 484bp) forms showed different levels of brain activation after colorectal distention. This functional gene polymorphism may partially predict the individual effect of long-lasting neural processing from visceral organs. Camilleri *et al*[20] also reported that genetic polymorphisms at the SERT promoter influence response to a 5-HT3 antagonist in D-IBS patients. Kohen *et al*[21] reported that the carriers of rare G allele in polymorphism rs25531 of SERT linked promoter region showed threefold increase in odds ratio of IBS compared to healthy controls.

The 5-HT transporter gene linked polymorphic regions (5-HTTLPR), which is a 43bp insertion/ deletion polymorphism in the 5' flanking promoter region. It is 1.2 kb upstream of the transcription start site. Jarrett *et al*[22] showed the functional polymorphism(insertion or deletion of 44bp) in the 5-HTTLPR that was associated with depression and anxiety traits[23]. Furthermore, the 5-HTTLPR short allele has been found associated with increased visceral sensitivity in IBS[24]. Moreover, the L/L genotype was significantly associated with IBS, IBS-C and IBS-M patients in Korean population[25]. These may suggest that 5-HTTLPR might play a key role in IBS by modulation of SERT at transcriptional level.

Serotonin modulates visceral sensitivity by its action on 5-HT3 receptors. 5-HTR3 receptor A subunit (5-HTR3A), playing a key role in receptor formation, has been associated with depression and anxiety related trait. A functional polymorphism in 5-HTR3A subunit C-42C>T(rs1062613) was associated with more severe dyspeptic symptoms[26], increased anxiety, amygdala responsiveness and severity of IBS[27].

The 5-HT2A receptor subunit A (5-HTR2A) was believed to play a significant role in the genesis of various neuropsychiatric diseases. 5-HTR2A was reported to be responsible in regulating the perception of abdominal pain and smooth muscle contraction in gastrointestinal tract[28,29]. Markoutsaki *et al*[30] reported that the carriers of A allele of the -1438(G/A) polymorphism[31] and homozygote C allele of the 102 T/C polymorphisms in 5-HTR2A had higher risks of IBS[31]. Pata *et al*[31] showed that T/T genotype of 102 T/C polymorphism in 5-HTR2A may be associated with more severe pain in patient with IBS.

Cholecystokinin

Cholecystokinin (CCK) is released by endocrine I cells within the duodenal and jejunal mucosa for stimulating protein and fat digestion. It also served as a hunger suppressant[32]. Elevated plasma CCK level was reported to be associated with patients with post-infectious IBS. Plasma CCK level was correlated with postprandial dyspeptic

symptoms in these patients[33]. Study by Park *et al*[34] showed that polymorphism in CCK receptor intron 1 (779 T>C) was associated with constipation predominant IBS(IBS-C) and mixed IBS(IBS-M) in Korean population.

Catechol-O-methyltransferase

Catechol-*O*-methyltransferase (COMT) is involved in the inactivation of the catecholamine neurotransmitters. Altered COMT activities by different polymorphisms were related to chronic pain conditions such fibromyalgia[35] whereas the COMT Val158Met polymorphism had been associated to panic disorder[36] as well as IBS (Val/Val carriers showed a trend of smaller proportion of hard stools and higher occurrence of postprandial defecation)[37].

Voltage-gated sodium channel

Voltage-gated sodium channel (Na_v) was present in gastrointestinal smooth muscles. These missense mutations were found in tetrodotoxin-resistant sodium channel (SCN5A) in 13 out of 584 patients with irritable bowel syndrome. It was more prevalent in Diarrhea -predominant IBS patients. And these mutations showed disruption in Na_v 1.5 function with decreased peak currents and mechanosensitivity[4,38].

Guanine nucleotide binding protein beta polypeptide 3

Guanine nucleotide binding protein (G-protein) beta polypeptide 3 (GNB3) encodes the beta3 subunit of heterotrimeric G-protein. G-protein is responsible for various functions such as ion channel, motility and contraction. Lee *et al*[39] reported that a polymorphism in C825T has been associated with IBS-C patients in South Korea. Although no association was found between C825T with the overlapping of IBS and FD patients by Kim *et al*[40], an association was reported between dyspeptic symptoms and homozygous 825T allele of GNB3 protein in the H Pylori-negative Japanese population[41]. Oshima *et al*[42] also revealed epigastric pain syndrome(EPS) was correlated with homozygous 825T allele in GNB3 protein of patients with FD. Moreover, Saito *et al*[43] showed that an interaction was found between GNB3 825T allele and gastrointestinal infection of IBS in western population.

Endocannabinoid system

Endocannabinoids serve as synaptic circuit breakers and regulate multiple physiological and pathological conditions including nociception (pain sensation), appetite, lipid metabolism, gastrointestinal motility, cardiovascular modulation, motor activity, mood, and memory. Cannabinoids suppress behavioral responses to noxious stimulation and nociceptive processing through activation of cannabinoid CB receptor 1 (CNR1) and CB receptor 2 (CNR2) subtypes[44]. Wong *et al*[45] reported polymorphism of rs806378 (CT/TT genotype) in CB(1) receptor was associated with IBS patients having a modest delay in colonic transit. Camilleri *et al*[46] also showed that TT group had the fastest colonic transit at 24 and 48 h. Besides, there was a significant association of CNR1 in rs806378 with sensation rating of gas, but not pain sensation in various IBS subtypes. Park *et al*[47] and colleagues found a different distribution of allelic frequency of AAT repeats in the *CNR1* gene between healthy controls and IBS patients. They also reported a significant association of CNR1 >10/>10 genotype with IBS.

Psychiatric distress

Research has implicated that a combination of genetic and environmental risk factors (*e.g.*, Early life adversity, traumatic experiences) in the pathogenesis of mood disorders such as depression[48]. While strong association was established between psychological distress and functional gastrointestinal diseases[49], an established biopsychosocial model was suggested where early life stress may predispose HPA axis dysfunction and develop functional gastrointestinal symptoms[50]. The prevalence of depression and anxiety disorder was 37.1% and 31.4% respectively in Indian population with IBS[51]. Lee *et al*[52] also reported that IBS is also strongly associated with generalized anxiety disorder in Chinese population. Chronic widespread pain related to fibromyalgia and chronic fatigue is associated with IBS and major depressive disorder. Sato *et al*[53] showed that TT genotype of rs7209436 and rs242924 in Corticotrophin-releasing hormone was significantly more common in patients with IBS than in healthy controls. Corticotrophin-releasing hormone carries a potential risk for depression. These polymorphisms were also associated with bowel pattern in these IBS patients. Besides, polymorphisms in 5-HTTLPR, intron 2 (STin2 VTNR) of SERT were also correlated with depressive episodes and IBS[22].

Neuropeptide S (NPS) is a 20 amino acids peptide that selectively binds and activates an orphan G-protein coupled receptor, Neuropeptide S receptor 1 (NPSR1). It is expressed on the intestinal epithelium. This neuropeptide S system is involved in stress responses, anxiety, and nociception through selectively inhibiting the evoked release of serotonin and norepinephrine the frontal cortex, by acting directly on serotonin and norepinephrine nerve terminals[54]. NPSR1 polymorphisms were reported to be associated with colonic transit rate (rs2609234, rs6972158 and rs1379928) and visceral pain(rs1379928)[55].

Cytokines

It has become increasingly clear that low-grade inflammation is implicated in the pathophysiology of IBS with subtle changes in pro-inflammatory or anti-inflammatory cytokines in blood or GI mucosa[56,57]. Studies reported significant associations between functional polymorphisms in these genes among IBS patients. Tumour necrosis factor alpha (TNFα) is a cytokine which involved in stimulating systemic inflammation and it is implicated in various diseases such as cancer, depression and inflammatory bowel disease. Although polymorphism in TNFα(-308 G/A) showed no difference in frequencies between Indian IBS patients and healthy volunteers[58], Barkhordari *et al*[59] showed that polymorphism of TNFα at position -308 and -238 were also

significantly higher in IBS patients. TNFSF15 is a member of the TNF (ligand) superfamily which codes for TL1A. It is expressed primarily in macrophage, T cells, and immune cells that are exposed to pro-inflammatory stimuli or microbes. TNFSF15 involves in the defense against pathogens and homeostatic interactions with commensal bacteria in the gut. Study by Zucchelli *et al*[60] showed the Crohn's disease risk allele rs4263839 G in TNFSF15 gene was significantly associated with increased risk of IBS and particularly in IBS-C patients. TNFRSF17 is expressed in mature B lymphocytes and development of B cells. Besides its involvement in inflammatory bowel disease (IBD), SNPs at position -1729G, -2445 and -2493 showed significantly distinct frequencies in patients with lower functional gastrointestinal disorders compared to healthy controls[61].

Interleukins are important modulators in inflammatory responses, they play a vital role in intestinal inflammation. Pro-inflammatory cytokines such as interleukin (IL)-6 and IL-8 has been up-regulated in IBS patients[56]. Although Camilleri *et al*[62] showed no difference in gene polymorphisms of IL-6 was found between subtypes of IBS patients and healthy individuals in American population, IL-6 G allele at position -174 showed higher frequencies in Iranian IBS patients[59]. Moreover, there was significant difference in frequencies shown in IL-8 G allele at position +396 and C allele +781 between IBS patients and healthy controls. Besides, the combinations of IL-8 ATCC haplotypes (at positions -251, +396, +781 and +1633) reported significant association with susceptibility to development of IBS[63]. In Mexican population, IL-8 T allele at position +781 was significantly overexpressed in patients with IBS and IL-8 G allele at position +396 was also associated with IBS[64].

Anti-inflammatory cytokines such as IL-10 and transforming growth factor (TGF) β1 also play an important role in the regulation of immune and inflammatory responses. Although no significant difference was found in colonic expression of TGFβ1 in IBS patients[65], Romero-Valdovinos *et al*[63] showed that IL-10 A allele at position 1082 were significantly increased in IBS Mexican patients. IL-10 can inhibit pro-inflammatory cytokines such as tumour necrosis factor beta (TNF-β). IL-10 ACC haplotypes (at positions -1082, -819 and -592) were also associated with development of IBS. IL-10 C allele at position -592 also showed association with higher risk in developing IBS in Mexican population[64].

CONCLUSION

IBS is a complex functional disorder that involves multiple interactions of genetic inheritance, environmental and psychosocial factors. This review summarized the recent discoveries on how genetics may influence on the symptoms severity and subtypes of IBS through modulation of gastrointestinal functions such as gut motility, immune activation and visceral sensation. Further studies are necessary to understand the mechanisms on how genetics may determine the clinical manifestations and therapeutic responses to subset of IBS patients. Besides, biomarker discovery for this complex heterogeneous disorder remains a big challenge. Future studies should be required to search for candidate genes with combinations of gene expression profiling for target treatment and diagnosis for specific subsets of IBS patients.

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