Intestinal barrier dysfunction in irritable bowel syndrome: a systematic review

Nikita Hanning, Adam L. Edwinson, Hannah Ceuleers, Stephanie A. Peters, Joris G. De Man, Leslie C. Hassett, Benedicte Y. De Winter and Madhusudan Grover

Ther Adv Gastroenterol

2021, Vol. 14: 1–31

DOI: 10.1177/1756284821993586

Abstract

Background and Aim: Irritable bowel syndrome (IBS) is a complex and heterogeneous disorder. Sensory, motor and barrier dysfunctions are the key physiological endophenotypes of IBS. Our aim is to review studies evaluating barrier dysfunction in adults and children with IBS, as well as to link those changes with IBS symptomatology and quality of life.

Methods: A comprehensive and systematic review of multiple databases was performed up to March 2020 to identify studies comparing intestinal permeability in IBS patients with healthy controls. Both *in vivo* and *in vitro* studies were considered.

Results: We identified 66 studies, of which 27 used intestinal probes to quantify barrier function. The prevalence of barrier dysfunction differed between PI-IBS (17–50%), IBS-D (37–62%) and IBS-C (4–25%). At a group level, permeability was increased compared with healthy controls in IBS-D (9/13 studies) and PI-IBS (4/4 studies), but only a minority of IBS-C (2/7 studies) and not in the only IBS-M study. All four studies in children with IBS demonstrated loss of barrier function. A heterogeneous set of tight junction genes were found to be altered in small and large intestines of adults with IBS, but these have not been evaluated in children. Positive associations were identified between barrier dysfunction and bowel disturbances (6/9 studies), abdominal pain (9/13 studies), overall symptom severity (1/6 studies), depression and anxiety (1/1 study) and quality of life (1/4 studies). Fecal slurry or supernatants of IBS patients were found to induce barrier disruption in animal models (5/6 studies).

Conclusions: Barrier dysfunction is present in a significant proportion of adult and all pediatric IBS studies, especially in the IBS-D and PI-IBS subtype. The majority of studies indicated a positive association between loss of barrier function and symptoms such as abdominal pain and changes in the bowel function.

Keywords: functional gastrointestinal disorders, immune cells, microbiome, occludin, zonula occludens

Received: 30 June 2020; revised manuscript accepted: 19 January 2021.

Correspondence to:
Madhusudan Grover
Department of Medicine and Physiology, Enteric NeuroScience Program, 200 First St SW, Rochester, MN 55905, USA
grover.madhusudan@mayo.edu

Benedicte Y. De Winter
Division of Gastroenterology, Laboratory of Experimental Medicine and Pediatrics, Universiteitsplein 1, Antwerp, 2610, Belgium;
Department of Gastroenterology and Hepatology, Antwerp University Hospital (UZA), Antwerp, Belgium
benedicte.dewinter@uantwerpen.be

Nikita Hanning
Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, Minnesota, USA;
Laboratory of Experimental Medicine and Pediatrics (LEMP) and Infla-Med, research consortium of excellence, University of Antwerp, Antwerp, Belgium

Adam L. Edwinson
Stephanie A. Peters
Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, Minnesota, USA

Hannah Ceuleers
Joris G. De Man
Laboratory of Experimental Medicine and Pediatrics (LEMP) and Infla-Med, research consortium of excellence, University of Antwerp, Antwerp, Belgium

Leslie C. Hassett
Mayo Clinic Libraries, Mayo Clinic, Rochester, Minnesota, USA

Introduction

Irritable bowel syndrome (IBS) is a chronic bowel disorder characterized by recurrent abdominal pain related to defecation and changes in bowel habits.[1] Clinically, IBS patients are characterized by their predominant aberrant bowel pattern as diarrhea-predominant (IBS-D), constipation-predominant (IBS-C) or mixed (IBS-M).[1] Increasing evidence points toward the presence of pathophysiological disturbances in subsets of IBS.[1,2] These include alterations in visceral sensitivity, gastrointestinal (GI) motility, intestinal permeability, the microbiome and the immune function.[1–3] Furthermore, several risk factors for the development of IBS have been identified, among which infectious gastroenteritis appears to

the most predominant.[4,5] However, the development of new therapeutics is hampered by heterogeneous presentation and difficulties in phenotypic characterization.[3]

With a surface area of up to 40 m^2, the digestive tract presents a large interface from which to interact with the external environment while serving many critical homeostatic functions.[6] The intestinal barrier protects the internal environment from a continuous exposure to pathogens and antigens, while at the same time being responsible for the uptake of nutrients and water.[7] To fulfill these conflicting functions, the gut has evolved into a complex system of multiple defensive layers, consisting of physical, biochemical and immune components.[8,9] First, intrinsic secretions of the GI tract as well as products of commensal microbes prevent the colonization of pathogens.[8,10–13] Second, the adherent mucus layer, a network consisting of mucin polymers produced by the goblet cells, coats the intestinal epithelium, providing a barrier between the host and the microbiome, while also entrapping pathogens.[14] Third and perhaps most importantly, the epithelial barrier itself, consisting of a single layer of epithelial cells interconnected by tight junctions, adherens junctions and desmosomes, provides the strongest physical defense against submucosal access of noxious luminal substances.[15,16] Fourth, the immune cells in the mucosa and in the lamina propria (e.g. dendritic cells, mast cells or macrophages) mount protective responses through the production of immunoglobulins, cytokines and many other critical immunomodulators.[17] In addition to physical and chemical components of the barrier, the propulsive motility of the gut also plays an important role in defending the internal environment.[18]

The intestinal epithelia also have important absorptive and secretory roles, necessitating the ability of ions, molecules and solutes to cross the intestinal epithelium. This can be accomplished *via* the transcellular or paracellular pathways.[19,20] Three distinct paracellular pathways have been proposed.[21] First is the pore pathway, a high-capacity size- and charge-selective pathway regulated by the members of the claudin (CLDN) family.[21,22] Second is the leak pathway, a non-selective low-capacity pathway predominantly regulated by zonula occludens-1 (ZO-1), occludin and myosin light chain kinase (MLCK).[23,24] Finally, the unrestricted pathway opens due to loss of tight junction complexes typically as a result of cell death, apoptosis or mucosal damage. This route can allow passage of large macromolecules and even microbes across the epithelium.[25] Barrier dysfunction has been linked to visceral hypersensitivity and pain in IBS, presumably due to exposure of submucosal neuronal and immune apparatus to the luminal microbes, antigens and other mediators. A recent animal and a human study demonstrated that inhibition or restoration of barrier dysfunction can correct visceral hypersensitivity[26] and pain[27] in IBS, respectively. However, direct evidence for an impaired barrier to causally result in visceral hypersensitivity is lacking. Moreover, the significance of small bowel *versus* colonic barrier dysfunction is poorly explored in IBS. It is possible that postprandial symptoms may be mediated by an impaired barrier in the proximal small bowel, whereas symptoms of lower abdominal pain and urgency are driven by colonic involvement.[28] Lastly, different measurements of barrier structure and function are often interpreted without appropriate context. Whereas *in vivo* studies such as those using saccharide administration reflect the end result of integrated host physiology including barrier function, studies with biopsies in Ussing chambers devoid of the neuro-vascular input and studies with luminal mediators or structural studies using electron microscopy provide significantly different pieces of information.

Previous narrative reviews on barrier dysfunction in IBS[29,30] provide few details on population characteristics, comorbidities such as psychological distress, and methodological details (*in vivo*, *ex vivo* and *in vitro*). Furthermore, associations of barrier dysfunction with IBS symptomatology and evidence for barrier dysfunction in children with IBS have not been summarized. We therefore performed a systematic review of studies investigating disturbances in intestinal permeability (*in vivo*) in IBS patients, evaluating the presence of *ex vivo* and *in vitro* barrier dysfunction in both children and adults and potential associations of barrier changes with IBS symptomatology and quality of life (QoL) measures.

Methods

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were followed while conducting and writing this systematic review.[31]

Selection criteria

We included peer-reviewed studies reporting on IBS defined by Rome criteria (I, II, III or IV) or by physician diagnosis. Studies that did not describe how the IBS diagnosis was determined were excluded. Studies across all age groups assessing *in vivo*, *ex vivo* and/or *in vitro* measurements of barrier function were included. Only studies comparing IBS patients with either a healthy control group or those using a predefined cut-off value of normality were included. Studies focusing on animal models were excluded unless human samples were used to modulate barrier function. Narrative reviews, guidelines, editorials, conference summaries, conference abstracts, case reports, study protocols and non-English studies were also excluded.

Data sources and search strategy

After an initial search by the authors, an experienced librarian (LCH) performed an extensive search to retrieve additional articles (last search conducted on 18 March 2020). The databases included MEDLINE and Epub Ahead of Print, In-Process & Other Non-Indexed Citations and Daily, Embase, Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic Reviews and Scopus. Combinations of subject headings and keywords were used to search for the primary concepts. Selected terms include: "irritable bowel," "irritable colon," "permeability," "tight junction," "adherens junction," "desmosomes," "claudin," "occludin," and "zona occludens." For the full search strategy, please refer to the Supplementary Materials. Identified records were imported into Endnote X9 software and combined to remove duplicates. Based upon title and abstract, one investigator (NH) excluded studies that did not focus on the research questions of interest. Subsequently, two investigators (NH and AE) independently reviewed the remaining full-text articles in more detail to assess whether they contained relevant information and met the inclusion criteria. Any disagreements in study selection were resolved by discussion and consensus with the senior investigators (BDW and MG). Finally, reference lists of all included studies were hand-searched to identify additional studies.

Data collection

One investigator (NH) extracted data using a standardized form in Microsoft Excel. The first author, the year of publication, the number of patients in the IBS and control groups and the diagnostic criteria used to identify IBS patients was abstracted. In addition, we extracted clinical characteristics of the studied populations including the IBS subtypes according to predominant stool pattern, age, gender, body mass index (BMI), psychological distress, symptom severity and QoL. For *in vivo* permeability studies, the details on methodology were abstracted (probes used, sample collection time, dietary restrictions, etc.). For *in vitro* permeability studies, the site of collected specimen and the experimental technique(s) were summarized. For interventional studies, only baseline parameters were extracted.

Assessment of quality and risk of bias

The risk of bias and the overall quality of all selected studies were assessed using the AXIS Tool, consisting of 20 items.[32] Two authors (NH and HC) independently reviewed all included studies. The inter-rater agreement between the two reviewers was 92%. Disagreements were resolved by one of the senior investigators (BDW), who scored all discrepant items.

Results

The search strategy resulted in the identification of 3350 unique records. After screening abstract and full text, a total of 66 unique articles met the inclusion criteria. Of these, one study was identified after screening reference lists. A flow chart summarizing the study screening selection is shown in Figure 1. Results of the Quality Assessment are shown in Supplementary Table 1. The median quality score for the included studies was 15 (range 10–19; <14 in nine studies, 14–16 in 48 studies and >16 in 10 studies). Owing to the large heterogeneity in the protocols followed to quantify intestinal permeability, a meta-analysis was not deemed feasible. Thus, the included studies are reviewed in a systematic way.

In vivo *measurements of intestinal permeability in adults*

Participant characteristics. Twenty-seven studies evaluated intestinal barrier function in adult IBS patients after the administration of permeability probes.[33–59] Ten studies were conducted in the United States, five in Italy, four in the United Kingdom, three in The Netherlands, two in

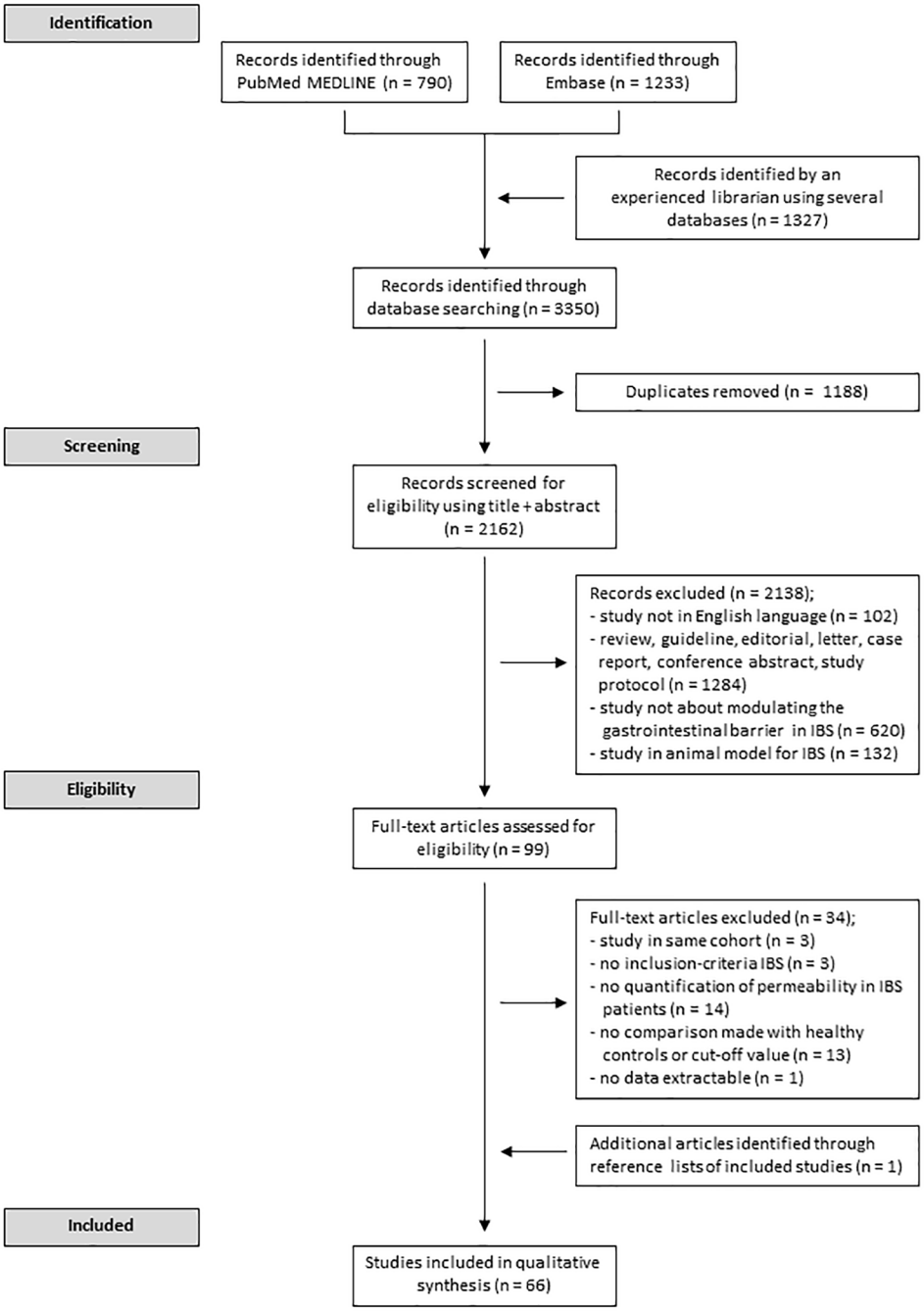

Figure 1. Study schematic. Flowchart describing process for screening and selection of studies included in the systematic review.

China, one in Canada, one in Hungary and one in South Korea. IBS was diagnosed according to the Rome criteria in 25 studies (two Rome I, six Rome II and 17 Rome III). In two studies, IBS diagnosis was based on a clinician evaluation. Of the 25 studies employing the Rome criteria, 11 included >1 subtype, nine only IBS-D, two only IBS-C and three did not report a subtype. Four studies specified including patients with post-infection IBS (PI-IBS). The proportion of women ranged between 34–100% (<60% females in seven studies; 60–80% in 10 studies; >80% in 10 studies). Twelve studies reported the BMI of their populations, which ranged between 22 and

34 kg m^{-2} (normal (<25 kg m^{-2}) in six studies; overweight (25–30 kg m^{-2}) in three studies; and obese (>30.0 kg m^{-2}) in three studies.

Methodological differences. Twenty-five studies quantified permeability by measuring the renal excretion of orally ingested and gastrointestinally absorbed probes and two quantified the probe in serum. Characteristics of the included studies are reported in Table 1, and additional demographic characteristics (country/region, gender, age, BMI, anxiety and depression scores, symptom and QoL scores) are in Supplementary Table 2. A number of probes, including mono- and disaccharides, ^{51}Chromium ethylene diamine tetra acetic acid (^{51}Cr-EDTA) and polyethylene glycol (PEG) polymers were used. An ideal probe molecule should not be degraded or metabolized in the human body or urine and cause no toxic effects. Furthermore, the molecule should not be present naturally (e.g. ingested *via* food) and fully and rapidly excreted *via* the urine. Finally, its measurement should be sensitive and accurate.[41,60,61] A large proportion of studies (22) combine the administration of a monosaccharide, such as mannitol, with the administration of a disaccharide, such as lactulose. With a diameter of 8 Å, mannitol is a smaller molecule than lactulose (13 Å). Mannitol can traverse the pore pathway as well as the larger leak and unrestricted pathways. Due to its larger size, lactulose can only move across the intestinal barrier *via* the leak pathway or through the unrestricted pathway.[62] Therefore, an increase in the lactulose-to-mannitol ratio (LMR) reflects a disruption of the epithelial barrier, normalized against the total paracellular transport. This becomes critical when comparing conditions such as celiac disease where there is a loss of absorptive surface area (and hence decreased mannitol excretion) in addition to increased leak through paracellular pathways (and, hence, increased lactulose excretion). Since sucrose is absorbed rapidly, it is thought to be a marker of gastric or gastroduodenal permeability.[50] Three studies investigated its absorption in IBS patients.[47,50,57] In contrast with the other sugars, the artificial disaccharide sucralose is not broken down by colonic bacteria, making it a more suitable marker for colonic permeability. Four studies reported the use of sucralose to reflect colonic barrier function.[38,47,50,53] Other less commonly used saccharides were raffinose, L-arabinose, and L-rhamnose.

Urine collection times varied between 2 h and 24 h post administration of the probes (15/25 collected for up to 24 h). In early studies, a urine collection period of 2 or 3 h was considered to represent gastroduodenal permeability, 5–6 h as a marker for the small intestinal permeability and ⩾8 h for colonic permeability. However, Rao *et al.*[45] observed that in healthy volunteers, 62% of the ingested liquid solution has already reached the colon at 2 h. In addition, alterations in intestinal transit like that seen in IBS-D and IBS-C will also affect the interpretation of the involved region in the GI tract. We believe that probe recovery after the first 2 h likely represents distal small bowel and colonic permeability, certainly in IBS-D patients. The migration and absorption of the probes throughout the GI lumen could be influenced by the intake of food or drinks.[64] In the majority of studies, probe solution was ingested after an overnight fast. In two studies, however, urine was collected overnight after drinking the probe solution in the evening. Participants were not allowed to consume solid foods after the ingestion of the probe solution in 13 studies, whereas in 14 studies, no dietary restrictions were reported. Standardized meals were provided in only three studies. The regulations for the intake of water were also quite variable among the studies (restricted for 2–3 h, *ad libitum* or no limitations). In three studies, the intake of probes accompanied a caloric drink.

Permeability measurements. Fourteen studies used a "normal" cut-off value to determine the percentage of IBS patients with an increased intestinal permeability.[33–36,38,40–42,44,51–53,57,58] These were either based on earlier experiments or newly recruited healthy controls. Cut-off values for the LMR ranged between 0.015 and 0.07.[40,42,51,52] The prevalence of increased permeability in IBS was highly variable, with 2–62% of the IBS subjects showing increased intestinal permeability *versus* 0–15% in controls.[33–36,38,40–42,44,51–53,57,58] An overview is shown in Figure 2. When assessing cumulative differences compared with controls, 14 studies using varying urine collection time points concluded increased intestinal permeability in IBS patients,[35–39,43–45,48,50,53,58,59,63] while no differences were detected in eight studies.[33,41,49,52,54–57] Remarkably, one study found a decreased colonic permeability in IBS patients.[47]

Assessing by IBS subtype, nine studies reported an increased permeability in IBS-D compared

Table 1. Studies comparing *in vivo* permeability in adult IBS patients with healthy controls or without controls.

Reference	Study population	Permeability probe	Dietary restrictions	Timing of urine or blood collection	Cut-off value of normality	Results	
						Comparison with healthy volunteers	Proportion of ↑ permeability
Studies with a control population							
Studies collecting urine over 0–8 h, representing proximal GI permeability							
Russo *et al.*[55]	IBS-D ($n=28$) HV ($n=19$)	10 g lactulose 5 g mannitol 100 mL of H_2O	Overnight fast: YES	0–5 h	NA	**Lactulose**: IBS-D 0.3% *versus* HV 0.4% (**$p=$ NS**) **Mannitol**: IBS-D 11.0% *versus* HV 12.2% (**$p=$ NS**) **LMR**: IBS-D 0.02 *versus* HV 0.03 (**$p=$ NS**)	NA
Lobley *et al.*[33]	IBS ($n=62$) HV ($n=40$)	2 g L-arabinose 20 g lactose 8 g raffinose 250 mL of H_2O	Overnight fast: YES Water *ad libitum* after 2 h, food not allowed	0–5 h	Ra/Ara >0.06	**L-arabinose**: IBS 15.1% *versus* HV 17.5% (**$p<0.01$**) **Raffinose**: IBS 0.2% *versus* HV 0.3% (**$p=$ NS**) **Ra/Ara**: IBS *versus* HV (**$p=$ NS**)	IBS 2% *versus* HV 0%
Mattioli *et al.*[44]	IBS-C ($n=32$) HV ($n=23$)	5 g lactulose 2 g D-mannitol 100 mL of H_2O	Overnight fast: YES Intake of 500 mL of H_2O after 30 min, fasting for the first 2 h of the collection period	0–5 h	LMR >0.052	**Lactulose**: IBS-C 0.6% *versus* HV 0.5% (**$p=$ NS**) **Mannitol**: IBS-C 17.1% *versus* HV 19.8% (**$p=$ NS**) **LMR**: IBS-C 0.04 *versus* HV 0.03 (**$p<0.05$**)	IBS-C 25% *versus* HV 9–13%
Del Valle-Pinero *et al.*[47]	IBS ($n=20$) HV ($n=39$)	10 g sucrose 5 g lactulose 1 g mannitol 0.1 g sucralose 100 mL of H_2O	Overnight fast: YES Water *ad libitum*, food not allowed	0–5 h	NA	**Sucrose**: IBS 0.03% *versus* HV 0.04% (**$p=0.118$**) **LMR**: IBS 0.01 *versus* HV 0.01 (**$p=0.45$**) **Sucralose**: IBS 5.4% *versus* HV 9.1% (**$p=0.011$**)	NA
Linsalata *et al.*[57]	IBS-D ($n=39$) HV ($n=20$)	40 g sucrose 10 g lactulose 5 g mannitol 100 mL of H_2O	Overnight fast: YES	0–5 h	LMR ⩾0.035	**Sucrose, lactulose, mannitol, LMR**: IBS-D *versus* HV (**$p=$ NS**)	IBS-D 46% *versus* HV 0%
Russo *et al.*[58]	IBS-D ($n=34$) HV ($n=17$)	40 g sucrose 10 g lactulose 5 g mannitol 100 mL of H_2O	Overnight fast: YES	0–5 h	LMR ⩾0.035	**Lactulose**: IBS-D 0.4% *versus* HV 0.2% (**$p=0.1212$**) **Mannitol**: IBS-D 11.0% *versus* HV 13.0% (**$p=0.0386$**) **LMR**:IBS-D 0.04 *versus* HV 0.02 (**$p=0.0091$**)	IBS-D 44%
Spiller *et al.*[35]	PI-IBS ($n=10$) HV ($n=10$)	5 g lactulose 2 g mannitol 100 mL of H_2O	Overnight fast: YES Test meal of 100 mL of Fortisip (200 kcal) before intake probes	0–6 h	LMR >0.03	**LMR**: PI-IBS 0.06 *versus* HV 0.009 (**$p=0.005$**)	PI-IBS 50% *versus* HV 0%

(Continued)

Table 1. (Continued)

Reference	Study population	Permeability probe	Dietary restrictions	Timing of urine or blood collection	Cut-off value of normality	Results	
						Comparison with healthy volunteers	Proportion of ↑ permeability
Kerckhoffs *et al.*[41]	IBS-A ($n=3$) IBS-C ($n=3$) IBS-D ($n=8$) HV ($n=15$)	40 g sucrose 5 g lactulose 2 g mannitol 100 mL of H_2O	Overnight fast: YES Water *ad libitum*, food not allowed	0–6 h	LMR >0.03	**LMR**: IBS 0.01 *versus* HV 0.01 (**$p=$ NS**)	IBS 21% *versus* HV 0%
Marshall *et al.*[36]	IBS ($n=132$), mostly PI-IBS HV ($n=86$)	100 g sucrose 5 g lactulose 2 g mannitol 500 mL of H_2O 1.5 g of flavored drink crystals	Overnight fast: NO	Overnight	LMR ⩾0.025	**LMR**: ↑ in IBS *versus* HV (**$p=0.007$**)	IBS 16% *versus* HV 8%
Park *et al.*[39]	IBS-A ($n=3$) IBS-C ($n=8$) IBS-D ($n=27$) HV ($n=12$)	PEG 400 PEG 3350	Overnight fast: ND	0–8 h	NA	**PEGR**: IBS 0.8 *versus* HV 0.4 (**$p<0.05$**) **PEGR**: IBS-A 0.8 *versus* IBS-C 0.7 *versus* IBS-D 0.9 (**$p=$ NS**)	NA
Valentin *et al.*[56]	IBS-D ($n=15$) HV ($n=12$)	1 g lactulose 0.1 g ^{13}C mannitol	Overnight fast: YES Water *ad libitum*, standardized breakfast (egg, toast, water) after 2 h, standardized lunch (chicken, potato and water) after 6 h	0–2 h 2–8 h	NA	**^{13}C mannitol**: IBS-D 0.2 *versus* HV (**$p=$ NS**) **LMR**: IBS-D *versus* HV (**$p=$ NS**) 2–8 h **^{13}C mannitol**: IBS-D 0.2 *versus* HV (**$p=$ NS**) **LMR**: IBS-D *versus* HV (**$p=$ NS**)	NA
Studies collecting urine over 0–24 h, representing distal or whole GI tract permeability							
Dunlop *et al.*[37]	IBS-C ($n=15$) PI-IBS-D ($n=15$) HV ($n=15$)	1.8 MBq of 100 µL of ^{51}Cr-EDTA 100 mL of H_2O 200 mL of Fortisip (300 kcal)	Overnight fast: YES Drinking allowed after 3 h, food after 5 h	0–3 h 3–5 h 5–24 h	NA	0–3 h PI-IBS-D 0.2% *versus* IBS-C 0.1% *versus* HV 0.1% (**$p=0.02$ overall, $p=0.037$ for PI-IBS-D *versus* HV, $p=0.004$ for PI-IBS *versus* IBS-C, $p=$ NS for IBS-C *versus* HV**) 3–5 h PI-IBS-D 0.2% *versus* IBS-C 0.1% *versus* HV 0.3% (**$p=0.08$**) 5–24 h PI-IBS-D 0.8% *versus* IBS-C 0.9% *versus* HV 1.0% (**$p=0.2$**)	NA

(Continued)

Table 1. (Continued)

Reference	Study population	Permeability probe	Dietary restrictions	Timing of urine or blood collection	Cut-off value of normality	Results	
						Comparison with healthy volunteers	Proportion of ↑ permeability
Dunlop *et al.*[37]	PI-IBS-D ($n=15$) nonPI-IBS-D ($n=15$) HV ($n=12$)	1.8 MBq of 100 μL of 51Cr-EDTA 100 mL of H_2O 200 mL of Fortisip (300 kcal)	Overnight fast: ND	0–6 h 6–24 h	NA	0–6 h Non-PI-IBS-D 0.8% *versus* PI-IBS-D 0.4% *versus* HV 0.3% (**$p=0.001$ overall, $p=0.028$ for nonPI-IBS-D *versus* HV, $p=0.001$ for PI-IBS-D *versus* HV, $p=0.004$ for non-PI-IBS-D *versus* PI-IBS-D**) 6–24 h Non-PI-IBS-D 1.2% *versus* PI-IBS-D 1.0% *versus* HV 0.8% (**$p=0.1$ overall, $p=0.04$ for non-PI-IBS-D *versus* HV, $p=0.5$ for PI-IBS-D *versus* HV**)	NA
Zeng *et al.*[38]	IBS-D ($n=29$) HV ($n=12$)	10 g lactulose 5 g mannitol 5 g sucralose 100 mL of H_2O	Overnight fast: YES Water and food allowed after 2 h	0–5 h 5–24 h	LMR >0.025 Sucralose ≥42.1 mg	0–5 h **LMR**: IBS-D 0.04 *versus* HV 0.02 (**$p=0.002$**) 0–24 h **Sucralose**: IBS-D 44.3 mg *versus* 31.4 mg (**$p=0.028$**)	LMR: IBS-D 62% Sucralose: IBS-D 52%
Zhou *et al.*[40]	IBS-D ($n=54$) HV ($n=22$)	5 g lactulose 2 g mannitol 100 mL of H_2O	Overnight fast: YES	0–24 h	LMR ⩾0.07	NA	IBS-D 39% *versus* HV 0%
Kerckhoffs *et al.*[41]	IBS-A ($n=3$) IBS-C ($n=3$) IBS-D ($n=8$) HV ($n=15$)	5 g PEG 400 1.5 g PEG 1500 5 g PEG 4000 10 g PEG 10000 100 mL of H_2O containing 0.1% sorbate	Overnight fast: YES Water *ad libitum*, food allowed after 6 h	0–2 h 2–4 h 4–6 h 6–8 h 8–10 h 10–12 h 12–14 h 14–16 h 16–24 h	NA	**PEG 400**: IBS 26.0% *versus* HV 27.9% (**$p=$ NS**) **PEG 1500**: IBS 1.0% *versus* HV 1.3% (**$p=$ NS**) **PEG 4000**: IBS 0.0% *versus* HV 0.02% (**$p=$ NS**)	NA
Zhou *et al.*[42]	IBS-D ($n=19$) HV ($n=10$)	5 g lactulose 2 g mannitol 100 mL of H_2O	Overnight fast: ND	0–5 h 6–24 h	LMR ⩾0.07	NA	0–5 h IBS-D 42% *versus* HV 0% 6–24 h IBS-D 42% *versus* HV 0%

(Continued)

Table 1. (Continued)

Reference	Study population	Permeability probe	Dietary restrictions	Timing of urine or blood collection	Cut-off value of normality	Results	
						Comparison with healthy volunteers	Proportion of ↑ permeability
Gecse *et al.*[43]	IBS-C ($n=12$) IBS-D ($n=18$) HV ($n=10$)	1.8 MBq of 100 µL of 51Cr-EDTA 100 mL of H_2O 200 mL of Fortisip (300 kcal)	Overnight fast: YES Drinking allowed after 3 h, food after 5 h	0–3 h 3–5 h 5–24 h	NA	0–3 h IBS-C 0.3% *versus* IBS-D 0.6% *versus* HV 0.6% (**$p<0.05$ for IBS-C *versus* HV, $p=$ NS for IBS-D *versus* HV**) 3–5 h IBS-C 0.4% *versus* IBS-D 0.6% *versus* HV 0.4% (**$p=$ NS**) 5–24 h IBS-C 0.7% *versus* IBS-D 2.7% *versus* HV 1.0% (**$p=$ NS for IBS-C *versus* HV, $p<0.05$ for IBS-D *versus* HV**) 0–24 h IBS-C 1.3% *versus* IBS-D 3.9% *versus* HV 2.0% (**$p=$ NS for IBS-C *versus* HV, $p<0.05$ for IBS-D *versus* HV**)	NA
Rao *et al.*[45]	IBS-D ($n=12$) HV ($n=12$)	1 g lactulose 0.2 g mannitol 250 mL of H_2O containing Tc-99m DTPA	Overnight fast: YES Water *ad libitum*, standardized breakfast (egg, toast, water) after 2 h, standardized lunch (chicken, potato and water) after 6 h, all food allowed after 8 h	0–0.5 h 0.5–1 h 1–1.5 h 1.5–2 h 2–4 h 4–6 h 6–8 h 8–24 h	NA	0–2 h **Mannitol**: ↑ IBS-D *versus* HV (**$p=0.056$**) **Lactulose, LMR**: IBS-D *versus* HV (**$p=$ NS**) 2–8 h **Mannitol**: ↑ IBS-D *versus* HV (**$p=0.0489$**) **Lactulose, LMR**: IBS-D *versus* HV (**$p=$ NS**) 8–24 h **Lactulose**: ↑ IBS-D *versus* HV (**$p=0.097$**) **Mannitol, LMR**: IBS-D *versus* HV (**$p=$ NS**)	NA
Rao *et al.*[45]	IBS-D ($n=12$) HV ($n=12$)	1 g lactulose 0.2 g mannitol Methacrylate-coated capsule in 250 mL of H_2O	Overnight fast: YES Water *ad libitum*, standardized breakfast (egg, toast, water) after 2 h, standardized lunch (chicken, potato and water) after 6 h, all food allowed after 8 h	0–0.5 h 0.5–1 h 1–1.5 h 1.5–2 h 2–4 h 4–6 h 6–8 h 8–24 h	NA	0–2 h **Lactulose, mannitol, LMR**: IBS-D *versus* HV (**$p=$ NS**) 2–8 h **Lactulose, mannitol, LMR**: IBS-D *versus* HV (**$p=$ NS**) 8–24 h **Lactulose, mannitol, LMR**: IBS-D *versus* HV (**$p=$ NS**)	NA
Vazquez-Roque *et al.*[63]	IBS-D ($n=45$) HV ($n=12$)	1 g lactulose 0.2 g mannitol	Overnight fast: YES Water *ad libitum*, standardized breakfast (egg, toast, water) after 2 h, standardized lunch (chicken, potato and water) after 6 h, all food allowed after 8 h	0–0.5 h 0.5–1 h 1–1.5 h 1.5–2 h 2–4 h 4–6 h 6–8 h 8–24 h	NA	0–2 h **Lactulose**: ↑ IBS-D *versus* HV (**$p<0.001$**) **Mannitol**: ↑ IBS-D *versus* HV (**$p<0.001$**) 8–24 h: **LMR**: ↑ IBS-D *versus* HV (**$p=0.106$**) **Lactulose, mannitol**: IBS-D *versus* HV (**$p=$ NS**)	NA

(Continued)

Table 1. (Continued)

Reference	Study population	Permeability probe	Dietary restrictions	Timing of urine or blood collection	Cut-off value of normality	Results	
						Comparison with healthy volunteers	Proportion of ↑ permeability
Swan *et al.*[48]	IBS-C ($n=18$) IBS-D ($n=37$) C. jejuni 6m post-enteritis HV ($n=26$)	1.8 MBq of 51Cr-EDTA 50 mL of H_2O 200 mL of Fortisip (150 kcal)	Overnight fast: YES Drinking allowed after 3 h, food after 5 h	0–3 h 3–6 h 6–24 h	NA	0–3 h PI-IBS *versus* IBS-C *versus* IBS-D *versus* HV (**$p=$ NS**) 3–6 h PI-IBS 0.7% *versus* IBS-C 0.6% *versus* IBS-D 0.6% *versus* HV 0.5% (**$p=0.025$ for PI-IBS *versus* HV, $p=0.9$ for IBS-C *versus* HV, $p=0.9$ for IBS-D *versus* HV**) 6–24 h PI-IBS *versus* IBS-C *versus* IBS-D *versus* HV (**$p=$ NS**)	NA
Camilleri *et al.*[59]	IBS-C ($n=30$) IBS-D ($n=64$) HV ($n=30$)	1 g lactulose 0.2 g mannitol 240 mL of H_2O	Overnight fast: ND	0–2 h 2–4 h 4–6 h 6–8 h 8–24 h	NA	0–2 h **Mannitol**: IBS-C 264.8 mg *versus* IBS-D 444.3 mg *versus* HV 355.2 mg (**$p=0.039$**) 8–24 h **Mannitol**: IBS-C 65.8 mg *versus* IBS-D 45.5 mg *versus* HV 43.6 mg (**$p=0.708$**)	NA
Mujagic *et al.*[50]	IBS-C ($n=21$) IBS-D ($n=34$) IBS-M ($n=30$) IBS-U ($n=6$) HV ($n=94$)	1 g sucrose 1 g lactulose 0.5 g L-rhamnose 1 g sucralose 1 g erythritol 150 mL of H_2O	Overnight fast: YES Water *ad libitum*, other drinks and food allowed after 5 h	0–5 h 5–24 h	NA	0–5 h **Sucrose**: IBS-C 7.4 μmol *versus* IBS-D 4.2 μmol *versus* IBS-M 6.6 *versus* HV 2.4 μmol (**$p=0.880$**) **LRR**: IBS-C 0.02 *versus* IBS-D 0.02 *versus* IBS-M 0.02 *versus* HV 0.01 (**$p=0.022$ for IBS-D *versus* HV, $p=$ NS for other groups**) 5–24 h **SER**: IBS-C 0.009 *versus* IBS-D 0.008 *versus* IBS-M 0.008 *versus* HV 0.010 (**$p=$ NS**) 0–24 h **SER**: IBS-C 0.008 *versus* IBS-D 0.009 *versus* IBS-M 0.010 *versus* HV 0.009 (**$p=$ NS**)	NA
Li *et al.*[53]	IBS-D ($n=40$) HV ($n=10$)	5 g lactulose 2 g mannitol 2 g sucralose 100 mL of H_2O	Overnight fast: YES	0–5 h 5–24 h	LMR > 0.025	0–5 h **LMR**: IBS-D 0.02 *versus* HV 0.02 (**$p=0.010$**) 0–24 h **Sucralose**: 23.3 mg *versus* HV 21.7 mg (**$p=0.574$**)	IBS-D 48% *versus* HV 10–20%

(Continued)

Table 1. (Continued)

Reference	Study population	Permeability probe	Dietary restrictions	Timing of urine or blood collection	Cut-off value of normality	Results	
						Comparison with healthy volunteers	Proportion of ↑ permeability
Peters *et al.*[54]	IBS-C ($n=19$) HV ($n=18$)	1 g lactulose 0.1 g ^{12}C mannitol 0.1 g ^{13}C mannitol 250 mL of H_2O	Overnight fast: ND	0–2 h 2–8 h 8–24 h	NA	0–2 h **Lactulose**: IBS-C 1.2 mg *versus* HV 1.0 mg (***p*** **= 0.53**) **^{13}C mannitol**: IBS-C 12.1 mg *versus* HV 13.2 mg (***p*** **= 0.39**) **LMR**: IBS-C 0.01 *versus* HV 0.007 (***p*** **= 0.25**) 8–24 h **Lactulose**: IBS-C 0.9 mg *versus* HV 0.5 mg (***p*** **= 0.75**) **^{13}C mannitol**: IBS-C 3.1 mg *versus* HV 3.9 mg (***p*** **= 0.08**) **LMR**: IBS-C 0.02 *versus* HV 0.01 (***p*** **= 0.87**)	NA
Studies collecting blood samples							
Keszthelyi *et al.*[49]	IBS-C ($n=5$) IBS-D ($n=7$) IBS-M ($n=3$) HV ($n=15$)	1 g lactulose 0.5 g L-rhamnose	Overnight fast: YES	1 hour	NA	**LRR**: IBS 12×10^{-3} *versus* HV 6.3×10^{-3} (***p*** **= 0.06**) No differences between IBS subtypes	NA
Paganelli *et al.*[34]	IBS ($n=14$) HV ($n=10$)	Fresh cow milk (10 mL/kg)	Overnight fast: YES	2 h 4 h	B-lactoglobulin ⩾0.3 ng/mL	NA	IBS 21%
Studies without a control population							
Zhou *et al.*[51]	IBS-C ($n=74$) IBS-D ($n=109$) HV ($n=36$)	5 g lactulose 2 g mannitol 100 mL of H_2O	Overnight fast: YES	0–24 h	LMR ⩾0.07	NA	IBS-C 4% *versus* IBS-D 37%
Jarrett *et al.*[52]	IBS-C ($n=11$) IBS-D ($n=27$) IBS-M ($n=38$) IBS-U ($n=4$)	6.375 g lactulose 1.275 g mannitol 127.5 mL of H_2O	Overnight fast: NO, but administration after a fasting period of 4 h, after the evening meal. Administration of probe solution directly followed by drinking 240 mL of H_2O	0–24 h	LMR >0.015	**LMR**: IBS-C 0.01 *versus* IBS-D 0.01 *versus* IBS-M 0.01 *versus* IBS-U 0.02 (***p*** **= 0.111**)	IBS 28%

^{51}Cr-EDTA, chromium-51-ethylenediamine tetraacetic acid; C, Campylobacter; HV, healthy volunteers; IBS, irritable bowel syndrome; IBS-A, irritable bowel syndrome with alternating stool pattern; IBS-C, irritable bowel syndrome with constipation; IBS-D, irritable bowel syndrome with diarrhea; IBS-M, irritable bowel syndrome with mixed stool pattern; IBS-U, unsubtypted irritable bowel syndrome; La/Ara, lactose-to-L-arabinose ratio; LMR, lactulose to mannitol ratio; LRR, lactulose to L-rhamnose ratio; NA, not applicable; ND, not described; PEG, polyethylene glycol; PEGR, polyethylene glycol 400 to polyethylene glycol 3350 ratio; PI-IBS, post-infection irritable bowel syndrome; Ra/Ara, raffinose to L-arabinose ratio; SER, sucralose-to-erythritol ratio.

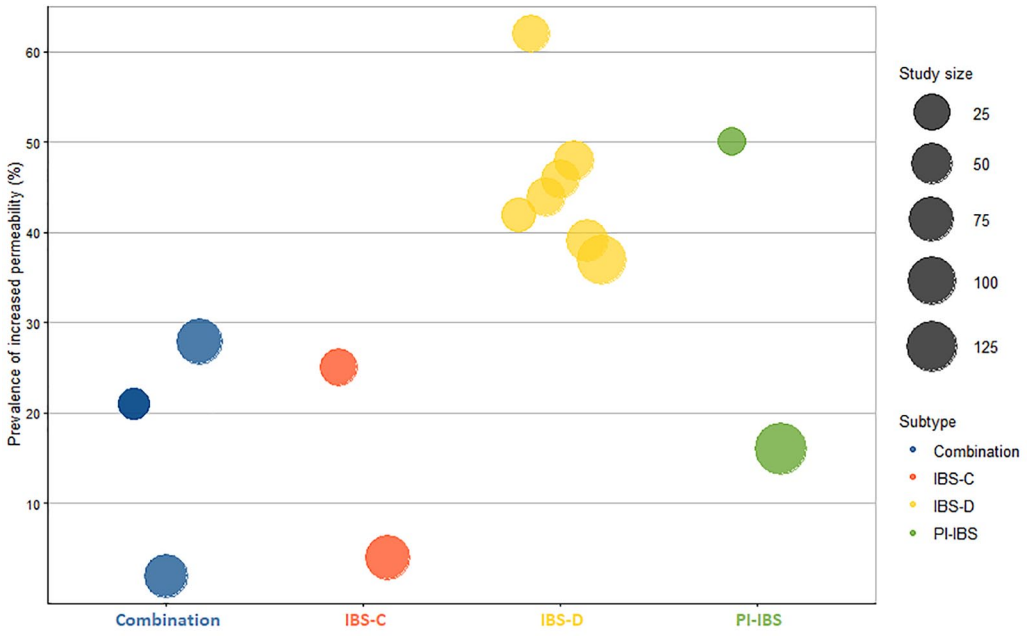

Figure 2. Proportion of patients with increased *in vivo* permeability in the different IBS subtypes. IBS-D represented by the highest number of studies, which show a much higher proportion of patients (39–62%) with increased permeability. Larger studies tend to have a lower proportion of patients with increased permeability compared with smaller studies.*

*Only studies that reported a proportion of IBS patients with increased permeability were included in this figure. Combination, ⩾1 subtype.

IBS-C, constipation-predominant irritable bowel syndrome; IBS-D, diarrhea-predominant irritable bowel syndrome; PI-IBS, post-infection irritable bowel syndrome.

with healthy controls,[37–39,43,45,50,53,58,63] whereas four studies did not.[48,56,57,55] The prevalence of increased permeability ranged between 37% and 62%.[38,40,42,51,53,57,58] In IBS-C, two studies[39,44] found increased permeability compared with healthy controls, whereas five studies showed no group differences.[37,48–50,54] Moreover, one study reported decreased gastroduodenal permeability, compared with controls.[43] The prevalence of increased permeability ranged between 4% and 25% compared with ~9% in controls.[44,51] Intestinal permeability was normal in one study investigating IBS-M patients.

Four studies focused on *in vivo* permeability in PI-IBS populations, all of which demonstrated increased permeability compared with controls.[35–37,48] A small study ($n=10$) found that 50% of PI-IBS patients had increased permeability,[35] while this dropped to 16% in a larger study from the Walkerton outbreak cohort ($n=132$).[36] The two other studies did not estimate the prevalence, but did show that cumulative small intestinal permeability was increased.[37,48]

Confocal laser endomicroscopy. Two studies used confocal laser endomicroscopy to visualize intercellular junctions *in vivo* in IBS patients.[65,66] The epithelial gap density was increased in the ileum of both IBS-C and IBS-D patients.[65] However, in the other study, no changes were seen in the rectosigmoid of IBS-D patients.[66] More studies are needed to evaluate the usefulness of the technique in evaluating permeability in the context of IBS.

In summary, 9/13 IBS-D studies and anywhere from one-third to two-thirds of the patients demonstrate increased intestinal permeability. On the contrary, IBS-C patients likely do not have increased intestinal permeability as studies are either negative or the proportion of patients with increased intestinal permeability is not much different from the controls.

Permeability studies in pediatric populations

Four studies have been performed in pediatric IBS populations, all using saccharide probes (Table 2).[67–70] Two of these studies also included

Table 2. Studies comparing *in vivo* permeability in pediatric IBS patients with healthy controls.

Reference	Study population	Permeability probe	Dietary restrictions	Timing of urine collection	Results	
					Comparison with healthy volunteers	Proportion of ↑ permeability
Studies with a control population						
Shulman *et al.*[67]	IBS or FAP ($n=93$) HV ($n=52$) Age IBS: 8.2 ± 1.4 Age HV: 8.5 ± 1.3	12.75 g sucrose 6.375 g lactulose 1.275 g mannitol 1.275 g sucralose 127.5 mL of H_2O	Overnight fast: YES 240 mL of H_2O directly after the probe ingestion, followed by 3 h of fasting	0–3 h	**Sucrose**: IBS/FAP 0.02% *versus* HV 0.02% (**$p=$ NS**) **Lactulose**: IBS/FAP 0.10% *versus* HV 0.09% (**$p=$ NS**) **Mannitol**: IBS/FAP 7.6% *versus* HV 7.6% (**$p=$ NS**) **Sucralose**: IBS/FAP 0.4% *versus* HV 0.4% (**$p=$ NS**) **SCLR**: IBS/FAP 0.6 *versus* HV 0.4 (**$p=0.001$**) **LMR**: IBS/FAP 0.06 *versus* HV 0.07 (**$p=$ NS**) **SALR**: IBS/FAP 1.0 *versus* HV 0.8 (**$p=0.05$**)	NA
Francavilla *et al.*[68]	IBS or FAP ($n=54$) HV ($n=55$) Age IBS: 6.4 ± 2.0 Age HV: range 5–12	5 g lactulose 2 g mannitol 150 mL of H_2O	Overnight fast: YES Water allowed after 30 min, food not allowed	0–5 h	**LMR**: IBS/FAP 0.04 *versus* HV 0.03 (**$p<0.01$**)	IBS/FAP 59%
Gervasoni *et al.*[69]	IBS-C ($n=2$) IBS-D ($n=8$) IBS-U ($n=5$) HV ($n=10$) Age IBS: range 5–16 Age HV: range 5–16	5 g lactulose 1 g mannitol 120 mL of H_2O	Overnight fast: YES	0–6 h	**Lactulose**: IBS *versus* HV (**$p=$ NS**) **Mannitol**: IBS *versus* HV (**$p=$ NS**) **LMR**: IBS 0.10 *versus* HV 0.01 (**$p<0.05$**) **LMR**: IBS-D 1.2 *versus* IBS-C + IBS-U 0.5 (**$p<0.05$**)	NA
Shulman *et al.*[70]	IBS ($n=95$) FAP ($n=25$) HV ($n=60$) Age IBS: 9.4 ± 1.5 Age FAP: 9.2 ± 1.7 Age HV: 9.7 ± 1.6	10 g sucrose 5 g lactulose 1 g mannitol 1 g sucralose (in a capsule, due to taste) 127.5 mL of H_2O	Overnight fast: YES 240 mL of H_2O directly after the probe ingestion	0–3 h 3–24 h	0–3 h **Sucrose**: IBS 0.06% *versus* FAP 0.08% *versus* HV 0.04% (**$p=0.49$ overall, $p=0.646$ for IBS *versus* FAP, $p=0.335$ for IBS *versus* HV, $p=0.328$ for FAP *versus* HV**) **Lactulose**: IBS 0.2% *versus* FAP 0.2% *versus* HV 0.1% (**$p=0.59$**) **Mannitol**: IBS 7.8% *versus* FAP 10.1% *versus* HV 9.3% (**$p=0.05$**) LMR: IBS 0.12 *versus* FAP 0.08 *versus* HV 0.07 (**$p=0.624$ for IBS *versus* FAP, $p=0.023$ for IBS *versus* HV, $p=0.178$ for FAP *versus* HV**) 0–24 h **Lactulose**: IBS 0.6% *versus* FAP 0.6% *versus* HV 0.5% (**$p=0.52$**) **Mannitol**: IBS 22.1% *versus* FAP 24.6% *versus* HV 25.0% (**$p=0.12$**) **LMR**: IBS 0.2 *versus* FAP 0.1 *versus* HV 0.1 (**$p=0.538$ for IBS *versus* FAP, $p=0.050$ for IBS *versus* HV, $p=0.315$ for FAP *versus* HV**) **Sucralose**: IBS 1.8% *versus* FAP 1.4% *versus* HV 1.8% (**$p=0.23$ overall, $p=0.279$ for IBS *versus* FAP, $p=0.248$ for IBS *versus* HV, $p=0.045$ for FAP *versus* HV**)	NA

BW, bodyweight; FAP, functional abdominal pain; HV, healthy volunteers; IBS, irritable bowel syndrome; IBS-C, irritable bowel syndrome with constipation; IBS-D, irritable bowel syndrome with diarrhea; IBS-U, unsubtyped irritable bowel syndrome; LMR, lactulose-to-mannitol ratio; SALR, sucralose to lactulose ratio; SCLR, sucrose-to-lactulose ratio.

children with functional abdominal pain (FAP), a functional GI disorder with abdominal pain but no bowel disturbances.[67,68] As many as 59% of children with IBS or FAP had increased intestinal permeability (based on LMR cut-off <0.034).[68] Shulman *et al.*[67] found increased colonic permeability, but did not detect changes in gastric and small intestinal permeability in the IBS/FAP population compared with healthy controls. A subsequent study found that small intestinal and whole-gut permeability was changed in children with IBS but not FAP.[70] Sex was found to have an effect on intestinal permeability in children with IBS.[70] Girls but not boys had an increased sucrose recovery, a marker of gastric barrier function. Conversely, boys but not girls had an increased 0–3 h LMR, suggesting increased small intestinal permeability. Lastly, colonic permeability was also only increased in boys.[70]

Two studies correlating intestinal permeability with symptoms found no significant associations with abdominal pain symptoms or stool characteristics.[67,68] Of note, McOmber *et al.*[71] showed that both siblings and parents of children with IBS had increased small bowel permeability, suggesting a possible role of genetic and/or environmental factors. At close to 60%, increased intestinal permeability may be more strongly associated in children with IBS than in adults. Additionally, it is clear that sex has an effect on barrier dysfunction in IBS in children, with males showing more prominent changes.

Ex vivo *and* in vitro *measurement of gastrointestinal barrier function in adults*

Thirty studies have assessed mucosal barrier properties using biopsy samples (12 IBS-D,[38,63,66,72–80] two IBS-C,[54,81] one IBS-M[82] and 15 >1 IBS subtypes[49,51,83–95]). An overview of these studies is given in Table 3. Furthermore, the results are summarized in Figure 3.

Functional studies. Nine studies assessed barrier function of biopsy samples in Ussing chambers (one IBS-C,[54] two IBS-D,[77,78] one IBS-M,[82] four multiple subtypes[84,86,88,91] and one without subtyping[97]). In the single IBS-C study, transepithelial electrical resistance measurements and the flux of probes across the mucosal biopsy were similar to controls in both the duodenum and the rectosigmoid colon.[54] The eight other studies all reported increased permeability in IBS patients compared with controls in biopsies taken in different locations along the GI tract.[77,78,82,84,86,88,91,97] In biopsies of the cecum[84,86] and the descending colon,[88] paracellular flux of FITC-labeled probes (0.4–4 kDa) was higher in IBS patients compared with healthy controls. As opposed to the study in IBS-C, the intestinal barrier was disrupted in the rectosigmoid colon of patients with IBS-D[77,78] and IBS-M.[82] Interestingly, one study noted increased translocation of *E. coli* and *S. typhimurium* across the epithelium, indicating that paracellular as well as transcellular transport might be affected in IBS.[91] Taken together, these studies suggest a functional disruption of the intestinal barrier in IBS patients, especially for the IBS-D and IBS-M subtypes. Although studies with mixed populations do not report on differences between IBS-C and the other subtypes, the negative studies in IBS-C likely indicate that permeability disturbances might be less relevant in this subtype.

Molecular and structural studies

Duodenum. Three studies (all multiple subtypes) evaluated duodenal epithelial barrier.[49,51,95] Occludin and ZO-1 protein expression were decreased but CLDN-2 expression was unchanged in study populations consisting of multiple IBS subtypes.[49,95] Furthermore, CLDN-1 expression was decreased in IBS-D patients, compared with healthy controls.[51] Hence, duodenal barrier function seems perturbed in IBS, although potential differences between subtypes require further exploration.

Jejunum. Four studies (all IBS-D) examined the jejunal mucosal barrier, all of which provided evidence for a disrupted epithelial barrier.[72–75] Transcriptomic studies described changes in tight junction and adherens junction signaling pathways,[72,98] while protein assessment revealed alterations in actin–cytoskeleton function and signaling.[75] An up-regulation of E-cadherin, catenin α1 and β1 and cingulin was seen.[74] The up-regulation of CLDN-2 but not CLDN-1, CLDN-3 and CLDN-4 was noted in two other studies.[73,74] Furthermore, contraction of the peri-junctional actinmyosin ring was seen in one study.[73] Several molecular targets within the jejunum that are important for maintaining proper barrier function appear to be affected in IBS patients.

Terminal ileum. Two studies investigated changes in the terminal ileum.[76,83] Electron microscopy

Table 3. Studies assessing *in vitro* or *ex vivo* gastrointestinal barrier function in adult IBS patients.

Reference	Study population	Findings
Duodenum		
Keszthelyi *et al.*[49]	IBS-C ($n=5$) IBS-D ($n=7$) IBS-M($n=3$) HV ($n=15$)	- PCR: ↓ occludin, ZO-1 in IBS *versus* HV - Immunohistochemistry: ↓ occludin, ZO-1 in IBS *versus* HV
Zhou *et al.*[51]	IBS-C ($n=74$) IBS-D ($n=109$) HV ($n=36$)	- PCR: ↑ mi-RNA-29a, mi-RNA-29b, mi-RNA-29c in IBS-D with ↑ permeability but not with = permeability or in IBS-C *versus* HV - Northern blot: ↑ mi-RNA-29a, mi-RNA-29b in IBS-D with ↑ permeability but not with = permeability *versus* HV - Immunoblot: ↓ CLDN-1, NKRF in IBS-D with ↑ permeability but not with = permeability *versus* HV
Peters *et al.*[54]	IBS-C ($n=19$) HV ($n=18$)	- Ussing chambers: = TER, flux of 4kDa FITC-dextran and translocation of E. coli in IBS-C *versus* HV
Fritscher-Ravens *et al.*[95]	IBS-C ($n=14$) IBS-D($n=52$) IBS-M ($n=42$) HV ($n=14$)	- PCR: = CLDN-2, occludin, ZO-1 in IBS *versus* HV - Immunohistochemistry: ↓ occludin, = CLDN-2 in IBS *versus* HV
Jejunum		
Martínez *et al.*[72]	IBS-D ($n=25$) HV ($n=23$)	- RNA microarray + IPA: tight junction signaling pathways are associated with IBS-D *versus* HV - PCR: ↓ ZO-1, ZO-3, = ZO-2 in IBS-D *versus* HV - Immunofluorescence: ↓ ZO-1, ZO-2, = ZO-3 in IBS-D *versus* HV
Martínez *et al.*[73]	IBS-D ($n=45$) HV ($n=30$)	- Immunofluorescence: ↑ MLCK, pMLC, ↓ PP1cδ, = ppMLC in IBS-D *versus* HV - Immunofluorescence: ↑ occludin staining in cytoplasm, ↓ occludin staining at tight junction complexes in IBS-D *versus* HV - Western blot: ↑ CLDN-2, ↓ p-occludin, = CLDN-1, CLDN-3, CLDN-4, occludin in IBS-D *versus* HV - EM: ↑ apical intercellular distance, proportion of dilated junctions, percentage of junctions with perijunctional cytoskeleton condensation
Martínez *et al.*[74]	IBS-D ($n=43$) HV ($n=26$)	- mRNA sequencing (exploration cohort): ↑ E-cadherin, catenin α1 + β1, cingulin, JAM-1, JAM-3, ↓ JAM-2 in IBS-D *versus* HV - nCounter RNA sequencing (validation cohort): ↑ E-cadherin, catenin α1 + β1, cingulin, JAM-1, = JAM-2, JAM-3 - mRNA sequencing + IPA: ↑ tight junction signaling, caveolar-mediated endocytosis signaling, actin cytoskeleton signaling, epithelial adherens junction signaling - PCR: ↓ has-miRNA-125b-5p, has-miRNA-16-5p in IBS-D *versus* HV - Western blot: ↑ cingulin, CLDN-2 in IBS-D *versus* HV
Rodiño-Janeiro *et al.*[75]	IBS-D ($n=15$) HV ($n=16$)	- Proteomics: ↓ pCFL1, TESK1, = CFL1 in IBS-D *versus* HV - Proteomics + IPA: alterations in actin cytoskeleton function, clathrin-mediated endocytosis signaling, actin cytoskeleton signaling, caveolar-mediated endocytosis signaling and integrin signaling
Ileum		
Turcotte *et al.*[65]	IBS-C ($n=4$) IBS-D ($n=12$) HV ($n=18$)	- CLE: ↑ epithelial gap density in IBS *versus* HV

(Continued)

Table 3. (Continued)

Reference	Study population	Findings
Cheng *et al*.[83]	IBS-C ($n=30$) IBS-D ($n=33$) HV ($n=30$)	- PCR: ↑ CLDN-1 in IBS-C *versus* HV, ↓ CLDN-1 in IBS-D *versus* HV - Western blot: ↑ CLDN-1 in IBS-C *versus* HV, ↓ CLDN-1 in IBS-D *versus* HV - Immunohistochemistry: ↑ CLDN-1 in IBS-C *versus* HV, ↓ CLDN-1 in IBS-D *versus* HV - EM: ↑ mucus secretion, mucus bubble fusion in goblet cells, tracer extravasation in IBS-C and IBS-D *versus* HV, ↑ width of gaps between tight junctions in 70% of patients in IBS-D *versus* HV, = intercellular tight junction structures in IBS-C *versus* HV
Ishimoto *et al*.[76]	IBS-D ($n=17$) HV ($n=20$)	- PCR: ↑ CLDN-2, = CLDN-1, CLDN-7, JAM-1, occludin, ZO-1 in IBS-D *versus* HV
Cecum		
Vivinus-Nébot *et al*.[84]	IBS-C ($n=10$) IBS-D ($n=13$) IBS-M ($n=11$) HV ($n=15$)	- Ussing chambers: ↑ flux of 4kDa FITC-dextran in IBS *versus* HV
Wilcz-Villega *et al*.[85]	IBS-A ($n=12$) IBS-D($n=22$) HV ($n=12$)	- Immunofluorescence: ↓ JAM-1 in IBS *versus* HV
Vivinus-Nébot *et al*.[86]	IBS-C ($n=15$) IBS-D ($n=18$) IBS-M ($n=18$) HV ($n=27$)	- Ussing chambers: ↑ flux of 0.4 kDa FITC-sulfonic acid in IBS *versus* HV - PCR: ↓ α-catenin, occludin, ZO-1 in IBS *versus* HV
Wilcz-Villega *et al*.[87]	IBS-A ($n=12$) IBS-D ($n=24$) HV ($n=12$)	- Immunofluorescence: ↓ E-cadherin, ZO-1, = CLDN-1 in IBS *versus* HV - Immunohistochemistry: = E-cadherin in IBS *versus* HV
Ishimoto *et al*.[76]	IBS-D ($n=17$) HV ($n=20$)	- PCR: = CLDN-1, CLDN-2, CLDN-7, JAM-1, occludin, ZO-1 in IBS-D *versus* HV
Ascending colon		
Cheng *et al*.[83]	IBS-C ($n=30$) IBS-D ($n=33$) HV ($n=30$)	- PCR: ↑ CLDN-1 in IBS-C *versus* HV, ↓ CLDN-1 in IBS-D *versus* HV - Western blot: ↑ CLDN-1 in IBS-C *versus* HV, ↓ CLDN-1 in IBS-D *versus* HV - Immunohistochemistry: ↑ CLDN-1 in IBS-C *versus* HV, ↓ CLDN-1 in IBS-D *versus* HV - EM: ↑ mucus secretion, mucus bubble fusion in goblet cells, tracer extravasation in IBS-C and IBS-D *versus* HV, ↑ width of gaps between tight junctions in 80% of patients in IBS-D *versus* HV, = intercellular tight junction structures in IBS-C *versus* HV
Descending colon		
Piche *et al*.[88]	IBS-A ($n=5$) IBS-C ($n=3$) IBS-D ($n=4$) HV ($n=5$)	- Ussing chambers: ↑ flux of FITC-sulfonic acid in IBS *versus* HV - PCR: ↓ ZO-1, = occludin in IBS *versus* HV - Caco-2 cell monolayers incubated with biopsy supernatant: ↑ flux of 4kDa FITC-dextran, ↓ TER in IBS *versus* HV - Caco-2 cell monolayers incubated with biopsy supernatant + PCR: ↓ ZO-1, = occludin in IBS *versus* HV
Coëffier *et al*.[89]	IBS-A ($n=4$) IBS-C ($n=8$) IBS-D ($n=13$) HV ($n=18$)	- PCR: = occludin in IBS *versus* HV - Western blot: ↓ occludin in IBS *versus* HV
Bertiaux-Vandaele *et al*.[90]	IBS-A ($n=15$) IBS-C ($n=14$) IBS-D ($n=19$) HV ($n=33$)	- PCR: = CLDN-1, occludin, ZO-1 in IBS *versus* HV - Western blot: ↓ CLDN-1, occludin, ZO-1 in IBS-D *versus* HV, ↓ ZO-1, = CLDN-1, occludin in IBS-A and IBS-C *versus* HV

(Continued)

Table 3. (Continued)

Reference	Study population	Findings
Vazquez-Roque *et al.*[63]	IBS-D ($n=25$) HV ($n=16$)	- PCR: ↓ occludin, ZO-1, = CLDN-1 in IBS-D *versus* HV
Barbaro *et al.*[94]	IBS-C ($n=8$) IBS-D ($n=9$) IBS-M ($n=11$) HV ($n=7$)	- Caco-2 cell monolayers incubated with biopsy supernatant: ↑ flux of FITC-sulfonic acid in IBS *versus* HV
Rectosigmoid colon		
Zeng *et al.*[38]	IBS-D ($n=30$) HV ($n=12$)	- PCR: ↓ occludin, ZO-1 in IBS-D *versus* HV - EM: Staining of junctional complexes among colonic enterocytes was faint and discontinuous in 33% of IBS-D, compared with HV
Lee *et al.*[77]	IBS-D ($n=20$) HV ($n=30$)	- Ussing chambers: ↑ flux of HRP in IBS-D *versus* HV
Lee *et al.*[78]	IBS-D ($n=16$) HV ($n=7$)	- Ussing chambers: ↑ flux of HRP in IBS-D *versus* HV
Camilleri *et al.*[80]	IBS-D ($n=9$) HV ($n=9$)	- RNA sequencing: ↑ RBP2, TFF1, ↓ FN1, WDR72, = CLDN-1, MMP1, MUC20, occludin, ZO-1 in IBS-D *versus* HV - PCR: ↓ FN1, = CLDN-1 occludin, RBP2, TFF1, ZO-1 in IBS-D *versus* HV
Camilleri *et al.*[93]	IBS-C ($n=10$) IBS-D ($n=47$) HV ($n=17$)	- RNA sequencing: ↓ CLDN-1, FN1, = ZO-1, OCLN, RBP2, TFF1 in IBS-D *versus* HV, ↓ OCLN, = ZO-1, CLDN-1, RBP2, FN1, TFF1 in IBS-C *versus* HV
Zhen *et al.*[79]	IBS-D ($n=42$) HV ($n=20$)	- Western blot: ↓ occludin in IBS-D *versus* HV - Immunohistochemistry: staining of occludin was faint and discontinuous in IBS-D *versus* HV
Ishimoto *et al.*[76]	IBS-D ($n=17$) HV ($n=20$)	- PCR: = CLDN-1, CLDN-2, CLDN-7, JAM-1, occludin, ZO-1 in IBS-D *versus* HV
Peters *et al.*[54]	IBS-C ($n=19$) HV ($n=18$)	- Ussing chambers: = TER, flux of 4kDa FITC-dextran and translocation of E. coli in IBS-C *versus* HV - PCR: = CLDN-1, CLDN-2, CLDN-3, CLDN-4, CLDN-5, CLDN-6, CLDN-7, CLDN-8, CLDN-9, CLDN-10, CLDN-11, CLDN-12, CLDN-14, CLDN-15, CLDN-16, CLDN-17, CLDN-18, CLDN-19, occludin, ZO-1, ZO-2, ZO-3 in IBS-C *versus* HV
Videlock *et al.*[92]	IBS-C ($n=10$) IBS-D ($n=10$) HV ($n=10$)	- Microarray profiling analysis: 1270 DETs for IBS-C *versus* HV (↑MUC-20, ↓ MYLK2, WDR72), no DETs meeting FDR <0.05 in IBS *versus* HV or IBS-D *versus* HV (= FN1, OCLN, TFF1, TJP1) - WGCNA: ↓ cell junction module in IBS-D but not IBS-C *versus* HV
Lee *et al.*[96]	IBS-C ($n=33$) IBS-D ($n=21$) IBS-M ($n=5$) HV ($n=36$)	- PCR: ↓ ZO-1 in females but not males, = CLDN-1, occludin in IBS-D *versus* HV (no differences in IBS-C and IBS-M) - Western blot: ↓ ZO-1 in IBS-D and IBS-M but not IBS-C *versus* HV
Zhao *et al.*[66]	IBS-D ($n=10$) HV ($n=10$)	- CLE: No differences in epithelial architecture, no fluorescein leakage into the lumen in IBS-D *versus* HV - EM: ↑ apical intercellular distance, percentage of dilated intercellular junctions, dilatation and destruction of adherens junctions and desmosomes in IBS-D *versus* HV
Katinios *et al.*[82]	IBS-M ($n=15$) HV ($n=15$)	- Ussing chambers: ↓ TER, ↑ flux of ^{51}Cr-EDTA in IBS-M *versus* HV

(Continued)

Table 3. (Continued)

Reference	Study population	Findings
Colon – unspecified location		
Annaházi *et al*.[81]	IBS-C ($n=14$) HV ($n=33$)	- Western blot: ↓ occludin in IBS-C *versus* HV
Zhou *et al*.[51]	IBS-C ($n=74$) IBS-D ($n=109$) HV ($n=36$)	- PCR: ↑ mi-RNA-29a, mi-RNA-29b with ↑ permeability but not with = permeability or in IBS-C *versus* HV, = mi-RNA-29c in IBS-D *versus* HV - Northern blot: ↑ mi-RNA-29a, mi-RNA-29b in IBS-D with ↑ permeability but not with = permeability *versus* HV - Western blot: ↓ CLDN-1, NKRF in IBS-D with ↑ permeability but not with = permeability *versus* HV - Immunoblot: ↓ CLDN-1, NKRF in IBS-D with ↑ permeability but not with = permeability *versus* HV
Tulic *et al*.[97]	IBS ($n=8$) HV ($n=6$)	- Ussing chambers: ↑ flux of FITC-sulfonic acid in IBS *versus* HV
Bednarska *et al*.[91]	IBS-C ($n=8$) IBS-D ($n=8$) IBS-M ($n=21$) HV ($n=15$)	- Ussing chambers: ↑ flux of ^{51}Cr-EDTA and translocation of bacteria, ↓ TER after 0-30-60 but not 90 min in IBS *versus* HV - Immunofluorescence: = occludin, ZO-1 in IBS *versus* HV

^{51}Cr-EDTA chromium-51-ethylenediamine tetraacetic acid; CFL1, cofilin 1; CLDN, claudin; CLE, confocal laser endomicroscopy; DET, differentially expressed transcript; E, Escherichia; EM, electron microscopy; FDR, false discovery rate; FITC, fluorescein isothiocyanate; FN1, fibronectin-1; HRP, horseradish peroxidase; HV, healthy volunteers; IBS, irritable bowel syndrome; IBS-A, irritable bowel syndrome with alternating stool pattern; IBS-C, irritable bowel syndrome with constipation; IBS-D, irritable bowel syndrome with diarrhea; IBS-M, irritable bowel syndrome with mixed stool pattern; IPA, ingenuity pathways analysis; JAM, junctional adhesion molecule; MLCK, myosin light chain kinase; MMP1, matrix metalloprotease-1; mRNA, messenger ribonucleic acid; MUC20, mucin 20; MYLK2, myosin light chain kinase 2; NKRF, NF-kappa-β repressing factor; OCLN, occludin isof. B precursor; pCFL1, phosphorylated cofilin 1; PCR, polymerase chain reaction; pMLC, phosphorylated myosin light chain; p-occludin, phosphorylated occludin; PP1cδ, protein phosphatase 1 catalytic subunit delta; ppMLC, di-phosphorylated myosin light chain; RBP2, retinoblastoma binding protein 2; RNA, ribonucleic acid; TER, transepithelial electrical resistance; TESK1, testis-associated actin remodeling kinase 1; TFF1, trefoil factor 1; TJP1, tight junction protein ZO-1 isof. A; WDR72, WD repeat domain 72; WGCNA, weighted gene coexpression network analysis; ZO, zona occludens.

showed increased mucus secretion and larger intercellular gaps in IBS-D.[83] Furthermore, CLDN-2 was up-regulated, while the expression of CLDN-7 and other regulatory tight junction molecules such as occludin, junctional adhesion molecule (JAM)-1 and ZO-1 was similar to controls.[76] One study found a decreased expression of CLDN-1 in IBS-D,[83] whereas the other study did not.[76] In IBS-C, CLDN-1 was up-regulated and electronic microscopy showed intact tight junction complexes.[83] Collectively, tight junction complexes in the terminal ileum are predominantly altered in IBS-D but unchanged in IBS-C.

Cecum. Four studies (one IBS-D, three multiple subtypes) determined changes in the cecum. Three of these found a disrupted barrier,[85–87] whereas one did not.[76] The expression of JAM-1 and E-cadherin was decreased in IBS-A and IBS-D, compared with healthy controls.[85,87] However, a study by Ishimoto and colleagues did not find any changes in the expression of tight junction molecules in IBS-D.[76] Paracellular permeability in the cecum is disrupted in IBS, but the associated molecular changes require further exploration.

Ascending colon. Only one study focused on the epithelial barrier in the ascending colon,[83] and found widened intercellular gaps in 80% of IBS-D patients and a decreased expression of CLDN-1 compared with controls. There was no evidence for disrupted tight junctions in IBS-C. However, mucus secretion in IBS-C was impaired, suggesting the mucus barrier could be dysfunctional.[83]

Descending colon. Five studies (one IBS-D, four mixed) investigated the descending colon, all of which found evidence for a disrupted barrier.[63,88–90,94] Exposure of Caco-2 monolayers to biopsy supernatant of IBS patients increased flux of probes.[88,94] Furthermore, two studies found a decreased protein expression of occludin in

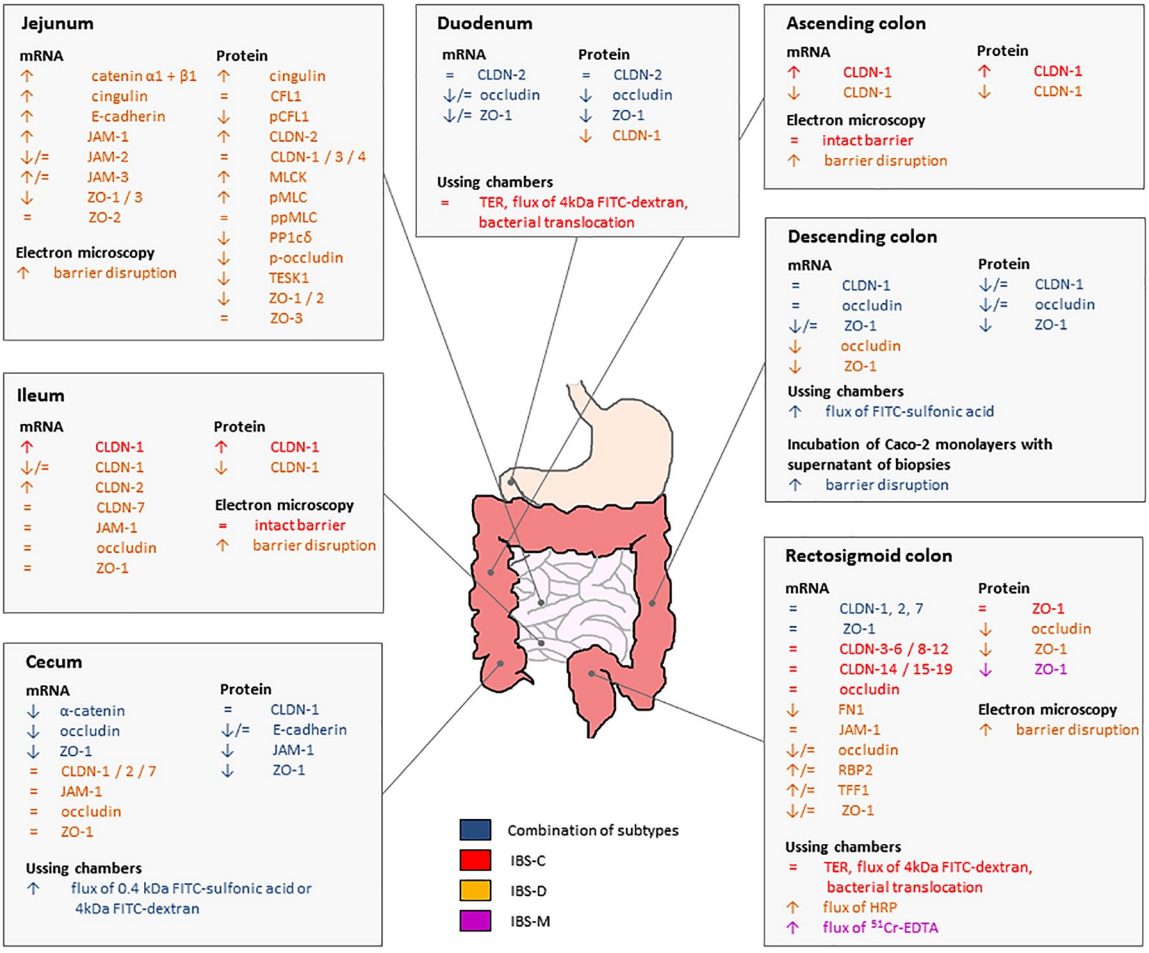

Figure 3. Overview of *in vitro* and *ex vivo* barrier function changes in the different parts of the gastrointestinal tract of IBS patients. Most studies are available from the rectosigmoid colon, jejunum and the cecum and studied an IBS-D population. There is a significant heterogeneity in the target proteins assessed and the methodology used for determination of changes *in vitro* and *ex vivo*.*

*Results of studies that did not specify the exact colonic region where biopsies were taken were not included in this figure, but are discussed in the main text.

colonic biopsies of IBS patients,[89,90] whereas differences in mRNA expression were conflicting across studies (for details, refer to Table 3).[63,89,90] Taken together, these studies clearly indicate the presence of increased intestinal permeability in the descending colon, although it remains unclear if differences exist between IBS subtypes.

Rectosigmoid colon. Being most easily accessible, intestinal barrier function in the rectosigmoid colon is most extensively described. Eight studies included an IBS-D population,[38,66,76,79,80,92,93,96] four IBS-C[54,92,93,96] and one IBS-M.[96] Seven of the ten studies found barrier dysfunction in IBS-D patients.[38,66,77–80,96] One found increased apical intercellular distances, as well as destruction of adherens junctions and desmosomes.[66] Evidence about the specific molecular changes involved is conflicting, but suggests a role for both occludin and ZO-1 (Table 3).[38,76,79,80,92,93,96] Five out of six studies examining the expression of several tight junction molecules found at least one significant change compared with healthy controls.[38,79,80,93,96] A study by Lee *et al.*[96] demonstrated that the differences in barrier function of IBS patients could be gender related, since they observed alterations in the expression of ZO-1 in females but not in males. Two studies in IBS-C did not find any alterations in the expression of tight junction proteins.[54,96] However, two other studies revealed alterations in the tight junction genes in IBS-C patients (Table 3).[92,93] Katinios and colleagues demonstrated an increased paracellular flux in sigmoid biopsies in IBS-M compared with

controls.[82] Although mRNA levels of tight junction molecules were similar to those in healthy controls, ZO-1 protein expression was decreased in a small study in IBS-M patients ($n=5$).[96]

The wealth of studies specifically examining the rectosigmoid colon has outlined that barrier function is mainly impaired in IBS-D and IBS-M, but not in IBS-C. This assertion, however, needs context as there is limited literature for IBS-C and IBS-M studies.

Colon (unidentified site). Three studies (one IBS-C and two multiple subtypes) investigated colonic permeability without presenting the sampling site in the colon,[51,81,91] two of which found changes in the expression of the tight junction molecules occludin and CLDN-1.[51,81] However, Bednarska and colleagues did not observe differences in the expression of occludin and ZO-1, even though they noted alterations in barrier function Ussing chamber experiments.[91]

Broadly, the rectosigmoid and descending colon are the most studied parts of the bowel and although preponderance of IBS-D studies demonstrates evidence of barrier dysfunction, the tight junction proteins involved and location along the GI tract are highly variable. Longitudinal sampling of the GI tract in the same individual may be able to provide a more complete understanding of barrier dysfunction in IBS. An example of such an attempt was duodenal and colonic assessment in IBS-C in our study, which showed unchanged barrier at both sites.[54] Furthermore, it should be noted that a change in the tight junction gene or protein expression or the ultrastructure does not necessarily imply an impaired barrier function.

Studies involving fecal samples. An overview of studies using IBS fecal samples to study the effects on permeability using a combination of *in vitro* and *in vivo* approaches is presented in Table 4.[81,99–104]

In vitro*: cell cultures.* T84 monolayers incubated with fecal supernatant (FSN) of IBS-C patients had an increased flux of 4kDa FITC-dextran and a loss of occludin as compared with supernatants from healthy controls.[81] Edogawa *et al.*[102] demonstrated that FSN from IBS patients (predominantly PI-IBS) with a high fecal proteolytic activity increased paracellular permeability in Caco-2 cell monolayers, compared with FSN of patients with a low fecal proteolytic activity. Additionally, high proteolytic activity-exposed monolayers had a lower expression of occludin and internalization of claudin-2, indicating likely involvement of both leak and pore pathways. Lastly, colonoids exposed to FSN of IBS-D patients were more permeable to 4kDa FITC-dextran than those exposed to FSN of healthy controls.[103]

In vivo*: colonic infusion of FSN in mice.* Infusion of FSN of IBS patients in the colon of mice resulted in an increased urinary excretion of ^{51}Cr-EDTA.[104] Another study demonstrated that mouse colonic epithelium exposed to FSN of IBS-D patients was more permeable to FITC-dextran in Ussing chambers, whereas epithelium exposed to FSN of IBS-C or IBS-M patients was not.[99] In contrast, FSN of IBS-C patients increased colonic flux to FITC-dextran in a study by Annaházi and colleagues.[81] At a molecular level, decreased colonic expression of occludin was also seen.[81] Furthermore, ZO-1 immunostaining showed intracellular internalization in response to IBS supernatants, which has been associated with weakening of the barrier.[99] Finally, colonic infusion of FSN resulted in an increase in the expression of phosphorylated myosin light chain, which has also been linked to a loss of barrier integrity.[99]

In vivo*: humanized rodent models.* Three studies have investigated the role of the microbiome in regulating intestinal permeability by gavaging human fecal slurry in germ-free rodents.[100–102] Three to six weeks later, barrier function was quantified. Urine excretion of orally administered ^{51}Cr-EDTA was unchanged in rats humanized with the fecal slurry from IBS-C patients. Although the gastrointestinal barrier was intact, these rats did display signs of visceral hypersensitivity.[100] In contrast, De Palma *et al.*[101] detected a disrupted *in vitro* colonic but not jejunal barrier following gavage with fecal slurry of IBS-D patients. In mice that were humanized with stool from IBS patients with a high proteolytic activity, creatinine but not 4kDa FITC-dextran or 70 kDa rhodamine-dextran crossed the barrier at a higher rate *in vivo*.[102] Proteolytic activity seems to be one of the important factors driving barrier dysfunction, since mice humanized with stool from an IBS patient with a low fecal proteolytic activity had an intact barrier similar to healthy volunteers.[102]

Table 4. Studies assessing effects of fecal slurries or fecal supernatant from adult IBS patients on barrier function in immortalized cell monolayers, organoids, intestinal tissue from rodents or germ-free mice.

Reference	Study population	Model	Findings
In vitro: cell cultures			
Annaházi *et al.*[81]	IBS-C, HV	*In vitro* assay	- Recombinant occludin degradation assay: ↑ occludin degradation by FSN from IBS-C *versus* HV
Annaházi *et al.*[81]	IBS-C, HV	T84 cell monolayer	- *In vitro* permeability: ↑ flux of 4kDa FITC-dextran in monolayers exposed to FSN from IBS-C *versus* HV
Edogawa *et al.*[102]	IBS with high FPA, IBS with low FPA	Caco-2 cell monolayer	- *In vitro* permeability: ↓ TER, ↑ flux of 4kDa Texas Red dextran in monolayers exposed to FSN from IBS with high FPA *versus* IBS with low FPA - Western blot + immunofluorescence: ↓ occludin, ↑ pMLC/MLC and co-localization with phalloidin, internalization of CLDN-2 in monolayers exposed to FSN from IBS with high FPA *versus* IBS with low FPA
Han *et al.*[103]	IBS-D, HV	Human colonoids	- *In vitro* permeability: ↓ retention of injected 4kDa FITC-dextran in colonoids exposed to FSN from IBS-D *versus* HV
In vivo: colonic infusion of FSN in mice			
Gecse *et al.*[99]	IBS-A, IBS-C, IBS-D, HV	C57BL/6J mice	- Ussing chambers: ↑ flux of 4kDa FITC-dextran in IBS-D *versus* HV but not IBS-C or IBS-A *versus* HV - Western blot: ↑ pMLC in IBS-D *versus* HV - Immunohistochemistry: pronounced and diffuse labeling of pMLC in epithelial cells and ZO-1 staining in the intracellular compartment, suggesting intensive internalization in IBS-D *versus* HV
Annaházi *et al.*[81]	IBS-C, HV	C57BL/6J mice	- *In vivo* probes: ↑ uptake of 4kDa FITC-dextran in the blood after 4 h but not 1 h in mice exposed to FSN from IBS-C *versus* HV - Western blot: ↓ occludin expression in colon from mice exposed to FSN from IBS-C *versus* HV
Nébot-Vivinus *et al.*[104]	IBS, HV	C57BL/6 mice	- *In vivo* probes: ↑ excretion of ^{51}Cr-EDTA *via* urine in mice exposed to FSN from IBS *versus* HV
In vivo: humanized rodent models			
Crouzet *et al.*[100]	IBS-C, HV	Humanized germ-free Fisher 344 albino rats	- *In vivo* probes: no difference in excretion of ^{51}Cr-EDTA *via* urine in rats humanized with stool from IBS-C *versus* HV
De Palma *et al.*[101]	IBS-D, HV	Humanized germ-free Swiss mice	- Ussing chambers: ↑ flux of ^{51}Cr-EDTA in colon tissue but = flux in jejunal tissue of mice humanized with stool from IBS-D *versus* HV
Edogawa *et al.*[102]	IBS with high FPA, IBS with low FPA, HV	Humanized germ-free Swiss Webster mice	- *In vivo* probes: ↑ uptake of creatinine in the blood in mice humanized with stool from IBS with high FPA *versus* HV, no differences in uptake of 4 kDa FITC-dextran and 70 kDa rhodamine-dextran

^{51}Cr-EDTA chromium-51-ethylenediamine tetraacetic acid; FITC-dextran, fluorescein isothiocyanate dextran; FPA, fecal proteolytic activity; FSN, fecal supernatant; HV, healthy volunteers; IBS, irritable bowel syndrome; IBS-A, irritable bowel syndrome with alternating stool pattern; IBS-C, irritable bowel syndrome with constipation; IBS-D, irritable bowel syndrome with diarrhea; pMLC, phosphorylated myosin light chain; ZO-1, zona occludens-1.

Collectively, *in vitro* cell culture and *in vivo* models using rodents have shed light on the potential mechanisms underlying barrier disruption in IBS patients. Again, greater evidence is available for IBS-D mediators to affect barrier compared with IBS-C and suggests an effect of proteases in mediation of barrier dysfunction by the luminal contents.

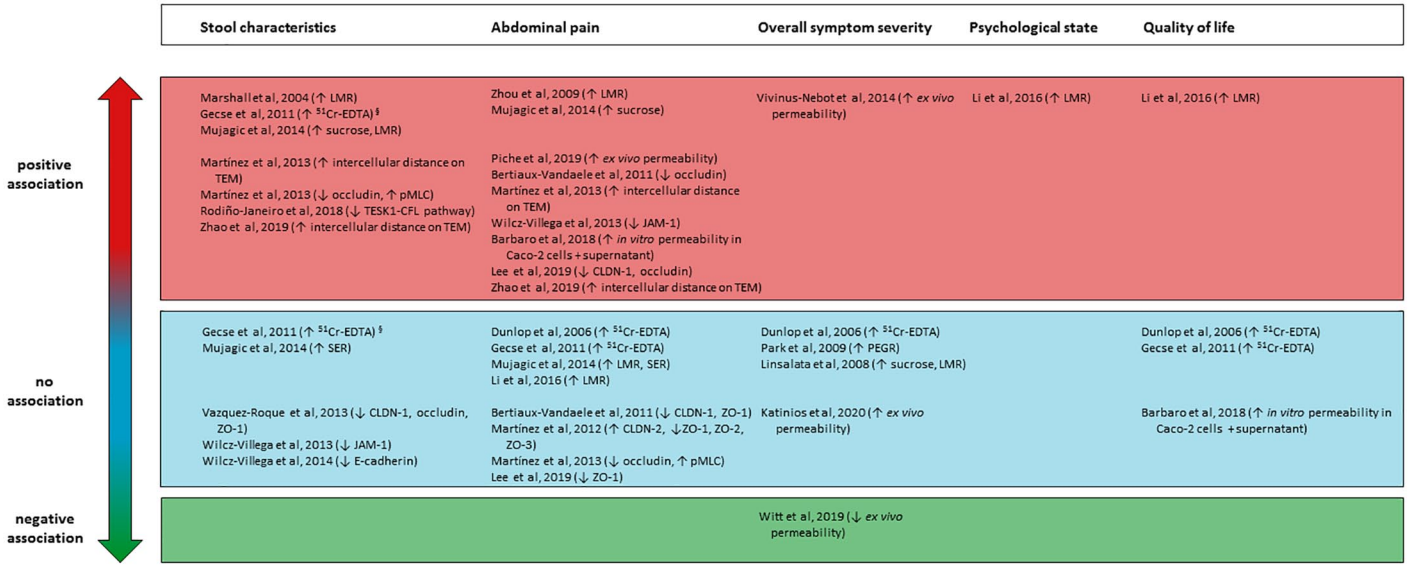

Figure 4. Overview of studies reporting associations between barrier function and stool characteristics, abdominal pain, overall symptom severity, psychological functioning and quality of life in IBS patients. A positive association (red color) indicates study concluded barrier dysfunction to be positively correlated with a more severe symptomatology in IBS patients *versus* no correlation (blue color) *versus* a negative correlation (green color).§

§Gecse and colleagues found an association between an increased intestinal permeability and stool frequency, but no association between stool consistency and increased intestinal permeability.

^{51}Cr-EDTA, 51Cr-EDTA, chromium-51-ethylenediamine tetraacetic acid; CFL, cofilin; CLDN, claudin; JAM-1, junctional adhesion molecule 1; LMR, lactulose-to-mannitol ratio; PEGR, polyethylene glycol 400 to polyethylene glycol 3350 ratio; pMLC, phosphorylated myosin light chain; SER, sucralose-to-erythritol ratio; TEM, transmission electron microscopy; TESK1, testis-associated actin remodeling kinase 1; ZO, zona occludens.

Barrier dysfunction and IBS symptomatology

Association with abdominal pain. One study found a modest but significant relationship between an increased *in vivo* intestinal permeability and severity of abdominal pain (Figure 4).[50] Zhou *et al.*[40] found that an increased LMR was associated with somatic hypersensitivity in response to thermal stimulation as well as visceral hypersensitivity to rectal distension. However, three other studies did not find correlations between abdominal pain and *in vivo* permeability.[37,43,53] In a small study visualizing the colonic mucosa ultrastructurally, intercellular gaps correlated with the frequency of abdominal pain[66] and similar findings were noted in jejunal[73] and colonic mucosa.[88] Lastly, *in vitro* barrier changes caused by colonic biopsy supernatants associated with both the severity and the frequency of abdominal pain.[94] The colonic expression of the tight junction molecules CLDN-1, ZO-1 and occludin was correlated with abdominal pain, although only the occludin expression remained significant in a multivariate analysis.[90] In a recent study, colonic mRNA expression of occludin and CLDN-1 showed a threefold and tenfold decrease in the patients experiencing more pain.[96] A lower cecal expression of JAM-1 was shown to be associated with more severe abdominal pain in IBS-M but not IBS-D patients.[85] However, CLDN-1 and ZO-1 expression in the cecum were not correlated with abdominal pain.[87] Furthermore, changes in zonula occludens 1–3, CLDN-2, occludin or pMLC expression in the jejunum could not be linked to either the intensity or the frequency of abdominal pain.[72,73]

Studies based on *in vivo* permeability have provided mixed results for the association with abdominal pain. However, ultrastructural as well as gene expression studies provide a more consistent association of barrier dysfunction with abdominal pain. Additional work needs to be done to better understand how changes in tight junction proteins mediate visceral pain in IBS.

Associations with stool characteristics. A positive correlation was found between the severity of diarrhea and both gastroduodenal and small intestinal permeability.[50] Stool frequency, but not stool consistency, was found to associate with whole-gut permeability in PI-IBS and colonic permeability in IBS-D patients.[36,43] Ultrastructural disruption

of tight junctions in jejunum and colorectum of IBS-D patients correlated with both greater stool frequency and looser consistency.[66,73] Furthermore, jejunal expression of occludin and pMLC correlated with the severity of diarrhea in IBS-D.[73] In female IBS-D patients, there was a downregulation of the TESK1/CFL pathway in the jejunum, which is involved in regulating cytoskeleton dynamics associated with bowel movements.[75] No significant correlations between stool characteristics and cecal expression of E-cadherin or JAM-1 could be detected[85,87] or with duodenal or colonic expression of ZO-1, claudin-1 and occludin in another study.[46] Although results were mixed, a greater number of studies associate small bowel changes with stool frequency.

Associations with overall severity scores. Vivinus-Nébot *et al.*[86] found a moderate positive correlation between IBS symptom severity and cecal paracellular permeability of IBS-C, IBS-D and IBS-M patients. However, an opposite result was found by Witt *et al.*,[105] who found a negative correlation between IBS severity scores and colonic paracellular permeability. Four other studies did not detect significant correlations between the overall symptom severity and barrier disruption, regardless of the IBS subtype.[37,39,57,82] IBS symptom severity is a composite measure of pain and bowel dissatisfaction which is probably driven by multiple factors, and changes in intestinal permeability may only partially drive the overall symptom severity.

Association with psychological functioning. IBS-D patients with an increased permeability scored higher on the Hospital Anxiety and Depression Scale for both anxiety and depression subscales.[53] In another study, the effect of anxiety and depression symptoms on barrier function was found to be small and not statistically significant.[50] Taken together, these studies suggest a potential for cross-talk between gut barrier function and psychological stress in IBS but also a lack of conclusive evidence for the same hypothesis.

Association with quality of life. One study associated an increased intestinal permeability with a decrease in QoL,[53] whereas three did not.[37,43,94] Li and colleagues observed a lower QoL in the subgroup of IBS-D patients with increased small intestinal permeability.[53] However, no significant correlations between intestinal permeability in IBS-C or PI-IBS patients and items from the IBS QoL questionnaire were found in a small study.[37] This was subsequently shown in other IBS subtypes as well.[43,94] Thus, there is overall weak evidence for permeability to explain overall QoL. This is not unexpected considering the complex and heterogeneous nature of the disease and the fact that only a variable subset of IBS patients have increased permeability, which likely results in weaker correlations for composite measures like symptom severity and QoL.

Discussion

This review provides several useful insights into barrier dysfunction in IBS. First, this is the largest systematic review and the first to be performed using PRISMA guidelines (includes 67 studies). Second, the criteria used to diagnose and subtype IBS were critically assessed in relation to the changes in the barrier function. Third, we comprehensively assessed methodologies (*in vivo*, *ex vivo* and *in vitro*) used to measure barrier function while also stratifying the studies according to the location along the GI tract. Fourth, barrier changes were associated with IBS symptomatology (abdominal pain, stool characteristics, symptom severity), psychological comorbidities and QoL. Finally, we included pediatric studies which, although limited in number, provide a reflection of barrier dysfunction in that population.

Increased intestinal permeability was present in 37–62% of IBS-D and 16–50% of PI-IBS patients. More IBS-C studies showed unchanged permeability compared with controls, and the ones showing increased permeability had smaller proportions of patients with increased permeability (4–25%). Unfortunately, the prevalence of barrier disruption in IBS-M remains unclear. Another important finding is that changes in the expression of tight junction genes or the ultrastructure were not specific to any particular region of the intestinal tract. However, only a limited number of studies examined different regions in the bowel.[51,54,76,83] In three studies, findings were consistent across the different regions,[51,76,83] whereas one study detected an increased expression of CLDN-2 in the ileum, but not in the cecum or the rectosigmoid colon.[76] The mucosal and luminal milieu, microbiome[106,107] and motility[108,109] exhibit biological differences both spatially as well as between individuals. This makes exploration of different regions of the gut within

the same volunteers essential to comprehensively understand changes in mucosal barrier function.

Demographic factors can influence barrier function as well. A recent study found that elderly IBS patients have greater disruption of small intestinal barrier compared with their younger counterparts.[110] Another study, however, found no effect of adjusting for age on permeability changes in IBS-D.[50] Furthermore, the expression of several tight junction (occludin, ZO-1 and CLDN-1) and adherens junction (JAM-1 and E-cadherin) molecules was not correlated with age in IBS patients.[49,85,87,90] There is an established female predominance in IBS. Some studies in healthy volunteers found a lower intestinal permeability in females than males,[111–113] although this was not confirmed by others.[114–116] In IBS patients, sex differences in barrier function were noted.[36,50,70,96] Sucrose excretion in IBS males was higher than females, indicating increased gastroduodenal permeability in males.[50] Furthermore, in the Walkerton cohort, IBS males had higher permeability.[36] In contrast with these findings, ZO-1 expression was decreased in female IBS-D patients, whereas this was unchanged in males.[96] Other *in vitro* studies did not find any sex differences in tight junction gene expression in the duodenum, cecum and descending colon.[49,85,87,90] Thus, the interaction between sex and permeability is still incompletely understood. Lastly, obesity has been associated with an impaired barrier function.[117–120] Two studies in IBS patients found a positive association between permeability and BMI.[50,90] BMI was a strong confounder of sucrose excretion in one study.[50] Furthermore, occludin expression was lower in obese patients ($BMI > 30\,kg\,m^{-2}$) compared with non-obese patients.[90] Compared with controls, IBS patients had a significantly higher BMI in two studies, which raises the question whether increased permeability in these particular studies is due to the effects of BMI.[45,59] Hence, we believe BMI should always be taken into consideration when designing and analyzing permeability studies.

We aimed to comprehensively document differences in the experimental protocols used to assess *in vivo* permeability. We observed large differences in the cut-off values (LMR > 0.015–0.07). However, cut-off values were mainly derived from prospectively enrolled healthy volunteers or historic controls from the same geographic region. When assessing future studies based on cut-off values, it is most ideal if those were obtained from a demographically and geographically similar population and ideally by the same investigators using identical protocols. The intake of food or drinks affects the passage of probe solutions throughout the GI lumen.[64] An overnight fasting period was reported in the majority of studies discussing dietary restrictions. Because 14/25 *in vivo* studies did not document restrictions imposed on their patients, it cannot be excluded that these affected the outcome. The urine collection time varied strongly across studies. Interestingly, studies using shorter collection periods also showed significant differences between IBS patients and controls, suggesting involvement of the proximal gut in the pathophysiology of IBS, which has been relatively underexplored with most studies focusing on the distal colon. We observed a positive correlation between barrier dysfunction and the diarrhea severity. Changes in claudin proteins can impair ionic fluxes which can perturb net absorption of water across the colonic epithelium. How these changes in tight junction proteins may lead to physiological changes contributing to diarrhea remains to be understood.[21,121] Psychological disturbances such as anxiety and depression are highly prevalent in IBS patients,[122,123] and can modulate intestinal barrier dysfunction, potentially *via* hypothalamic–pituitary adrenal axis-induced mast cell activation.[124,125] Acute stress in healthy volunteers has been shown to increase intestinal permeability as well.[124] Chronic anxiety and depression can by itself impair barrier function.[126] Although there is some suggestion that IBS patients with anxiety and depression have greater impairment of barrier functions, understanding the precise role of psychological factors will require cohorts of IBS patients with and without these psychological factors.[53,127] One such cohort is our PI-IBS cohort that is fairly low in anxiety and depression scores but still demonstrates a high prevalence of impaired barrier function.[102] A recent randomized clinical trial by Zhou and colleagues demonstrated that glutamine supplementation in PI-IBS (IBS-D) patients with increased intestinal permeability resulted in improvement of stool frequency, consistency, abdominal pain, overall IBS symptom severity scores, and a reduction of intestinal permeability.[27] This suggests intestinal permeability can be specifically targeted resulting in an improvement of barrier function and clinical symptoms.

Both biopsy and fecal supernatants from IBS patients impair barrier *in vitro*, suggesting a role

of peripheral mediators. The exact mediators and their mechanism of action have yet to be fully unraveled, but food antigens,[128] proteases,[81,99,102] bile acids[129] and short-chain fatty acids[130] are among the top targets. The recent development of humanized animal models as well as organoids provides unique platforms to study the effects of specific patient-derived mediators on host environment that more closely resemble the complexity of humans. Although evidence in children with IBS is limited, it points to permeability as a relevant pathophysiological mechanism in approximately 60% of the patients.[68] Research in children is mainly hampered by the lack of structural investigations, and mainly focuses on the non-invasive *in vivo* permeability assays. McOmber and colleagues found an increased permeability in healthy siblings and parents of children with IBS, indicating a familial predisposition toward the development of barrier disruption.[71] Several genetic variations in IBS patients have been described, some of which, like CDH-1, have been associated with increased permeability.[131–134]

We recognize our review has limitations. Heterogeneity in experimental protocols used to assess *in vivo* permeability in regard to the type and amount of permeability probes used, dietary restrictions for patients and the timing of urine collections make it hard to reach concrete conclusions. Therefore, we summarized the differences in the protocols used and accounted for them when interpreting the results. Similarly, tight junction gene expression studies are hard to interpret, due to differences in genes, examined sites, and methodology. Clinical characteristics such as age, BMI and gender can confound permeability assays and, ideally, should be accounted for in the analysis and interpretation of individual studies. Unfortunately, most studies reviewed did not use multivariable statistics so the differences observed might be influenced, either positively or negatively, by any of these variables. The clinical diversity of IBS populations resulted in a low number of studies per IBS subtype, making it difficult to formally assess the presence of publication bias. Finally, most studies did not provide IBS severity and psychiatric comorbidities, which limited comprehensive assessment of their associations with intestinal permeability.

Conclusion

Barrier dysfunction is present in a significant proportion of patients with IBS, especially in the IBS-D and PI-IBS subtypes. Future studies should attempt to use standardized experimental protocols to increase reproducibility. Furthermore, potential confounders like age, BMI, sex, psychological factors and diet should be adjusted for before drawing conclusions. The mechanisms underlying barrier dysfunction in IBS need to be studied but several studies have pointed to potential drivers. These include mast cell activation,[78,91] microbiome changes,[102] diet[46] and mediators such as vasoactive intestinal polypeptide,[91] serotonin,[49] serine proteases[49] and cysteine proteases.[81] Further research is necessary to identify specific therapeutic targets for addressing increased permeability and assays to determine patient subsets that are most likely to benefit from those targets, underscoring the need for personalized treatment of IBS.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: NH is supported by a grant from the Flemish Institute for Research (FWO-SBO grant nr. S001017) and a grant from the University of Antwerp (TOP grant nr. 35018). MG is supported by NIDDK K23 103911 and R03 120745

Conflict of interest statement

The authors declare that there is no conflict of interest.

ORCID iDs

Benedicte Y. De Winter https://orcid.org/0000-0003-0327-6304

Madhusudan Grover https://orcid.org/0000-0001-5092-0831

Supplemental material

Supplemental material for this article is available online.

References

1. Ford AC, Lacy BE and Talley NJ. Irritable bowel syndrome. *N Engl J Med* 2017; 376: 2566–2578.
2. Holtmann GJ, Ford AC and Talley NJ. Pathophysiology of irritable bowel syndrome. *Lancet Gastroenterol Hepatol* 2016; 1: 133–146.
3. Simrén M and Tack J. New treatments and therapeutic targets for IBS and other functional

bowel disorders. *Nat Rev Gastroenterol Hepatol* 2018; 15: 589–605.

4. Sibelli A, Chalder T, Everitt H, *et al.* A systematic review with meta-analysis of the role of anxiety and depression in irritable bowel syndrome onset. *Psychol Med* 2016; 46: 3065–3080.
5. Klem F, Wadhwa A, Prokop LJ, *et al.* Prevalence, risk factors, and outcomes of irritable bowel syndrome after infectious enteritis: a systematic review and meta-analysis. *Gastroenterology* 2017; 152: 1042–1054.e1.
6. Helander HF and Fändriks L. Surface area of the digestive tract - revisited. *Scand J Gastroenterol* 2014; 49: 681–689.
7. Maynard CL, Elson CO, Hatton RD, *et al.* Reciprocal interactions of the intestinal microbiota and immune system. *Nature* 2012; 489: 231–241.
8. Johansson ME, Phillipson M, Petersson J, *et al.* The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proc Natl Acad Sci U S A* 2008; 105: 15064–15069.
9. Ehmann D, Wendler J, Koeninger L, *et al.* Paneth cell α-defensins HD-5 and HD-6 display differential degradation into active antimicrobial fragments. *Proc Natl Acad Sci U S A* 2019; 116: 3746–3751.
10. Corr SC, Li Y, Riedel CU, *et al.* Bacteriocin production as a mechanism for the antiinfective activity of Lactobacillus salivarius UCC118. *Proc Natl Acad Sci U S A* 2007; 104: 7617–7621.
11. Kommineni S, Bretl DJ, Lam V, *et al.* Bacteriocin production augments niche competition by enterococci in the mammalian gastrointestinal tract. *Nature* 2015; 526: 719–722.
12. Kamada N, Kim YG, Sham HP, *et al.* Regulated virulence controls the ability of a pathogen to compete with the gut microbiota. *Science* 2012; 336: 1325–1329.
13. Johansson ME and Hansson GC. Immunological aspects of intestinal mucus and mucins. *Nat Rev Immunol* 2016; 16: 639–649.
14. Pelaseyed T and Hansson GC. Membrane mucins of the intestine at a glance. *J Cell Sci* 2020; 133: jcs240929.
15. Otani T, Nguyen TP, Tokuda S, *et al.* Claudins and JAM-A coordinately regulate tight junction formation and epithelial polarity. *J Cell Biol* 2019; 218: 3372–3396.
16. Schlegel N, Meir M, Heupel W-M, *et al.* Desmoglein 2-mediated adhesion is required for intestinal epithelial barrier integrity. *Am J Physiol Gastrointest Liver Physiol* 2010; 298: G774–G783.
17. Allaire JM, Morampudi V, Crowley SM, *et al.* Frontline defenders: goblet cell mediators dictate host-microbe interactions in the intestinal tract during health and disease. *Am J Physiol Gastrointest Liver Physiol* 2018; 314: G360–G377.
18. Benguettat O, Jneid R, Soltys J, *et al.* The DH31/CGRP enteroendocrine peptide triggers intestinal contractions favoring the elimination of opportunistic bacteria. *PLoS Pathog* 2018; 14: e1007279.
19. Watson CJ, Rowland M and Warhurst G. Functional modeling of tight junctions in intestinal cell monolayers using polyethylene glycol oligomers. *Am J Physiol Cell Physiol* 2001; 281: C388–C397.
20. Krug SM, Amasheh S, Richter JF, *et al.* Tricellulin forms a barrier to macromolecules in tricellular tight junctions without affecting ion permeability. *Mol Biol Cell* 2009; 20: 3713–3724.
21. Tsai PY, Zhang B, He WQ, *et al.* IL-22 upregulates epithelial claudin-2 to drive diarrhea and enteric pathogen clearance. *Cell Host Microbe* 2017; 21: 671–681.e674.
22. Weber CR, Raleigh DR, Su L, *et al.* Epithelial myosin light chain kinase activation induces mucosal interleukin-13 expression to alter tight junction ion selectivity. *J Biol Chem* 2010; 285: 12037–12046.
23. Marchiando AM, Shen L, Graham WV, *et al.* Caveolin-1-dependent occludin endocytosis is required for TNF-induced tight junction regulation in vivo. *J Cell Biol* 2010; 189: 111–126.
24. Turner JR, Buschmann MM, Romero-Calvo I, *et al.* The role of molecular remodeling in differential regulation of tight junction permeability. *Semin Cell Dev Biol* 2014; 36: 204–212.
25. Odenwald MA and Turner JR. The intestinal epithelial barrier: a therapeutic target? *Nat Rev Gastroenterol Hepatol* 2017; 14: 9–21.
26. Long Y, Du L, Kim JJ, *et al.* MLCK-mediated intestinal permeability promotes immune activation and visceral hypersensitivity in PI-IBS mice. *Neurogastroenterol Motil* 2018; 30: e13348.
27. Zhou Q, Verne ML, Fields JZ, *et al.* Randomised placebo-controlled trial of dietary glutamine supplements for postinfectious

irritable bowel syndrome. *Gut* 2019; 68: 996–1002.

28. Vanheel H, Vicario M, Vanuytsel T, *et al.* Impaired duodenal mucosal integrity and low-grade inflammation in functional dyspepsia. *Gut* 2014; 63: 262–271.
29. Camilleri M, Lasch K and Zhou W. Irritable bowel syndrome: methods, mechanisms, and pathophysiology. The confluence of increased permeability, inflammation, and pain in irritable bowel syndrome. *Am J Physiol Gastrointest Liver Physiol* 2012; 303: G775–G785.
30. Bischoff SC, Barbara G, Buurman W, *et al.* Intestinal permeability - a new target for disease prevention and therapy. *BMC Gastroenterology* 2014; 14: 189.
31. Moher D, Liberati A, Tetzlaff J, *et al.* Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009; 6: e1000097.
32. Downes MJ, Brennan ML, Williams HC, *et al.* Development of a critical appraisal tool to assess the quality of cross-sectional studies (AXIS). *BMJ Open* 2016; 6: e011458.
33. Lobley RW, Burrows PC, Warwick R, *et al.* Simultaneous assessment of intestinal permeability and lactose tolerance with orally administered raffinose, lactose and L-arabinose. *Clin Sci (Lond)* 1990; 79: 175–183.
34. Paganelli R, Fagiolo U, Cancian M, *et al.* Intestinal permeability in irritable bowel syndrome. Effect of diet and sodium cromoglycate administration. *Ann Allergy* 1990; 64: 377–380.
35. Spiller RC, Jenkins D, Thornley JP, *et al.* Increased rectal mucosal enteroendocrine cells, T lymphocytes, and increased gut permeability following acute Campylobacter enteritis and in post-dysenteric irritable bowel syndrome. *Gut* 2000; 47: 804–811.
36. Marshall JK, Thabane M, Garg AX, *et al.* Intestinal permeability in patients with irritable bowel syndrome after a waterborne outbreak of acute gastroenteritis in Walkerton, Ontario. *Aliment Pharmacol Ther* 2004; 20: 1317–1322.
37. Dunlop SP, Hebden J, Campbell E, *et al.* Abnormal intestinal permeability in subgroups of diarrhea-predominant irritable bowel syndromes. *Am J Gastroenterol* 2006; 101: 1288–1294.
38. Zeng J, Li YQ, Zuo XL, *et al.* Clinical trial: effect of active lactic acid bacteria on mucosal barrier function in patients with diarrhoea-predominant irritable bowel syndrome. *Aliment Pharmacol Ther* 2008; 28: 994–1002.
39. Park JH, Park DI, Kim HJ, *et al.* The relationship between small-intestinal bacterial overgrowth and intestinal permeability in patients with irritable bowel syndrome. *Gut Liver* 2009; 3: 174–179.
40. Zhou Q, Zhang B and Verne GN. Intestinal membrane permeability and hypersensitivity in the irritable bowel syndrome. *Pain* 2009; 146: 41–46.
41. Kerckhoffs AP, Akkermans LM, de Smet MB, *et al.* Intestinal permeability in irritable bowel syndrome patients: effects of NSAIDs. *Dig Dis Sci* 2010; 55: 716–723.
42. Zhou Q, Souba WW, Croce CM, *et al.* MicroRNA-29a regulates intestinal membrane permeability in patients with irritable bowel syndrome. *Gut* 2010; 59: 775–784.
43. Gecse K, Roka R, Sera T, *et al.* Leaky gut in patients with diarrhea-predominant irritable bowel syndrome and inactive ulcerative colitis. *Digestion* 2011; 85: 40–46.
44. Mattioli F, Fucile C, Marini V, *et al.* Assessment of intestinal permeability using sugar probes: influence of urinary volume. *Clin Lab* 2011; 57: 909–918.
45. Rao AS, Camilleri M, Eckert DJ, *et al.* Urine sugars for in vivo gut permeability: validation and comparisons in irritable bowel syndrome-diarrhea and controls. *Am J Physiol Gastrointest Liver Physiol* 2011; 301: G919–G928.
46. Vazquez-Roque MI, Camilleri M, Smyrk T, *et al.* A controlled trial of gluten-free diet in patients with irritable bowel syndrome-diarrhea: effects on bowel frequency and intestinal function. *Gastroenterology* 2013; 144: 903–911.e903.
47. Del Valle-Pinero AY, Van Deventer HE, Fourie NH, *et al.* Gastrointestinal permeability in patients with irritable bowel syndrome assessed using a four probe permeability solution. *Clin Chim Acta* 2013; 418: 97–101.
48. Swan C, Duroudier NP, Campbell E, *et al.* Identifying and testing candidate genetic polymorphisms in the irritable bowel syndrome (IBS): association with TNFSF15 and TNFα. *Gut* 2013; 62: 985–994.
49. Keszthelyi D, Troost FJ, Jonkers DM, *et al.* Serotonergic reinforcement of intestinal barrier function is impaired in irritable bowel syndrome. *Aliment Pharmacol Ther* 2014; 40: 392–402.
50. Mujagic Z, Ludidi S, Keszthelyi D, *et al.* Small intestinal permeability is increased in diarrhoea predominant IBS, while alterations in

gastroduodenal permeability in all IBS subtypes are largely attributable to confounders. *Aliment Pharmacol Ther* 2014; 40: 288–297.

51. Zhou Q, Costinean S, Croce CM, *et al.* MicroRNA 29 targets nuclear factor-κB-repressing factor and Claudin 1 to increase intestinal permeability. *Gastroenterology* 2015; 148: 158–169.e158.
52. Jarrett ME, Cain KC, Barney PG, *et al.* Balance of autonomic nervous system predicts who benefits from a self-management intervention program for irritable bowel syndrome. *J Neurogastroenterol Motil* 2016; 22: 102–111.
53. Li L, Xiong L, Yao J, *et al.* Increased small intestinal permeability and RNA expression profiles of mucosa from terminal ileum in patients with diarrhoea-predominant irritable bowel syndrome. *Dig Liver Dis* 2016; 48: 880–887.
54. Peters SA, Edogawa S, Sundt WJ, *et al.* Constipation-predominant irritable bowel syndrome females have normal colonic barrier and secretory function. *Am J Gastroenterol* 2017; 112: 913–923.
55. Russo F, Chimienti G, Linsalata M, *et al.* The obestatin/ghrelin ratio and ghrelin genetics in adult celiac patients before and after a gluten-free diet, in irritable bowel syndrome patients and healthy individuals. *Eur J Gastroenterol Hepatol* 2017; 29: 160–168.
56. Valentin N, Camilleri M, Carlson P, *et al.* Potential mechanisms of effects of serum-derived bovine immunoglobulin/protein isolate therapy in patients with diarrhea-predominant irritable bowel syndrome. *Physiol Rep* 2017; 5: e13170.
57. Linsalata M, Riezzo G, D'Attoma B, *et al.* Noninvasive biomarkers of gut barrier function identify two subtypes of patients suffering from diarrhoea predominant-IBS: a case-control study. *BMC Gastroenterol* 2018; 18: 167.
58. Russo F, Chimienti G, Riezzo G, *et al.* Adipose tissue-derived biomarkers of intestinal barrier functions for the characterization of diarrhoea-predominant IBS. *Dis Markers* 2018; 2018: 1827937.
59. Camilleri M, Shin A, Busciglio I, *et al.* Validating biomarkers of treatable mechanisms in irritable bowel syndrome. *Neurogastroenterol Motil* 2014; 26: 1677–1685.
60. Chadwick VS, Phillips SF and Hofmann AF. Measurements of intestinal permeability using low molecular weight polyethylene glycols (PEG 400). I. Chemical analysis and biological properties of PEG 400. *Gastroenterology* 1977; 73: 241–246.
61. Grover M, Camilleri M, Hines J, *et al.* (13) C mannitol as a novel biomarker for measurement of intestinal permeability. *Neurogastroenterol Motil* 2016; 28: 1114–1119.
62. Ordiz MI, Davitt C, Stephenson K, *et al.* EB 2017 article: interpretation of the lactulose:mannitol test in rural Malawian children at risk for perturbations in intestinal permeability. *Exp Biol Med (Maywood)* 2018; 243: 677–683.
63. Vazquez-Roque MI, Camilleri M, Smyrk T, *et al.* Association of HLA-DQ gene with bowel transit, barrier function, and inflammation in irritable bowel syndrome with diarrhea. *Am J Physiol Gastrointest Liver Physiol* 2012; 303: G1262–G1269.
64. Jones RB, Dockray GJ and Thompson DG. The effects of fasting duration on gastric emptying in man, an exploration of the role of the endocannabinoid system and inter-individual responsiveness. *Neurogastroenterol Motil* 2012; 24: 928–e461.
65. Turcotte JF, Kao D, Mah SJ, *et al.* Breaks in the wall: increased gaps in the intestinal epithelium of irritable bowel syndrome patients identified by confocal laser endomicroscopy (with videos). *Gastrointest Endosc* 2013; 77: 624–630.
66. Zhao DY, Qi QQ, Long X, *et al.* Ultrastructure of intestinal mucosa in diarrhea-predominant irritable bowel syndrome. *Physiol Int* 2019; 106: 225–235.
67. Shulman RJ, Eakin MN, Czyzewski DI, *et al.* Increased gastrointestinal permeability and gut inflammation in children with functional abdominal pain and irritable bowel syndrome. *J Pediatr* 2008; 153: 646–650.
68. Francavilla R, Miniello V, Magistà AM, *et al.* A randomized controlled trial of Lactobacillus GG in children with functional abdominal pain. *Pediatrics* 2010; 126: e1445–e1452.
69. Gervasoni J, Schiattarella A, Giorgio V, *et al.* Validation of an LC-MS/MS method for urinary lactulose and mannitol quantification: results in patients with irritable bowel syndrome. *Dis Markers* 2016; 2016: 5340386.
70. Shulman RJ, Devaraj S and Heitkemper M. Gut permeability is affected by sex and increased in children with irritable bowel syndrome but not in functional abdominal pain. *Neurogastroenterol Motil* 2020; 32: e13765.

71. McOmber M, Rafati D, Cain K, *et al*. Increased gut permeability in first-degree relatives of children with irritable bowel syndrome or functional abdominal pain. *Clin Gastroenterol Hepatol* 2020; 18: 375–384.e1.

72. Martínez C, Vicario M, Ramos L, *et al*. The jejunum of diarrhea-predominant irritable bowel syndrome shows molecular alterations in the tight junction signaling pathway that are associated with mucosal pathobiology and clinical manifestations. *Am J Gastroenterol* 2012; 107: 736–746.

73. Martínez C, Lobo B, Pigrau M, *et al*. Diarrhoea-predominant irritable bowel syndrome: an organic disorder with structural abnormalities in the jejunal epithelial barrier. *Gut* 2013; 62: 1160–1168.

74. Martínez C, Rodiño-Janeiro BK, Lobo B, *et al*. miR-16 and miR-125b are involved in barrier function dysregulation through the modulation of claudin-2 and cingulin expression in the jejunum in IBS with diarrhoea. *Gut* 2017; 66: 1537–1538.

75. Rodiño-Janeiro BK, Martínez C, Fortea M, *et al*. Decreased TESK1-mediated cofilin 1 phosphorylation in the jejunum of IBS-D patients may explain increased female predisposition to epithelial dysfunction. *Sci Rep* 2018; 8: 2255.

76. Ishimoto H, Oshima T, Sei H, *et al*. Claudin-2 expression is upregulated in the ileum of diarrhea predominant irritable bowel syndrome patients. *J Clin Biochem Nutr* 2017; 60: 146–150.

77. Lee H, Park JH, Park DI, *et al*. Mucosal mast cell count is associated with intestinal permeability in patients with diarrhea predominant irritable bowel syndrome. *J Neurogastroenterol Motil* 2013; 19: 244–250.

78. Zhen Y, Chu C, Zhou S, *et al*. Imbalance of tumor necrosis factor-α, interleukin-8 and interleukin-10 production evokes barrier dysfunction, severe abdominal symptoms and psychological disorders in patients with irritable bowel syndrome-associated diarrhea. *Mol Med Rep* 2015; 12: 5239–5245.

79. Camilleri M, Carlson P, Acosta A, *et al*. RNA sequencing shows transcriptomic changes in rectosigmoid mucosa in patients with irritable bowel syndrome-diarrhea: a pilot case-control study. *Am J Physiol Gastrointest Liver Physiol* 2014; 306: G1089–G1098.

80. Annaházi A, Ferrier L, Bézirard V, *et al*. Luminal cysteine-proteases degrade colonic tight junction structure and are responsible for abdominal pain in constipation-predominant IBS. *Am J Gastroenterol* 2013; 108: 1322–1331.

81. Katinios G, Casado-Bedmar M, Walter SA, *et al*. Increased colonic epithelial permeability and mucosal eosinophilia in ulcerative colitis in remission compared with irritable bowel syndrome and health. *Inflamm Bowel Dis* 2020; 26: 974–984.

82. Cheng P, Yao J, Wang C, *et al*. Molecular and cellular mechanisms of tight junction dysfunction in the irritable bowel syndrome. *Mol Med Report* 2015; 12: 3257–3264.

83. Vivinus-Nébot M, Dainese R, Anty R, *et al*. Combination of allergic factors can worsen diarrheic irritable bowel syndrome: role of barrier defects and mast cells. *Am J Gastroenterol* 2012; 107: 75–81.

84. Wilcz-Villega EM, McClean S and O'Sullivan MA. Mast cell tryptase reduces junctional adhesion molecule-a (JAM-A) expression in intestinal epithelial cells: implications for the mechanisms of barrier dysfunction in irritable bowel syndrome. *Am J Gastroenterol* 2013; 108: 1140–1151.

85. Vivinus-Nébot M, Frin-Mathy G, Bzioueche H, *et al*. Functional bowel symptoms in quiescent inflammatory bowel diseases: role of epithelial barrier disruption and low-grade inflammation. *Gut* 2014; 63: 744–752.

86. Wilcz-Villega E, McClean S and O'Sullivan M. Reduced E-cadherin expression is associated with abdominal pain and symptom duration in a study of alternating and diarrhea predominant IBS. *Neurogastroenterol Motil* 2014; 26: 316–325.

87. Piche T, Barbara G, Aubert P, *et al*. Impaired intestinal barrier integrity in the colon of patients with irritable bowel syndrome: involvement of soluble mediators. *Gut* 2009; 58: 196–201.

88. Coëffier M, Gloro R, Boukhettala N, *et al*. Increased proteasome-mediated degradation of occludin in irritable bowel syndrome. *Am J Gastroenterol* 2010; 105: 1181–1188.

89. Bertiaux-Vandaele N, Youmba SB, Belmonte L, *et al*. The expression and the cellular distribution of the tight junction proteins are altered in irritable bowel syndrome patients with differences according to the disease subtype. *Am J Gastroenterol* 2011; 106: 2165–2173.

90. Bednarska O, Walter SA, Casado-Bedmar M, *et al*. Vasoactive intestinal polypeptide and mast cells regulate increased passage of colonic bacteria in patients with irritable

bowel syndrome. *Gastroenterology* 2017; 153: 948–960.e3.

91. Videlock EJ, Mahurkar-Joshi S, Hoffman JM, *et al*. Sigmoid colon mucosal gene expression supports alterations of neuronal signaling in irritable bowel syndrome with constipation. *Am J Physiol Gastrointest Liver Physiol* 2018; 315: G140–G157.

92. Camilleri M, Carlson P, Acosta A, *et al*. Colonic mucosal gene expression and genotype in irritable bowel syndrome patients with normal or elevated fecal bile acid excretion. *Am J Physiol Gastrointest Liver Physiol* 2015; 309: G10–G20.

93. Barbaro MR, Fuschi D, Cremon C, *et al*. Escherichia coli Nissle 1917 restores epithelial permeability alterations induced by irritable bowel syndrome mediators. *Neurogastroenterol Motil*. Epub ahead of print 28 June 2018. DOI: 10.1111/nmo.13388.

94. Fritscher-Ravens A, Pflaum T, Mösinger M, *et al*. Many patients with irritable bowel syndrome have atypical food allergies not associated with immunoglobulin E. *Gastroenterology* 2019; 157: 109–118.e5.

95. Tulic MK, Vivinus-Nebot M, Rekima A, *et al*. Presence of commensal house dust mite allergen in human gastrointestinal tract: a potential contributor to intestinal barrier dysfunction. *Gut* 2016; 65: 757–766.

96. Wohlfarth C, Schmitteckert S, Härtle JD, *et al*. miR-16 and miR-103 impact 5-HT_4 receptor signalling and correlate with symptom profile in irritable bowel syndrome. *Sci Rep* 2017; 7: 14680.

97. Lee JY, Kim N, Park JH, *et al*. Expression of neurotrophic factors, tight junction proteins, and cytokines according to irritable bowel syndrome subtype and sex. *J Neurogastroenterol Motil* 2020; 26: 106–116.

98. Lee JW, Park JH, Park DI, *et al*. Subjects with diarrhea-predominant IBS have increased rectal permeability responsive to tryptase. *Dig Dis Sci* 2010; 55: 2922–2928.

99. Gecse K, Róka R, Ferrier L, *et al*. Increased faecal serine protease activity in diarrhoeic IBS patients: a colonic lumenal factor impairing colonic permeability and sensitivity. *Gut* 2008; 57: 591–599.

100. Crouzet L, Gaultier E, Del'Homme C, *et al*. The hypersensitivity to colonic distension of IBS patients can be transferred to rats through their fecal microbiota. *Neurogastroenterol Motil* 2013; 25: e272–e282.

101. De Palma G, Lynch MDJ, Lu J, *et al*. Transplantation of fecal microbiota from patients with irritable bowel syndrome alters gut function and behavior in recipient mice. *Sci Transl Med* 2017; 9: eaaf6397.

102. Edogawa S, Edwinson AL, Peters SA, *et al*. Serine proteases as luminal mediators of intestinal barrier dysfunction and symptom severity in IBS. *Gut*. Epub ahead of print 28 March 2019. DOI: 10.1136/gutjnl-2018-317416.

103. Han X, Lee A, Huang S, *et al*. Lactobacillus rhamnosus GG prevents epithelial barrier dysfunction induced by interferon-gamma and fecal supernatants from irritable bowel syndrome patients in human intestinal enteroids and colonoids. *Gut Microbes* 2019; 10: 59–76.

104. Nébot-Vivinus M, Harkat C, Bzioueche H, *et al*. Multispecies probiotic protects gut barrier function in experimental models. *World J Gastroenterol* 2014; 20: 6832–6843.

105. Witt ST, Bednarska O, Keita ÅV, *et al*. Interactions between gut permeability and brain structure and function in health and irritable bowel syndrome. *Neuroimage Clin* 2019; 21: 101602.

106. Duan R, Zhu S, Wang B, *et al*. Alterations of gut microbiota in patients with irritable bowel syndrome based on 16S rRNA-targeted sequencing: a systematic review. *Clin Transl Gastroenterol* 2019; 10: e00012.

107. Sundin J, Aziz I, Nordlander S, *et al*. Evidence of altered mucosa-associated and fecal microbiota composition in patients with irritable bowel syndrome. *Sci Rep* 2020; 10: 593.

108. Camilleri M, McKinzie S, Busciglio I, *et al*. Prospective study of motor, sensory, psychologic, and autonomic functions in patients with irritable bowel syndrome. *Clin Gastroenterol Hepatol* 2008; 6: 772–781.

109. Deiteren A, Camilleri M, Burton D, *et al*. Effect of meal ingestion on ileocolonic and colonic transit in health and irritable bowel syndrome. *Dig Dis Sci* 2010; 55: 384–391.

110. Wilms E, Troost FJ, Elizalde M, *et al*. Intestinal barrier function is maintained with aging - a comprehensive study in healthy subjects and irritable bowel syndrome patients. *Sci Rep* 2020; 10: 475.

111. Suenaert P, Bulteel V, Den Hond E, *et al*. The effects of smoking and indomethacin on small intestinal permeability. *Aliment Pharmacol Ther* 2000; 14: 819–822.

112. Edogawa S, Peters SA, Jenkins GD, *et al*. Sex differences in NSAID-induced perturbation

of human intestinal barrier function and microbiota. *FASEB J* 2018; 32: fj201800560R.

113. Suenaert P, Bulteel V, Den Hond E, *et al*. In vivo influence of nicotine on human basal and NSAID-induced gut barrier function. *Scand J Gastroenterol* 2003; 38: 399–408.

114. Prytz H, Benoni C and Tagesson C. Does smoking tighten the gut? *Scand J Gastroenterol* 1989; 24: 1084–1088.

115. Blomquist L, Bark T, Hedenborg G, *et al*. Evaluation of the lactulose/mannitol and 51Cr-ethylenediaminetetraacetic acid/14C-mannitol methods for intestinal permeability. *Scand J Gastroenterol* 1997; 32: 805–812.

116. Shaikh M, Rajan K, Forsyth CB, *et al*. Simultaneous gas-chromatographic urinary measurement of sugar probes to assess intestinal permeability: use of time course analysis to optimize its use to assess regional gut permeability. *Clin Chim Acta* 2015; 442: 24–32.

117. Wilbrink J, Bernards N, Mujagic Z, *et al*. Intestinal barrier function in morbid obesity: results of a prospective study on the effect of sleeve gastrectomy. *Int J Obes (Lond)* 2020; 44: 368–376.

118. Damms-Machado A, Louis S, Schnitzer A, *et al*. Gut permeability is related to body weight, fatty liver disease, and insulin resistance in obese individuals undergoing weight reduction. *Am J Clin Nutr* 2017; 105: 127–135.

119. Genser L, Aguanno D, Soula HA, *et al*. Increased jejunal permeability in human obesity is revealed by a lipid challenge and is linked to inflammation and type 2 diabetes. *J Pathol* 2018; 246: 217–230.

120. Rainone V, Schneider L, Saulle I, *et al*. Upregulation of inflammasome activity and increased gut permeability are associated with obesity in children and adolescents. *Int J Obes (Lond)* 2016; 40: 1026–1033.

121. Tang Y, Clayburgh DR, Mittal N, *et al*. Epithelial NF-kappaB enhances transmucosal fluid movement by altering tight junction protein composition after T cell activation. *Am J Pathol* 2010; 176: 158–167.

122. Whitehead WE, Palsson O and Jones KR. Systematic review of the comorbidity of irritable bowel syndrome with other disorders: what are the causes and implications? *Gastroenterology* 2002; 122: 1140–1156.

123. Fond G, Loundou A, Hamdani N, *et al*. Anxiety and depression comorbidities in irritable bowel syndrome (IBS): a systematic review and meta-analysis. *Eur Arch Psychiatry Clin Neurosci* 2014; 264: 651–660.

124. Vanuytsel T, van Wanrooy S, Vanheel H, *et al*. Psychological stress and corticotropin-releasing hormone increase intestinal permeability in humans by a mast cell-dependent mechanism. *Gut* 2014; 63: 1293–1299.

125. Alonso C, Guilarte M, Vicario M, *et al*. Acute experimental stress evokes a differential gender-determined increase in human intestinal macromolecular permeability. *Neurogastroenterol Motil* 2012; 24: 740–746, e348–e349.

126. Stevens BR, Goel R, Seungbum K, *et al*. Increased human intestinal barrier permeability plasma biomarkers zonulin and FABP2 correlated with plasma LPS and altered gut microbiome in anxiety or depression. *Gut* 2018; 67: 1555–1557.

127. Shulman RJ, Jarrett ME, Cain KC, *et al*. Associations among gut permeability, inflammatory markers, and symptoms in patients with irritable bowel syndrome. *J Gastroenterol* 2014; 49: 1467–1476.

128. Aguilera-Lizarraga J, Mondelaers S, Florens M, *et al*. 245 evidence for a new mechanism underlying persistent visceral hypersensitivity and increased permeability in a model of post-infectious IBS. *Gastroenterology* 2015; 148: S-55.

129. Camilleri M, Busciglio I, Acosta A, *et al*. Effect of increased bile acid synthesis or fecal excretion in irritable bowel syndrome-diarrhea. *Am J Gastroenterol* 2014; 109: 1621–1630.

130. Sun Q, Jia Q, Song L, *et al*. Alterations in fecal short-chain fatty acids in patients with irritable bowel syndrome: a systematic review and meta-analysis. *Medicine (Baltimore)* 2019; 98: e14513.

131. Zucchelli M, Camilleri M, Andreasson AN, *et al*. Association of TNFSF15 polymorphism with irritable bowel syndrome. *Gut* 2011; 60: 1671–1677.

132. Camilleri M, Carlson P, McKinzie S, *et al*. Genetic susceptibility to inflammation and colonic transit in lower functional gastrointestinal disorders: preliminary analysis. *Neurogastroenterol Motil* 2011; 23: 935–e398.

133. Wouters MM, Lambrechts D, Knapp M, *et al*. Genetic variants in CDC42 and NXPH1 as susceptibility factors for constipation and diarrhoea predominant irritable bowel syndrome. *Gut* 2014; 63: 1103–1111.

134. Villani AC, Lemire M, Thabane M, *et al*. Genetic risk factors for post-infectious irritable bowel syndrome following a waterborne outbreak of gastroenteritis. *Gastroenterology* 2010; 138: 1502–1513.