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Intestinal Chemosensitivity in Irritable Bowel Syndrome Associates with Small Intestinal TRPV Channel Expression

Short title: Intestinal Chemosensitivity in IBS

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Abbreviations: BOP, basal operating pressure; DEG, differentially expressed gene; GI, gastrointestinal; FC, fold change; FDR, false discovery rate; HAD, Hospital Anxiety and Depression; HV, healthy volunteers; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; IBS-C, constipation-predominant IBS; IBS-D, diarrhea-predominant IBS; pCLE, probe-based confocal laser endomicroscopy; PI-IBS, post-infection IBS.

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SUMMARY

Background: Irritable bowel syndrome (IBS) patients often experience meal-associated symptoms. However, the underlying mechanisms are unclear.

Aim: ~~Our objective was t~~To determine small intestinal mechanisms of lipid-induced symptoms and rectal hypersensitivity in IBS.

Methods: We recruited 26 IBS patients (12 IBS-C, 14 IBS-D) and 15 healthy volunteers (HV). *In vivo* permeability was assessed using saccharide excretion assay. Rectal sensitivity was assessed using a barostat before and after ~~a 1 hour~~ small bowel lipid infusion and symptoms assessed throughout. Next, an extended upper endoscopy with probe-based confocal laser endomicroscopy (pCLE) was performed with changes induced by ~~intraluminal~~ lipids. Duodenal and jejunal mucosal biopsies were obtained for transcriptomics.

Results: Following lipid infusion, ~~in~~ compared~~ison~~ to IBS patients, higher proportion of HV reported no pain, no nausea, no fullness and no urgency (P<0.05 for all). In a model adjusted for sex and anxiety, IBS-C and IBS-D patients had lower thresholds for first rectal sensation (P=0.0007) and pain (P=0.004) than HV. *In vivo* small intestinal permeability and mean pCLE scores were similar between IBS and HV. Post-lipid, pCLE scores were higher than pre-lipid, but were not different among the groups. Baseline duodenal transient receptor potential vanilloid (TRPV) 1 and 3 expression was increased in IBS-D and TRPV3 in IBS-C. Duodenal TRPV1 expression correlated with abdominal pain (r=0.51, FDR=0.01) and inversely with first rectal sensation (r=-0.48, FDR=0.01) and pain (r=-0.41, FDR=0.02) thresholds.

Conclusion: Lipid infusion elicits greater symptom response in IBS compared with HV, which associates with small intestinal expression of TRPV channels. TRPV-mediated small intestinal chemosensitivity may mediate post-meal symptoms in IBS.

Keywords: intestinal barrier; visceral hypersensitivity; postprandial symptoms; confocal laser endomicroscopy; pain; ion channels

INTRODUCTION

Irritable bowel syndrome (IBS) is a common disorder of gut-brain interaction that causes substantial morbidity, impairment in quality of life and decreased work productivity[1]. Patients experience chronic or recurrent abdominal pain associated with changes in stool frequency and/or form[2]. Although the pathophysiology of IBS is incompletely understood, visceral hypersensitivity and impaired barrier function are phenotypes associated with it[3, 4]. IBS symptoms often initiate or worsen with meal ingestion[5, 6], with self-reported food intolerances known to cause significant impairment in quality of life[7]. Infusion of nutrients, particularly fats, into the duodenum increases rectal sensitivity in IBS patients[8, 9]. This response was initially found to be limited to diarrhea-predominant IBS (IBS-D)[10]; however, subsequent larger studies demonstrated it regardless of the predominant bowel habit[9]. Although attributed to exaggerated sensory component of the gastrocolic response[10], the mechanisms of nutrient-induced symptom exacerbation or rectal hypersensitivity are not clear.

A subset of IBS patients, especially IBS-D and post-infection IBS (PI-IBS), have increased intestinal permeability[11, 12]. Some studies have associated increased intestinal permeability with symptom severity[12, 13]. Conventionally, intestinal permeability has been measured using urinary excretion of orally administered, poorly-metabolized probe molecules (e.g. lactulose, mannitol, sucrose)[14]. Novel saccharides, such as ^{13}C mannitol have been demonstrated to perform better due to lower contamination at baseline[15, 16]. More recently, probe-based confocal laser endomicroscopy (pCLE), a novel *in-vivo* method that allows cross-sectional imaging of the epithelium at a resolution of ~1 µm has allowed the assessment of epithelial gaps, in addition to providing architectural details[17, 18]. In IBS patients, pCLE demonstrated an increase in intraepithelial

lymphocytes, epithelial breaks, and increased intervillous spaces when dietary antigens were applied to the duodenum[19]. These findings were driven by a subset of IBS patients that demonstrated a visible reaction to the food antigens. Terminal ileal pCLE identified more epithelial gaps in IBS patients compared to healthy volunteers (HV)[20]. These observations suggest that pCLE evaluation of the intestinal architecture may be a helpful diagnostic tool in evaluating the epithelial barrier and changes in response to nutrients or antigens. However, it is not clear if the description of gaps alone correlates with increased permeability either by other parameters on pCLE or using conventional measures of *in vivo* permeability assessment.

A number of studies have assessed changes in colonic gene expression in IBS, mostly in the rectosigmoid[21]. Small bowel signaling is likely more important in understanding meal-induced symptoms in IBS. Most studies in the small intestine have assessed limited gene expression panels showing changes in mast cells, Toll-like receptors and tight junction signaling[16, 22-24]. Small intestinal mucosal transcriptome has not been assessed in IBS to date. Our aims were to determine (a) small intestinal permeability in IBS using conventional methods of *in vivo* saccharide excretion as well as pCLE; (b) changes in symptoms, rectal sensitivity and pCLE features in response to small bowel lipid infusion; and (c) changes in duodenal and jejunal transcriptome as well as signaling pathways in these cohorts of IBS patients, along with associations with symptoms and rectal sensation induced by small bowel lipid infusion.

MATERIALS AND METHODS

Study Design and Participants

We enrolled 26 IBS patients (12 IBS-C and 14 IBS-D; defined by Rome III criteria) and 15 HV. Mayo Clinic Institutional Review Board approved the study (IRB # 14-000837), and all subjects provided written informed consent before participation. All authors had access to the study data and reviewed and approved the final manuscript. The study was registered as Clinical trials.gov NCT02358564. Participants underwent screening using the validated Bowel Disease Questionnaire and the Hospital Anxiety and Depression Scale (HADS) questionnaire to ensure they did not have other chronic gastrointestinal (GI) conditions or significant psychiatric disorders[25]. Those with anxiety and/or depression score of >18 on HADS were excluded. All subjects enrolled in the study underwent a complete history and physical examination before enrollment. Subjects completed a 7-day symptom and bowel diary. Symptom severity of abdominal pain/discomfort, bloating and urgency were scored every evening using a 6-point scale ranging from 0 (none) to 5 (very severe), and the time and consistency of bowel movements were reported using the Bristol Stool Form Scale, ranging from 1 (hard, lumpy) to 7 (watery, diarrhea). Further details on exclusion criteria and dietary and medication restrictions are provided in **Supplemental Methods**.

In vivo Permeability

In vivo permeability was assessed using a lactulose/mannitol excretion assay. Briefly, volunteers were asked to ingest a solution of lactulose (1000 mg), mannitol (100 mg, regular or ^{12}C mannitol)

and radiolabeled ^{13}C mannitol (100 mg) in 250 mL of water[15]. Water (500 mL) was given at 30 minutes post-sugar administration to aid in urine collection. Urine was collected at baseline and 0-2 hours (to represent small bowel permeability)[14]. Urine volumes were recorded, and the concentration of each sugar was measured via high performance liquid chromatography-tandem mass spectrometry. The total milligram of each sugar was calculated using urine volume and concentration per time point. The concentration of ^{13}C mannitol was adjusted for the percentage of ^{13}C in ^{12}C mannitol as previously described[16].

Rectal Barostat

Following an overnight fast, a feeding catheter (Dobhoff feeding tube) was placed into the duodenum with fluoroscopic assistance. Once positioned, participant underwent bowel preparation (Fleet® phosphate enema). Thirty minutes after enema, a barostat manometry catheter (customized rectal barostat catheter, part no. C7-2CB-R-22F; MUI Scientific, Mississauga, Ontario, Canada), to which a polyethylene bag (pillow type rectal barostat balloon, part no. CT-BP600R; length, 22 cm; diameter, 15 cm; capacity 600 mL; MUI Scientific) was attached, was placed in the rectum. Further details on the barostat procedure are provided in **Supplemental Methods**.

Extended Upper Endoscopy and Probe-based Confocal Laser Endomicroscopy

During a sedated, extended upper endoscopy, probe-based confocal imaging was performed with the Cellvizio ColoFlex UHD probe prior to biopsy of the small bowel to assess mucosal and barrier morphology. The endoscope was positioned within the jejunum in the first 10-20 cm distal to the ligament of Treitz. Immediately prior to confocal imaging, if no macroscopic mucosal inflammation was present, 4 mL of 10% fluorescein solution (AK Fluor, Akorn Pharmaceuticals,

Buffalo Grove, IL) were administered intravenously over a 30-second push. Images were obtained within the first 8 to 20 minutes after intravenous fluorescein injection for best imaging results.[26] Continuous confocal images of the jejunum were obtained for 3 minutes. The scope was then pulled back to the third portion of the duodenum, approximately 10 cm proximal to the ligament of Treitz, and images were obtained for a further 3 minutes. On completion of the baseline (pre-lipid) imaging, a 3 ml bolus of lipid (Microlipid 50% fat emulsion diluted as 2 kcal/mL, Nestlé Health Science, Bridgewater, NJ) was infused into the duodenum over 5 seconds, and then the scope advanced to the jejunum. A second 3 ml bolus of lipid was then infused into the jejunum over 5 seconds. Repeat jejunal imaging and duodenal imaging were then carried out. These videos were scored for presence of gaps (1=yes; 0=no), fluorescein leakage (1=yes; 0=no), cellular infiltrate (1= increased; 0 = normal), vessels (1=dilated; 0=normal), goblet cells (1=reduced; 0=normal), and widening of intervillous space (1=yes; 0=no). A total score was calculated from the sum of these individual scores. Additional details are provided in **Supplemental Methods**.

RNA sequencing (RNA-seq)

After pCLE imaging, the duodenum and jejunum mucosa were biopsied using large capacity (2.8 mm) biopsy forceps (no pin). The duodenum was sampled in all volunteers, and while the jejunum was sampled in the majority of volunteers, some (4 IBS-C and 3 IBS-D) were not, due to technical difficulties getting endoscopic access. Biopsies were immediately placed in vials containing AllProtect Tissue Reagent (Qiagen) and were flash frozen in liquid nitrogen. Samples were then stored at -80°C until further analysis. Further details on the sample processing and sequencing are provided in **Supplemental Methods**.

Bioinformatics: The sequencing raw data and gene expression counts were analyzed by MAPRSeq[27] (v3.1.3) with reference of GRCh38. Following standard procedures, gene counts were normalized to account for technical variability, and statistical assessment of differential expression of each gene was performed using glmLRT procedure[28]. Resulting differential expression p-values were corrected using the Benjamini-Hochberg method. Genes with an adjusted p-value ≤0.05 and an absolute log2 fold change of ≥1 were considered as differentially expressed for further analyses. Independently, gene ranks were computed for each group comparison using GSEA PreRanked procedure and gene set enrichment analysis was performed, using GSEA v4.0.0 software[29], to identify differentially regulated pathways between any two experimental groups. Pathways that had a corrected false discovery rate of ≤0.05 were used for further analysis. Gene expression data has been uploaded to GEO with the reference number **GSE166869 (Token: clghiswenxyjdcz)** and further details are provided in **Supplementary Methods.**

Statistical Analysis

Means and standard deviations (SD) are reported for continuous variables, while frequencies and percentages are reported for categorical variables. Comparisons of demographic and clinical characteristics, *in vivo* permeability, pCLE score, rectal sensitivity thresholds between the 3 groups (HV, IBS-C and IBS-D) were made using Kruskal-Wallis test with Dunn's test for post-hoc analysis. Matched two-way ANOVA test was used to assess changes after lipid infusion within the same study group. Sex and anxiety adjusted multivariate models of symptom severity and rectal sensation thresholds were used. Analysis of categorical variables was done with Chi-squared method. Spearman correlations were used to investigate the relationship between gene expression and the severity of symptoms at the end of lipid infusion and rectal sensitivity. Analyses were

made using GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA). A *P*-value of <0.05 was considered statistically significant unless otherwise specified. The sample size was calculated based on pCLE measurements by Turcotte et al., which showed a roughly two-fold increase in epithelial gap density in IBS compared with controls[20]. This magnitude of difference is similar to that seen in permeability measured using urinary excretion of probe molecules[11, 13]. With 13 IBS-D (or 13 IBS-C) vs 13 controls, the study has an 80% power (at the 5% significance level) to detect the effect size of 0.82 between the groups. Similarly, using pooled data from IBS and HV for 0-2 hr excretion of lactulose, an n=12/group provides 80% power to detect effect size of 1.04 between the groups

RESULTS

Demographic and Clinical Characteristics

Participants' demographics and clinical characteristics are shown in **Table 1**. The three groups were similar in age. 57-67% participants were males in the three groups. IBS subgroups had higher HADS anxiety and depression scores than HV. Seven-day symptom and bowel diary showed that IBS-C and IBS-D have higher average severity scores for abdominal pain (1.7 vs 2.0 vs 0.1; $P<0.001$), bloating (2.2 vs 1.7 vs 0.1; $P<0.001$) and urgency (1.1 vs 1.7 vs 0.4; $P=0.002$) than HV. HV had 1.5 bowel movements (BM) per day, whereas IBS-C 1.2 and IBS-D 2.1 ($P=0.08$). The average Bristol stool type was 3.9 for HV, 2.7 for IBS-C and 5.0 for IBS-D ($P<0.001$).

Saccharide Excretion-based in vivo Permeability

Baseline lactulose was undetectable in all volunteers. Baseline ^{13}C mannitol was detectable in two IBS-D patients at low concentration; however, means were not statistically different between the 3 groups (**Supplemental Figure 1A, B**). Cumulative 0-2 hr secretion of lactulose [HV: 1.08 (0.84) mg, n=3; IBS-C: 1.79 (1.52) mg, n=10; and IBS-D 1.21 (0.87) mg, n=10; $P=0.68$],^{13}C mannitol [HV: 10.48 (2.13) mg, n=3; IBS-C: 12.08 (9.71) mg, n=10; and IBS-D: 12.87 (4.66) mg, n=10; p=0.46 (ANOVA)], and lactulose/^{13}C mannitol excretion ratio [HV: $1.04\text{x}10^{-2}$ ($7.67\text{x}10^{-3}$) mg, n=3; IBS-C: $3.00\text{x}10^{-2}$ ($3.87\text{x}10^{-2}$) mg, n=10; and IBS-D: $9.31\text{x}10^{-3}$ ($5.73\text{x}10^{-3}$), n=10; $P=0.2$] was similar between the three groups (**Figure 1A-C**). Considering only a small number of HV underwent ^{13}C mannitol excretion, we used 0-2 hr ^{13}C mannitol excretion from a larger database in our program[12, 16, 30] that showed cumulative excretion of 11.97 (0.54) mg, n=45, which is similar to the values observed in current cohort.

Symptoms During Small Bowel Lipid Infusion

A summary of symptoms in a model adjusted for sex is presented in **Figure 2**. At the end of 60-minute lipid infusion, 83% HV had no pain, whereas only 30% IBS-C and 33% IBS-D reported no pain (P=0.02). Bloating, nausea, fullness and urgency were not different at start of the lipid infusion protocol between the 3 groups. However, at the end of lipid infusion, bloating was higher in IBS-C and IBS-D compared to HV (P=0.04). At the end of lipid infusion, 83% HV reported no nausea, whereas only 20% IBS-C and 33% IBS-D had no nausea (P=0.006); 67% HV had no fullness, while only 20% of IBS-C and 25% IBS-D had no fullness (P=0.04); 75% of HV had no urgency while only 10% IBS-C and 8.3% of IBS-D had no urgency (P<0.001). There was no difference in the severity of any symptom between IBS-C and IBS-D at any timepoint during the infusion. The proportion of patients with no symptoms and with moderate to very severe symptoms throughout lipid infusion is shown in **Supplemental Figure 2**.

Rectal Sensation Thresholds

Basal operating pressure and baseline anxiety scores were similar between groups (P=0.06 and P=0.91, respectively). In a model adjusted for sex and anxiety (**Supplemental Table 1**), first sensation before lipid infusion was significantly associated with the group status (P=0.0007). While both IBS-C and IBS-D patients had a lower threshold for first sensation than HV, the two IBS subgroups did not differ from each other (P=0.85). First sensation after lipid infusion followed a similar pattern for group status (P=0.015), and there was no difference between IBS-C and IBS-D patients (P=0.93). Pre-lipid pain threshold was significantly associated with the group status (P=0.004) with both IBS-C and IBS-D patients having a lower pain threshold than HV, but no difference between the IBS subtypes (P=0.44). Post-lipid, pain threshold followed a similar pattern

for group status (P=0.017) while there was no difference between IBS-C and IBS-D patients (P=0.90). The change in first sensation and pain thresholds after lipid infusion were not significantly different between the IBS and HV groups (**Supplemental Figure 3**).

Probe-based Confocal Laser Endomicroscopy

Representative pCLE images before and after small bowel lipid infusion are shown in **Figure 3**. A summary of individual scores and score totals of pCLE is presented in **Supplemental Table 2**. Pre-lipid mean duodenal score was similar among the three groups (HV 0.6, IBS-C 1.0, IBS-D 0.9; P=0.46). Widening of intervillous spaces (pre-lipid) was more common in IBS-C than HV and IBS-D. Post-lipid duodenal score was significantly higher than the pre-lipid (P<0.001) but not different among the three groups (HV 4.2, IBS-C 3.8, IBS-D 3.3; P=0.30) (**Figure 3C**). In the jejunum, pre-lipid score for widening of intervillous spaces as well as the cumulative score was higher in IBS-C compared to IBS-D, but both were not statistically different from HV (HV 0.8, IBS-C 1.1, IBS-D 0.0; P=0.008). Pre-lipid dilated vessels were more common in HV than the IBS groups. No differences in total score was noted post-lipid in the jejunum (HV 3.9, IBS-C 3.3, IBS-D 3.3; P=0.23) **(Figure 3F**).

Duodenal Transcriptome

Differentially expressed genes: There were 49 upregulated and 92 downregulated genes in IBS-D when compared with HV (log2FC>1.0, *FDR*<0.05). Top five overexpressed genes in IBS-D are XIST, SMIM5, NEAT1, SLC19A1 and NR1D1 (log2FC>1.25 for all). Top five underexpressed genes are C6orf58, GP2, GAST, FMOD and LYZ (log2FC<-1.25 for all). The DEG are shown in **Figure 4A** (trimmed to top 50 by *FDR* when >50 genes were differentially expressed). Transient

receptor potential vanilloid receptor 3 (TRPV3) was found to be overexpressed in IBS-D (log2FC=1.4, *FDR*<0.001). Two additional TRP channel genes, TRPV1 (ENSG00000196689, log2FC=0.87, *FDR*<0.01) and TRPM4 (log2FC=0.71, *FDR*<0.05), were also found to be upregulated in IBS-D compared to HV. Interlectin 1 or intestinal lactoferrin receptor gene expression was decreased in IBS-D (log2FC=-1.37, *FDR*<0.05). A complete list of DEG between IBS-D and HV can be found in **Supplemental Table 3**.

In IBS-C, 17 genes were upregulated and 64 downregulated compared to HV (log2FC>1.0, *FDR*<0.05; **Figure 4C**). Top overexpressed genes in IBS-C are PLCH2 and WASH7P (log2FC>1.25 for both). Top underexpressed genes in IBS-C are SPINK4 and TXNDC5 (log2FC<-1.25 for both). Claudin-3 was found to be overexpressed in IBS-C (log2FC=1.06), which has been previously demonstrated in IBS-C as well[31]. Additionally, similar to changes seen in IBS-D, TRPV3 was found to be overexpressed in IBS-C patients (log2FC=1.04, *FDR*<0.05), though no differences were seen in other TRP channels. A complete list of DEG between IBS-C and HV can be found in **Supplemental Table 3**. No duodenal gene expressions were significantly different between IBS-D and IBS-C.

Association with symptoms: Since TRP channels have been linked to GI pain and hypersensitivity in IBS [32] and other conditions [33], TRP gene expression data from volunteers was correlated with clinical symptoms reported at the end of intraluminal lipid infusion into the small bowel. TRPV1 (ENSG00000196689) gene expression was found to have significant positive correlation with pain/discomfort (Spearman r=0.51, Benjamini-Hochberg corrected *FDR*=0.01), bloating (r=0.46, *FDR*=0.01), nausea (r=0.50, *FDR*=0.01), fullness (r=0.45, *FDR*=0.01) and urgency (r=0.49,

FDR=0.01) (**Figure 5A-C**). A significantly positive correlation was also seen between TRPV3 expression and post-lipid infusion pain (Spearman r=0.45, *FDR*=0.02), bloating (r=0.43, *FDR*=0.02), and urgency (r=0.58, *FDR*=0.002) (**Figure 5D-F**). Additionally, TRPM4 expression also modestly correlated with pain during lipid infusion (Spearman correlation r=0.38, *P*=0.03). As an exploratory analysis to determine sex-differences, TRPV1 expression associated with symptoms only in males (pain: Spearman r=0.62, *P*=0.002; bloating: Spearman r=0.47, *P*=0.03; nausea: Spearman r=0.47, *P*=0.03; urgency: Spearman r=0.53, *P*=0.01). In contrast, TRPV3 expression associated with symptoms only in females (pain: Spearman r=0.66, *P*=0.03; bloating: Spearman r=0.73, *P*=0.01; urgency: Spearman r=0.75, *P*=0.01).

Pathway analysis: GSEA analysis suggested that 65 pathway terms were significantly downregulated in IBS-D while none were significantly upregulated (*FDR* threshold <0.1). Top downregulated Reactome pathways focused around amino acid metabolism: SRP-dependent cotranslational protein targeting to membrane, selenoamino acid metabolism, response of EIF2AK4 GCN2 to amino acid deficiency and metabolism of amino acid derivatives. The pathway involved in cellular responses to external stimuli was also downregulated. Additionally, translation-related pathways were downregulated (eukaryotic translation initiation, eukaryotic translation elongation, translation). Impaired translation in IBS was noted in a prior study as well[34]. TNFα signaling via NF-KB and peroxisomal lipid metabolism were upregulated at a lower threshold for statistical significance (*FDR*=0.15 for both). 117 pathways were downregulated in IBS-C while none were upregulated. Downregulated pathways include amino acid metabolism translation and cellular responses to external stimuli pathways seen in IBS-D. An interesting downregulated pathway was “Defective CFTR causing cystic fibrosis” which involves genes

associated with chloride transport, the major anionic secretion in the intestinal epithelium which has previously been implicated in IBS-C[16]. IFNα, IFNγ, complement cascade, epithelial to mesenchymal interactions, cell adhesion molecules and integrin cell surface interactions were also downregulated in IBS-C compared with HV. A complete list of duodenal GSEA pathways for IBS-D and IBS-C compared to HV is available in **Supplemental Table 4**.

Jejunal Transcriptome

Differentially expressed genes: There were 53 upregulated and 211 downregulated genes in IBS-D when compared to HV (log2FC>1, *FDR*<0.05). Top five overexpressed genes in IBS-D are XIST, MT-RNR 1,2, RN7SL2, CYP4F11 and MUC12 (log2FC>1.5 for all). Top five under expressed genes are C3, EVI2B, LY6E, C7, and APOE (log2FC<-1.5 for all). The DEGs are shown in **Figure 4B** (trimmed to top 50 by *FDR*). Like in the duodenum, various TRPV channels were overexpressed in IBS-D: TRPV1 (ENSG0000196689, log2FC=1.02, *FDR*<0.001), TRPV3 (log2FC=1.13, *FDR*<0.005) and TRPM6 (log2FC=0.83, *FDR*<0.05). A complete list of DEG in jejunum between IBS-D and healthy can be found in **Supplemental Table 5**.

In contrast, downregulated PHLDA1 (log2FC=-1.31, *FDR*<0.01) and upregulated CYP2B7P (log2FC=1.43, *FDR*<0.01) were the only two DEGs between IBS-C and HV (**Figure 4D**). PHLDA1 is an anti-apoptotic nuclear protein, and CYP2B7P is a cytochrome P450 family gene, though neither has been previously described in IBS. When comparing IBS-D with IBS-C, two genes were differentially expressed (both upregulated in IBS-D), SLC16A4 (log2FC=1.48, *FDR*<0.01) and MUC12 (log2FC=2.00, *FDR*<0.01). SLC16A4 encodes for a monocarboxylate

transporter protein (MCT5) and MUC12 encodes for mucosa associated mucin. While MUC12 has not previously been associated with IBS, it has been found to be involved in epithelial protection from bacterial infection *in vitro*[35], and has also been studied in inflammatory bowel disease (IBD) [36], though no clear differences in expression were seen.

Association with symptoms: TRPV3 gene expression correlated positively with urgency during rectal sensation (Spearman correlation r=0.41, *P*=0.04).

Pathway analysis: In IBS-D, 27 pathways were upregulated, and 160 gene sets downregulated (*FDR*<0.1). The most significantly upregulated gene set in IBS-D was Reactome pathway "ion channel transport" (*FDR*=0.004), with TRPV1 and TRPV3 as leading-edge genes (**Figure 4E**). Both TRPV1 and TRPV3 have been shown to express in colonic mucosal biopsies with stronger cytoplasmic TRPV1 expression than TRPV3[33]. Other leading-edge genes in the "ion channel transport" pathway include TRPM4, TRPM6, chloride voltage gated ion channel 2 (CLCN2), and ANO9. However, the expression of these genes was not altered in IBS-D making their significance unclear [37].. Reactome pathway "stimuli-sensing channels" was also unregulated (*FDR*=0.012). Lastly, bile acid metabolism (*FDR*=0.016, Reactome) and transport (*FDR*=0.05, Reactome) pathways were upregulated. Similar to the duodenum, translation pathways (initiation, elongation, SRP dependent membrane targeting) and amino acid pathways (response of EIF2AK4 GCN2 to amino acid deficiency, selenoamino acid metabolism) were downregulated. Integrin cell surface interactions and cell adhesion molecules pathway were also downregulated. Lastly, signaling related to complement cascade and interferon α and γ responses were downregulated in IBS-D.

In IBS-C compared to HV, two pathways were upregulated and 27 down regulated (*FDR*<0.01). "Stimuli sensing channels" and "TRP channels" were the two upregulated gene sets (**Figure 4F**). Leading edge genes for the TRP channel gene set included TRPM4, TRPV1, TRPV3, and TRPV6. In the colon, TRP channel genes have been implicated in IBS pathophysiology, with regards to visceral nociception and hypersensitivity[38]. Leading edge genes for the stimuli sensing gene set included TRPM4, TRPV1, CLCN2, and TRPV3, and various sodium channels. Downregulated pathways include IFNα, IFNγ, complement cascade, epithelial to mesenchymal interactions, cell adhesion molecules and integrin cell surface interactions. For the entire list of GSEA data in jejunum for IBS-D and IBS-C compared to healthy, see **Supplemental Table 6**.

TRPV Gene Expressions Associated with Rectal Sensation

Selected TRP gene expressions were correlated with baseline rectal barostat data. Duodenal TRPV1 and TRPV3 expressions inversely correlated with threshold for first rectal sensation (r=-0.48, *FDR*=0.01 for TRPV1 and r=-0.39, *FDR*=0.02 for TRPV3) and pain (r=-0.41, *FDR*=0.02 for TRPV1 and r=-0.39, *FDR*=0.02 for TRPV3) (**Figure 6**). Additionally, TRPV6 gene expression was found to moderately correlate with pain symptom (r=-0.38, *P*=0.03). Additionally, in the jejunum, TRPV3 gene expression inversely correlated with threshold for pain (r=-0.42, *P*<0.05).

DISCUSSION

The pathophysiology of post-prandial GI symptoms in IBS is poorly understood. Experimental studies using small bowel lipid administration have revealed evidence of exaggerated symptoms, as well as rectal visceral hypersensitivity in IBS patients[8, 9]. Mucosal barrier of small bowel, neurohormonal reflexes as well as small bowel microbiome have all been implicated in meal-induced symptomatology in IBS[10, 39, 40]. Our study demonstrates that IBS patients experience more

GI symptoms upon lipid administration in the small bowel. There was no difference in baseline *in vivo* intestinal permeability or small bowel architecture examined using pCLE between IBS patients and HV. In contrast, small intestinal TRPV channel expression was increased in IBS patients, and correlated strongly with symptoms during lipid infusion. Similarly, TRPV channel expression inversely correlated with pain thresholds. Additionally, gene set enrichment pathways for "ion channel transport", "stimuli sensing channels" and "TRP channels" were significantly upregulated in IBS patients. These findings were present in both IBS-D and IBS-C patients, which aligns with abdominal pain being the defining and central feature of IBS, regardless of the subtype.

Most studies examining the intestinal barrier in IBS have focused on the colon. *In vivo* permeability assessments have largely used a 5-6 hour time frame following administration of oral probes for characterizing permeability of the proximal GI tract. However, Rao et al. observed that in healthy volunteers, 62% of the ingested liquid solution has already reached the colon at 2 hours[14]. We believe that probe recovery beyond the first 2 hours likely represents distal small bowel and colonic permeability. When assessing within the first 2-3 hours and using saccharide excretion, many[11, 14, 41] but not all[42, 43] studies have found evidence of increased permeability in IBS-D. In contrast, no evidence of increased small bowel permeability was noted in IBS-C in prior studies by us and others[11, 16]. One study found a modest but significant relationship between an increased *in vivo* intestinal permeability and severity of abdominal pain[44]. Zhou *et al.* found that an increased lactulose to mannitol excretion ratio was associated with somatic hypersensitivity in response to thermal stimulation as well as visceral hypersensitivity to rectal distension[45]. However, 3 other studies did not find correlations between abdominal pain and *in vivo* permeability[11, 46, 47]. In a small study visualizing the colonic mucosa ultrastructurally, intercellular gaps correlated with the

frequency of abdominal pain[48] and similar findings were noted in jejunal mucosa[49]. pCLE has also demonstrated increased small intestinal epithelial gaps in IBS patients[20]. In a subsequent study, application of diluted food antigens (cow's milk, wheat, yeast, and soy) to duodenal mucosa resulted in increased intraepithelial lymphocytes, formation of epithelial gaps and widening of intervillous spaces in about 60% of IBS patients[19]. These findings were replicated in a recent larger study by the same group, with 61% of IBS patients demonstrating changes on pCLE to wheat[50]. In our study, widening of intervillous spaces was observed in duodenum from IBS-C patients compared to HV and IBS-D patients. Following lipid application, goblet cells were reduced in HV but not in IBS groups. Some changes were also seen in jejunum in IBS-C patients (widening of intervillous spaces). However, when cumulative scores were calculated, no robust differences were seen between the three groups at baseline or post-lipid administration. Thus, although small bowel architectural changes occur in response to lipid administration, these were observed in both HV and IBS and hence unlikely to explain the significantly greater post-prandial symptom response in IBS patients compared with healthy volunteers.

Although observed in a subset of patients, rectal visceral hypersensitivity is considered a pathognomonic feature of IBS. We also observed that both IBS-D and IBS-C patients had rectal hypersensitivity compared to HV. Several prior studies have shown that small bowel lipid administration exacerbates rectal hypersensitivity in IBS[8-10]. However, this was not observed in the current study. This could be plausibly related to a greater proportion of males in our study compared to previous studies that have been female predominant. The differences in thresholds for first sensation and pain between IBS patients and HV observed in this study are greater than those observed in prior studies. Possibly, this cohort of IBS patients was overly hypersensitive at

baseline (40% of the healthy threshold for first sensation), that lipid administration did not increase it any further. Additional possibilities include differences in rectal barostat protocol used in the current study and location of testing (rectum vs sigmoid colon in prior studies). The mechanisms of lipid-induced rectal hypersensitivity are unclear. One study found IBS-C patients to have lower corticotropin-releasing hormone levels compared with IBS-D; however, similar changes were noted with saline infusion[10]. Furthermore, saline infusion induced greater motilin levels compared to lipid infusion. Another study found that 5HT3 antagonists reduced lipid-induced colonic hypersensitivity[39]. A recent study using an animal model of ovalbumin challenge and *Citrobacter* infection showed induction of colonic hypersensitivity to be dependent on mast cell activation[6]. Histamine as well as colonic supernatants from IBS were found to mediate neuronal excitability in a TRPV1-dependent manner[6, 51, 52]. Altered expression, modulation or sensitization of TRP cation channels on visceral afferents has been widely determined to mediate visceral hypersensitivity[32]. Rectosigmoid biopsies from IBS patients as well as quiescent IBD patients with IBS-like symptoms were shown to have higher numbers of TRPV1 immunoreactive fibers[53, 54]. Although TRP channels have not been studied in the proximal small bowel in the context of IBS, an interesting study demonstrated IBS patients to experience worse abdominal symptoms within 2 hours of chili consumption in an experimental protocol suggesting the role of capsaicin-sensitive visceral nociception[55]. Animal studies have also shown that TRPV1 plays an essential role in development of post inflammatory visceral pain following colitis[56]. The role of TRPV3 is less well characterized. In the mouse, distal colonic but not duodenal mucosa expressed TRPV3, predominantly in the epithelial cells[57]. TRPV3 expression has also been detected in human colonic mucosa[33, 58]. In another study, mice jejunal and ileal mucosa was shown to express TRPV3[59]. We found that in duodenum, TRPV3 expression was increased in both IBS-D and IBS-C while TRPV1

was only increased in IBS-D. In contrast, in jejunum, only IBS-D patients should increase in expression of TRPV1 and 3 channels. Additionally, there may be sex-specific differences in pathophysiology of lipid-induced symptoms as TRPV1 expression associated with the symptoms of pain, bloating and urgency in males and TRPV3 in females.

We also observed greater expression of PDZ domain-containing protein 3 (PDZD3) in jejunum of IBS-D patients compared to healthy volunteers. The PDZ protein associates with guanylate cyclase C and regulates cGMP production, which mediates chloride secretion and has previously been found to be overexpressed in rectosigmoid mucosa from IBS-D patients[60]. Tryptophan hydroxylase-1 (Tph1) expression was decreased in the duodenum of IBS-D patients. Serotonin availability as well as Tph1 has been found to be decreased in IBS in other studies[61, 62]. Lastly, we observed downregulation of amino acid metabolism pathways in IBS-D duodenum and jejunum. Purine metabolism has recently been identified to be an important host-microbial signaling pathway for IBS pathophysiology[63]. Interestingly, in this deep sequencing analysis, no duodenal genes and only two jejunal genes were differentially expressed between IBS-D and IBS-C. Limitations of the study include smaller representation of females, lack of control (saline) group for lipid administration studies, absence of protein expression and localization data, and limitations of the sample size that can allow identification of subsets within IBS patients with unique physiological or transcriptional profiles.

In conclusion, this study provides a complementary assessment of small intestinal permeability and architecture using confocal endomicroscopy with response to locally applied lipids, small intestinal transcriptomics and rectal sensitivity with response to intraduodenal lipid infusion. Although meal and lipid-induced sensitivity has been described in IBS, this study is the first to

associate it with expression of TRPV channels in the small bowel. Further studies will need to mechanistically understand the localization of these channels as well as mechanisms of sensitization with lipids and potential ways to inhibit it. Lipids induced mucosal changes were detectable on confocal endomicroscopy. Automation and other advancements in image analysis can help identify patterns that distinguish responses in IBS from controls and responses to broader range of nutrients. Overall, these strategies will lay foundation for further assessment of postprandial symptoms and visceral hypersensitivity in IBS patients. *Clinical trials.gov registration NCT02358564*

AUTHORSHIP STATEMENT

Data Availability Statement: Transcriptomic and de-identified demographic metadata has been shared on GEO reference number GSE166869 (Token: clghiswenxyjdcz).

Guarantor of Article: Madhusudan Grover

Specific Author Contributions: MG: study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; statistical analysis; study supervision. AB: acquisition of data; analysis and interpretation of data; drafting of the manuscript; statistical analysis. SP: acquisition of data; analysis and interpretation of data; drafting of the manuscript; statistical analysis. TW: acquisition of data, analysis and interpretation of data; critical revision of the manuscript. MBL: patient recruitment; acquisition of data; critical revision of the manuscript. WSH: analysis and interpretation of data; statistical analysis; critical revision of the manuscript. IB: acquisition of data; critical revision of the manuscript. DB: acquisition of data; critical revision of the manuscript. MVR: acquisition of data; critical revision of the manuscript. KDV: acquisition of data; critical revision of the manuscript. MC: acquisition of data; critical revision of the manuscript. MW: acquisition of data; critical revision of the manuscript. SD: acquisition of data; analysis and interpretation of data; critical revision of the manuscript. HN: acquisition of data; analysis and interpretation of data; critical revision of the manuscript. LAH: study concept and design; acquisition of data; study supervision, critical revision of the manuscript. MG and LAH jointly shared responsibilities.

All authors approved the final version of the article, including the authorship list.

REFERENCES

1. Black CJ, Ford AC. Global burden of irritable bowel syndrome: trends, predictions and risk factors. Nat Rev Gastroenterol Hepatol 2020;17:473-486.
2. Mearin F, Lacy BE, Chang L, Chey WD, Lembo AJ, Simren M, Spiller R. Bowel Disorders. Gastroenterology 2016.
3. Bouin M, Plourde V, Boivin M, Riberdy M, Lupien F, Laganiere M, Verrier P, Poitras P. Rectal distention testing in patients with irritable bowel syndrome: sensitivity, specificity, and predictive values of pain sensory thresholds. Gastroenterology 2002;122:1771-7.
4. Mertz H, Naliboff B, Munakata J, Niazi N, Mayer EA. Altered rectal perception is a biological marker of patients with irritable bowel syndrome. Gastroenterology 1995;109:40-52.
5. Ragnarsson G, Bodemar G. Pain is temporally related to eating but not to defaecation in the irritable bowel syndrome (IBS). Patients' description of diarrhea, constipation and symptom variation during a prospective 6-week study. Eur J Gastroenterol Hepatol 1998;10:415-21.
6. Aguilera-Lizarraga J, Florens MV, Viola MF, Jain P, Decraecker L, Appeltans I, Cuende-Estevez M, Fabre N, Van Beek K, Perna E, Balemans D, Stakenborg N, Theofanous S, Bosmans G, Mondelaers SU, Matteoli G, Ibiza Martinez S, Lopez-Lopez C, Jaramillo-Polanco J, Talavera K, Alpizar YA, Feyerabend TB, Rodewald HR, Farre R, Redegeld FA, Si J, Raes J, Breynaert C, Schrijvers R, Bosteels C, Lambrecht BN, Boyd SD, Hoh RA, Cabooter D, Nelis M, Augustijns P, Hendrix S, Strid J, Bisschops R, Reed DE, Vanner SJ, Denadai-Souza A, Wouters MM, Boeckxstaens GE. Local immune response to food antigens drives meal-induced abdominal pain. Nature 2021.

7. Bohn L, Storsrud S, Tornblom H, Bengtsson U, Simren M. Self-reported food-related gastrointestinal symptoms in IBS are common and associated with more severe symptoms and reduced quality of life. Am J Gastroenterol 2013;108:634-41.

8. Simren M, Agerforz P, Bjornsson ES, Abrahamsson H. Nutrient-dependent enhancement of rectal sensitivity in irritable bowel syndrome (IBS). Neurogastroenterol Motil 2007;19:20-9.

9. Simren M, Abrahamsson H, Bjornsson ES. Lipid-induced colonic hypersensitivity in the irritable bowel syndrome: the role of bowel habit, sex, and psychologic factors. Clin Gastroenterol Hepatol 2007;5:201-8.

10. Simren M, Abrahamsson H, Bjornsson ES. An exaggerated sensory component of the gastrocolonic response in patients with irritable bowel syndrome. Gut 2001;48:20-7.

11. Dunlop SP, Hebden J, Campbell E, Naesdal J, Olbe L, Perkins AC, Spiller RC. Abnormal intestinal permeability in subgroups of diarrhea-predominant irritable bowel syndromes. Am J Gastroenterol 2006;101:1288-94.

12. Edogawa S, Edwinson AL, Peters SA, Chikkamenahalli LL, Sundt W, Graves S, Gurunathan SV, Breen-Lyles M, Johnson S, Dyer R, Graham R, Chen J, Kashyap P, Farrugia G, Grover M. Serine proteases as luminal mediators of intestinal barrier dysfunction and symptom severity in IBS. Gut 2020;69:62-73.

13. Zhou Q, Zhang B, Verne GN. Intestinal membrane permeability and hypersensitivity in the irritable bowel syndrome. Pain 2009;146:41-6.

14. Rao AS, Camilleri M, Eckert DJ, Busciglio I, Burton DD, Ryks M, Wong BS, Lamsam J, Singh R, Zinsmeister AR. 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:G919-28.

15. Grover M, Camilleri M, Hines J, Burton D, Ryks M, Wadhwa A, Sundt W, Dyer R, Singh RJ. (13) C mannitol as a novel biomarker for measurement of intestinal permeability. Neurogastroenterol Motil 2016;28:1114-9.

16. Peters SA, Edogawa S, Sundt WJ, Dyer RB, Dalenberg DA, Mazzone A, Singh RJ, Moses N, Smyrk TC, Weber C, Linden DR, MacNaughton WK, Turner JR, Camilleri M, Katzka DA, Farrugia G, Grover M. Constipation-Predominant Irritable Bowel Syndrome Females Have Normal Colonic Barrier and Secretory Function. Am J Gastroenterol 2017;112:913-923.

17. Kiesslich R, Goetz M, Angus EM, Hu Q, Guan Y, Potten C, Allen T, Neurath MF, Shroyer NF, Montrose MH, Watson AJ. Identification of epithelial gaps in human small and large intestine by confocal endomicroscopy. Gastroenterology 2007;133:1769-78.

18. Robles-Medranda C, Oleas R, Valero M, Puga-Tejada M, Soria-Alcivar M, Ospina J, Alvarado-Escobar H, Munoz-Jurado G, Baquerizo-Burgos J, Pitanga-Lukashok H. Confocal laser endomicroscopy detects colonic inflammation in patients with irritable bowel syndrome: a prospective study. Endosc Int Open 2020;8:E550-E557.

19. Fritscher-Ravens A, Schuppan D, Ellrichmann M, Schoch S, Rocken C, Brasch J, Bethge J, Bottner M, Klose J, Milla PJ. Confocal endomicroscopy shows food-associated changes in the intestinal mucosa of patients with irritable bowel syndrome. Gastroenterology 2014;147:1012-20 e4.

20. Turcotte JF, Kao D, Mah SJ, Claggett B, Saltzman JR, Fedorak RN, Liu JJ. 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;77:624-30.

21. Kasim A, Shkedy Z, Lin D, Van Sanden S, Abrahantes JC, Gohlmann HW, Bijnens L, Yekutieli D, Camilleri M, Aerssens J, Talloen W. Translation of disease associated gene signatures across tissues. Int J Data Min Bioinform 2015;11:301-13.

22. Martinez C, Vicario M, Ramos L, Lobo B, Mosquera JL, Alonso C, Sanchez A, Guilarte M, Antolin M, de Torres I, Gonzalez-Castro AM, Pigrau M, Saperas E, Azpiroz F, Santos J. 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;107:736-46.

23. Martinez C, Rodino-Janeiro BK, Lobo B, Stanifer ML, Klaus B, Granzow M, Gonzalez-Castro AM, Salvo-Romero E, Alonso-Cotoner C, Pigrau M, Roeth R, Rappold G, Huber W, Gonzalez-Silos R, Lorenzo J, de Torres I, Azpiroz F, Boulant S, Vicario M, Niesler B, Santos J. 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.

24. Camilleri M, Carlson P, Valentin N, Acosta A, O'Neill J, Eckert D, Dyer R, Na J, Klee EW, Murray JA. Pilot study of small bowel mucosal gene expression in patients with irritable bowel syndrome with diarrhea. Am J Physiol Gastrointest Liver Physiol 2016;311:G365-76.

25. Talley NJ, Phillips SF, Wiltgen CM, Zinsmeister AR, Melton LJ, 3rd. Assessment of functional gastrointestinal disease: the bowel disease questionnaire. Mayo Clin Proc 1990;65:1456-79.

26. Becker V, von Delius S, Bajbouj M, Karagianni A, Schmid RM, Meining A. Intravenous application of fluorescein for confocal laser scanning microscopy: evaluation of contrast dynamics and image quality with increasing injection-to-imaging time. Gastrointest Endosc 2008;68:319-23.

27. Kalari KR, Nair AA, Bhavsar JD, O'Brien DR, Davila JI, Bockol MA, Nie J, Tang X, Baheti S, Doughty JB, Middha S, Sicotte H, Thompson AE, Asmann YW, Kocher JP. MAP-RSeq: Mayo Analysis Pipeline for RNA sequencing. BMC Bioinformatics 2014;15:224.

28. McCarthy DJ, Chen Y, Smyth GK. Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. Nucleic Acids Res 2012;40:4288-97.

29. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005;102:15545-50.

30. Edogawa S, Peters SA, Jenkins GD, Gurunathan SV, Sundt WJ, Johnson S, Lennon RJ, Dyer RB, Camilleri M, Kashyap PC, Farrugia G, Chen J, Singh RJ, Grover M. Sex differences in NSAID-induced perturbation of human intestinal barrier function and microbiota. FASEB J 2018:fj201800560R.

31. Kong WM, Gong J, Dong L, Xu JR. [Changes of tight junction claudin-1,-3,-4 protein expression in the intestinal mucosa in patients with irritable bowel syndrome]. Nan Fang Yi Ke Da Xue Xue Bao 2007;27:1345-7.

32. Balemans D, Boeckxstaens GE, Talavera K, Wouters MM. Transient receptor potential ion channel function in sensory transduction and cellular signaling cascades underlying visceral hypersensitivity. Am J Physiol Gastrointest Liver Physiol 2017;312:G635-G648.

33. Rizopoulos T, Papadaki-Petrou H, Assimakopoulou M. Expression Profiling of the Transient Receptor Potential Vanilloid (TRPV) Channels 1, 2, 3 and 4 in Mucosal Epithelium of Human Ulcerative Colitis. Cells 2018;7.

34. Rodino-Janeiro BK, Pardo-Camacho C, Santos J, Martinez C. Mucosal RNA and protein expression as the next frontier in IBS: abnormal function despite morphologically intact small intestinal mucosa. Am J Physiol Gastrointest Liver Physiol 2019;316:G701-G719.

35. Liévin-Le Moal V, Servin AL, Coconnier-Polter M-H. The increase in mucin exocytosis and the upregulation of MUC genes encoding for membrane-bound mucins induced by the thiol-activated exotoxin listeriolysin O is a host cell defence response that inhibits the cell-entry of Listeria monocytogenes. Cellular Microbiology 2005;7:1035-1048.

36. Yamamoto-Furusho JK, Ascano-Gutierrez I, Furuzawa-Carballeda J, Fonseca-Camarillo G. Differential Expression of MUC12, MUC16, and MUC20 in Patients with Active and Remission Ulcerative Colitis. Mediators Inflamm 2015;2015:659018.

37. Camilleri M. Intestinal secretory mechanisms in irritable bowel syndrome-diarrhea. Clin Gastroenterol Hepatol 2015;13:1051-7; quiz e61-2.

38. Beckers AB, Weerts Z, Helyes Z, Masclee AAM, Keszthelyi D. Review article: transient receptor potential channels as possible therapeutic targets in irritable bowel syndrome. Aliment Pharmacol Ther 2017;46:938-952.

39. Simren M, Simms L, D'Souza D, Abrahamsson H, Bjornsson ES. Lipid-induced colonic hypersensitivity in irritable bowel syndrome: the role of 5-HT3 receptors. Aliment Pharmacol Ther 2003;17:279-87.

40. Saffouri GB, Shields-Cutler RR, Chen J, Yang Y, Lekatz HR, Hale VL, Cho JM, Battaglioli EJ, Bhattarai Y, Thompson KJ, Kalari KK, Behera G, Berry JC, Peters SA, Patel R, Schuetz AN, Faith JJ, Camilleri M, Sonnenburg JL, Farrugia G, Swann JR, Grover M, Knights D, Kashyap PC. Small intestinal microbial dysbiosis underlies symptoms associated with functional gastrointestinal disorders. Nat Commun 2019;10:2012.

41. Vazquez-Roque MI, Camilleri M, Smyrk T, Murray JA, O'Neill J, Carlson P, Lamsam J, Eckert D, Janzow D, Burton D, Ryks M, Rhoten D, Zinsmeister AR. 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:G1262-9.

42. Valentin N, Camilleri M, Carlson P, Harrington SC, Eckert D, O'Neill J, Burton D, Chen J, Shaw AL, Acosta A. Potential mechanisms of effects of serum-derived bovine immunoglobulin/protein isolate therapy in patients with diarrhea-predominant irritable bowel syndrome. Physiol Rep 2017;5.

43. Camilleri M, Shin A, Busciglio I, Carlson P, Acosta A, Bharucha AE, Burton D, Lamsam J, Lueke A, Donato LJ, Zinsmeister AR. Validating biomarkers of treatable mechanisms in irritable bowel syndrome. Neurogastroenterol Motil 2014;26:1677-85.

44. Mujagic Z, Ludidi S, Keszthelyi D, Hesselink MA, Kruimel JW, Lenaerts K, Hanssen NM, Conchillo JM, Jonkers DM, Masclee AA. 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;40:288-97.

45. Zhou Q, Zhang B, Nicholas Verne G. Intestinal membrane permeability and hypersensitivity in the irritable bowel syndrome. Pain 2009;146:41-46.

46. Gecse K, Roka R, Sera T, Rosztoczy A, Annahazi A, Izbeki F, Nagy F, Molnar T, Szepes Z, Pavics L, Bueno L, Wittmann T. Leaky Gut in Patients with Diarrhea-Predominant Irritable Bowel Syndrome and Inactive Ulcerative Colitis. Digestion 2011;85:40-46.

47. Li L, Xiong L, Yao J, Zhuang X, Zhang S, Yu Q, Xiao Y, Cui Y, Chen M. 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;48:880-7.

48. Zhao DY, Qi QQ, Long X, Li X, Chen FX, Yu YB, Zuo XL. Ultrastructure of intestinal mucosa in diarrhea-predominant irritable bowel syndrome. Physiol Int 2019;106:225-235.

49. Martínez C, Lobo B, Pigrau M, Ramos L, González-Castro AM, Alonso C, Guilarte M, Guilá M, de Torres I, Azpiroz F, Santos J, Vicario M. Diarrhoea-predominant irritable bowel syndrome: an organic disorder with structural abnormalities in the jejunal epithelial barrier. Gut 2013;62:1160-8.

50. Fritscher-Ravens A, Pflaum T, Mosinger M, Ruchay Z, Rocken C, Milla PJ, Das M, Bottner M, Wedel T, Schuppan D. Many Patients With Irritable Bowel Syndrome Have Atypical Food Allergies Not Associated With Immunoglobulin E. Gastroenterology 2019;157:109-118 e5.

51. Balemans D, Aguilera-Lizarraga J, Florens MV, Jain P, Denadai-Souza A, Viola MF, Alpizar YA, Van Der Merwe S, Vanden Berghe P, Talavera K, Vanner S, Wouters MM, Boeckxstaens GE. Histamine-mediated potentiation of transient receptor potential (TRP)

ankyrin 1 and TRP vanilloid 4 signaling in submucosal neurons in patients with irritable bowel syndrome. Am J Physiol Gastrointest Liver Physiol 2019;316:G338-G349.

52. Wouters MM, Balemans D, Van Wanrooy S, Dooley J, Cibert-Goton V, Alpizar YA, Valdez-Morales EE, Nasser Y, Van Veldhoven PP, Vanbrabant W, Van der Merwe S, Mols R, Ghesquiere B, Cirillo C, Kortekaas I, Carmeliet P, Peetermans WE, Vermeire S, Rutgeerts P, Augustijns P, Hellings PW, Belmans A, Vanner S, Bulmer DC, Talavera K, Vanden Berghe P, Liston A, Boeckxstaens GE. Histamine Receptor H1-Mediated Sensitization of TRPV1 Mediates Visceral Hypersensitivity and Symptoms in Patients With Irritable Bowel Syndrome. Gastroenterology 2016;150:875-87 e9.

53. Akbar A, Yiangou Y, Facer P, Walters JR, Anand P, Ghosh S. Increased capsaicin receptor TRPV1-expressing sensory fibres in irritable bowel syndrome and their correlation with abdominal pain. Gut 2008;57:923-9.

54. Akbar A, Yiangou Y, Facer P, Brydon WG, Walters JR, Anand P, Ghosh S. Expression of the TRPV1 receptor differs in quiescent inflammatory bowel disease with or without abdominal pain. Gut 2010;59:767-74.

55. Gonlachanvit S, Mahayosnond A, Kullavanijaya P. Effects of chili on postprandial gastrointestinal symptoms in diarrhoea predominant irritable bowel syndrome: evidence for capsaicin-sensitive visceral nociception hypersensitivity. Neurogastroenterol Motil 2009;21:23-32.

56. Lapointe TK, Basso L, Iftinca MC, Flynn R, Chapman K, Dietrich G, Vergnolle N, Altier C. TRPV1 sensitization mediates postinflammatory visceral pain following acute colitis. Am J Physiol Gastrointest Liver Physiol 2015;309:G87-99.

57. Ueda T, Yamada T, Ugawa S, Ishida Y, Shimada S. TRPV3, a thermosensitive channel is expressed in mouse distal colon epithelium. Biochem Biophys Res Commun 2009;383:130-4.

58. Toledo Maurino JJ, Fonseca-Camarillo G, Furuzawa-Carballeda J, Barreto-Zuniga R, Martinez Benitez B, Granados J, Yamamoto-Furusho JK. TRPV Subfamily (TRPV2, TRPV3, TRPV4, TRPV5, and TRPV6) Gene and Protein Expression in Patients with Ulcerative Colitis. J Immunol Res 2020;2020:2906845.

59. De Petrocellis L, Orlando P, Moriello AS, Aviello G, Stott C, Izzo AA, Di Marzo V. Cannabinoid actions at TRPV channels: effects on TRPV3 and TRPV4 and their potential relevance to gastrointestinal inflammation. Acta Physiol (Oxf) 2012;204:255-66.

60. Camilleri M, Carlson P, Acosta A, Busciglio I, Nair AA, Gibbons SJ, Farrugia G, Klee EW. 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:G1089-98.

61. Coates MD, Mahoney CR, Linden DR, Sampson JE, Chen J, Blaszyk H, Crowell MD, Sharkey KA, Gershon MD, Mawe GM, Moses PL. Molecular defects in mucosal serotonin content and decreased serotonin reuptake transporter in ulcerative colitis and irritable bowel syndrome. Gastroenterology 2004;126:1657-64.

62. Kerckhoffs AP, ter Linde JJ, Akkermans LM, Samsom M. SERT and TPH-1 mRNA expression are reduced in irritable bowel syndrome patients regardless of visceral sensitivity state in large intestine. Am J Physiol Gastrointest Liver Physiol 2012;302:G1053-60.

63. Mars RAT, Yang Y, Ward T, Houtti M, Priya S, Lekatz HR, Tang X, Sun Z, Kalari KR, Korem T, Bhattarai Y, Zheng T, Bar N, Frost G, Johnson AJ, van Treuren W, Han S, Ordog T, Grover M, Sonnenburg J, D'Amato M, Camilleri M, Elinav E, Segal E, Blekhman R, Farrugia G, Swann JR, Knights D, Kashyap PC. Longitudinal Multi-omics Reveals Subset-Specific Mechanisms Underlying Irritable Bowel Syndrome. Cell 2020;182:1460-1473 e17.

Table 1. Demographic and clinical characteristics of study participants

	HV (n=15)	**IBS-C (n=12)**	**IBS-D (n=14)**	***P* value**
Age, y, mean ± SD	33.9 ± 9.0	32.8 ± 7.8	32.6 ± 11.4	0.40
Female sex, n (%)	5 (33.3)	5 (41.7)	6 (42.9)	0.85
Caucasian, n (%)	14 (93.3)	12 (100.0)	10 (76.9)	0.14
BMI, kg/m^2, mean ± SD	28.6 ± 5.5	25.1 ± 4.0	28.2 ± 4.9	0.26
HADS anxiety score, mean ± SD	3.5 ± 2.6	6.3 ± 6.2	5.7 ± 4.6	0.50
HADS depression score, mean ± SD	1.5 ± 2.0	3.5 ± 3.1	2.9 ± 3.0	0.12
Abdominal pain/discomfort (>25% time)[¥], n (%)	3 (25.0)	8 (66.7)	13 (92.9)	**0.002**
-Before meals or when hungry	3 (25.0)	3 (25.0)	0 (0.0)	0.13
-Made better by eating	2 (16.7)	1 (8.3)	1 (7.1)	0.70
-Immediately post-meal (0-30 minutes)	1 (8.3)	6 (50.0)	9 (69.2)	**0.008**
-Post-meal (30-120 minutes)	0 (0.0)	6 (50.0)	11 (78.6)	**<0.001**
-Triggered by a specific food/drink	1 (8.3)	5 (41.7)	6 (42.9)	0.11
7-day symptom and bowel diary[€], mean ± SD				
Average abdominal pain/discomfort	0.1 ± 0.2	1.7 ± 1.0	2.0 ± 1.0	**<0.001**[†]
Average abdominal bloating	0.1 ± 0.2	2.2 ± 1.1	1.7 ± 1.4	**<0.001**[†]
Average urgency	0.4 ± 0.5	1.1 ± 0.8	1.7 ± 0.9	**0.002**[†]
Average BMs per day	1.5 ± 0.8	1.2 ± 0.7	2.1 ± 1.1	0.088
Average Bristol stool type	3.9 ± 0.8	2.7 ± 0.9	5.0 ± 0.9	**<0.001**[†]
Straining during defecation	0.4 ± 0.4	1.9 ± 0.87	1.2 ± 0.8	**<0.001**[†]
Urgency during defecation	0.4 ± 0.5	1.5 ± 0.7	2.1 ± 1.0	**<0.001**[†]

NOTE. Data were missing for the number of HV, IBS-C, and IBS-D, respectively, in the following variables: Caucasian, 0, 0 and 1; abdominal pain/discomfort, 3, 0 and 0; before meals or when hungry, 3, 0 and 0; made better by eating, 3, 0 and 0; immediately post-meal (0-30 minutes); 3, 0 and 1; post-meal (30-120 minutes); triggered by a specific food/drink, 3, 0 and 0; all variables of the 7-day symptom and bowel diary, 2, 0 and 3.

Continuous and categorical variables analyzed using Kruskal Wallis test and Chi-Square test, respectively. Abbreviations: BM, bowel movement; BMI, body mass index; HADS, hospital anxiety and depression scale.

[¥]Based on the Bowel Disease Questionnaire (BDQ). Positive response meaning the symptom occurred "often" (>25% of the time).

[€]Severity scale: 0 "none", 1 "very mild", 2 "mild", 3 "moderate", 4 "quite severe", 5 "very severe".

[†]Post-hoc Dunn's multiple comparisons test ($P<0.05$): Average abdominal pain/discomfort, HV vs IBS-C, HV vs IBS-D; Average abdominal bloating, HV vs IBS-C, HV vs IBS-D; Average urgency, HV vs IBS-D; Average Bristol stool type, HV vs IBS-C, IBS-C vs IBS-D; Straining during defecation, HV vs IBS-C; Urgency during defecation, HV vs IBS-C, HV vs IBS-D.