Randomized, Double Blinded, Placebo Controlled Trial To Understand Efficacy Of Colesevelam In Diarrhea Predominant IBS Patients With Bile Acid Malabsorption

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Study Title: Randomized, Double Blinded, Placebo controlled trial to understand efficacy of Colesevelam in diarrhea predominant IBS patients with Bile Acid Malabsorption

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SPECIFIC AIM

Irritable bowel syndrome (IBS) is the most common lower gastrointestinal (GI) disorder that affects 11% of adults (1). Currently, IBS and subgroups diarrhea-predominant IBS (IBS-D), and IBS-constipation (IBS-C), are defined by symptoms alone. Apart from central hypervigilance and psychological disturbances in IBS (2),48% of IBS-D patients have accelerated colonic transit, 21% have increased rectal sensation (3), and others have increased intestinal permeability, reduced barrier protein expression (4) and abnormal bile acid (BA) synthesis or excretion (5). These findings suggest that colonic "irritation" may be mediated by *extrinsic factors* (e.g. stress or prior GI infections) and *intraluminal mediators* [e.g. BAs and short chain fatty acids (SCFA)] which alter mucosal function, stimulate serotonin release, alter mucosal permeability, induce immune activation, and increase colonic motility.

We have previously shown that colonic **mucosal mRNA expression** (first in 9 patients, then confirmed in 47 other patients) showed that IBS-D is associated with changes in transcriptome which regulates mucosal functions, such as neurotransmitters (P2RY4 and VIP), ion channels [GUC2AB, and PDZD3) cytokines (CCL20), and immune function (C4BPA).

Building on these preliminary observations, our **overarching goal** is to examine effects of BA sequestration in IBS-D with increased BA excretion on quantitative traits and mucosal mRNA expression in rectosigmoid colon.

Hypothesis: BA sequestration normalizes colonic *functions* in IBS-D with increased fecal BA excretion.

Specific Aim: To compare with a randomized trial *(n=15 per treatment group), effects of* colesevelam 1.875g bid and placebo treatment, on colonic transit, bowel functions, *permeability and tight junction expression in rectosigmoid mucosa* of IBS-D with Bile Acid Malabsorption.

This protocol addresses the third specific aim of a program on IBS-D with increased fecal BA excretion

ABBREVIATIONS used: **ASBT**: apical, Na-dependent bile acid transporter; ASIC: acid-sensing ion channel; ATP2C2: annotated as splice-site acceptor motif, and involved in Ca2+ encoding secretory pathway; BA: bile acids; C4BPA: complement component 4 binding protein, alpha; CA: cholic acid; CCL20: chemokine (C-C motif) ligand 20 CDCA chenodeoxycholic acid; CDH-1: cadherin 1; CLDN: claudin;CNR1: cannabinoid type 1 receptor; C11orf30: chromosome 11, open-reading frame 30; CYP, cytochrome P450; DCA deoxycholic acid; FAAH: fatty acid amide hydrolase; FABP6: fatty acid binding protein 6; **FDR**: false discovery rate; **FXR**: farnesoid X receptor; **FGF19**: fibroblast growth factor 19; **FGFR4**: FGF receptor 4; FN1: fibronectin 1; **GpBAR1**:G protein coupled bile acid receptor 1; **GUC**: guanylate cyclase; 5-HT: serotonin; 5-HTTLPR: 5-HT transporter, long polymorphic repeat; IL: interleukin; IBS: irritable bowel syndrome; IFIT3: interferon-induced protein with tetratricopeptide repeats 3; **KLB**: klotho B;LRH1: liver receptor homolog-1 ; MAF: minor allele frequency; NaV: voltage gated sodium channel; **NR1H4**: Nuclear receptor subfamily 1, group H, member 4 ;OCLN: occluding; ORMDL3: Orosomucoid like 3; OST: organic solute transporter; OTU: operational taxonomic units; PRDM1: positive regulatory domain I-binding factor; **P2RY4**: purinergic receptor P2Y, G-protein coupled, 4; **PDZD3**: postsynaptic density-95, disks large, zonula occludens-1, domain 3; QTL: quantitative trait loci; RBP2: retinal-binding protein 2;SHP: Small Heterodimer Partner; **SLC** solute carrier C; **SNP**: single nucleotide polymorphism; **SNV**: Single nucleotide variants;Th17: IL-17-producing $CD4^+$ T cells; **TGR5 =GpBAR1**; TFF1: trefoil factor 1 TJ: tight junctions; TNFSF15: tumor necrosis factor, superfamily 15; TLR9: toll-like receptor 9; TRPV: transient receptor potential vanilloid; VIP: vasoactive intestinal peptide; XAF-1: X-Linked inhibitor of apoptosis-associated factor-1; ZO: zonula occludens

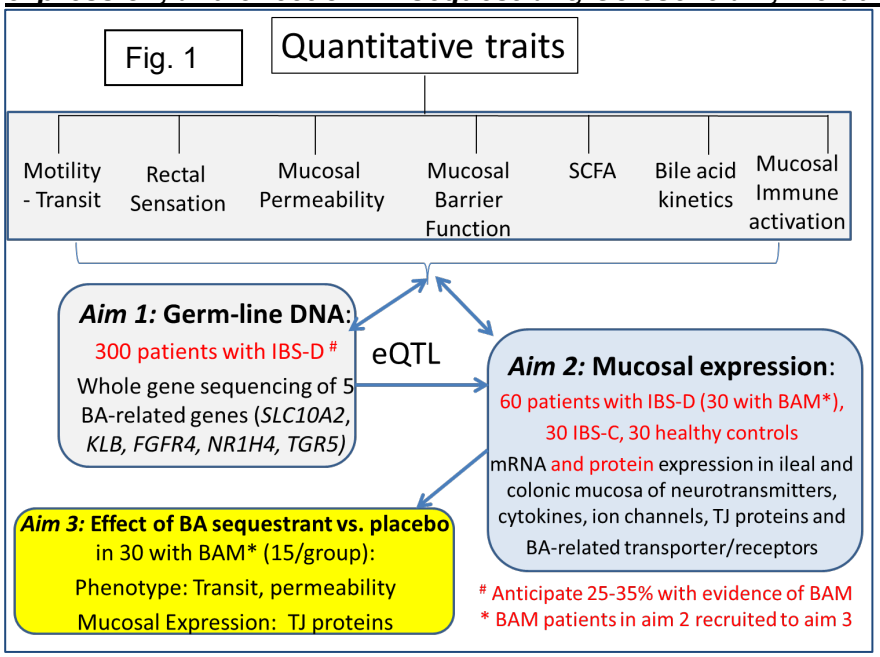
A. INTRODUCTION

A.1. Significance of the Problem. IBS affects ~11% and IBS-D affects ~5% of the US population (1) and often impairs quality of life (13). IBS is currently defined by symptoms (i.e., abdominal discomfort associated with bowel disturbances) in the absence of organic disease on routine testing. The few available therapeutic agents for IBS-D are of very limited efficacy. In contrast to this "one size fits all" approach, there is a large body of evidence **that IBS-D symptoms are manifestations of peripheral mechanisms that perturb motor, mucosal and sensory functions** (3,14). This application is founded on the concept that *better understanding of the phenotypes in IBS-D will identify biomarkers that ultimately will support diagnosis and targeted (individualized) therapy* for the disorder, significantly enhancing health.

Current concepts on IBS focus on disturbances of colonic transit, mucosal immune function, visceral hypersensitivity, and central nervous system (CNS) hypervigilance (2,14,15). The **current proposal focuses on IBS-D**: in our series of 119 patients with IBS-D (3), 48% had accelerated transit, while 20.5% had increased and 16.5% had reduced rectal sensation. Since the pathophysiology and, possibly, genetic and expression mechanisms controlling (for example) motor and secretory functions, may differ significantly in IBS-D and IBS-C, this proposal focuses on IBS-D. Recent research, detailed in *New England Journal of Medicine* (14) and *Journal of Physiology* (16), summarizes peripheral mechanisms in IBS: intraluminal irritants (such as mal-digested carbohydrates (producing SCFA) or fats, excess of BAs, bioactive amines [e.g. serotonin (17)], mucosal barrier function (TJ proteins, e.g. ref. 18), immune activation, increased small intestinal and colonic permeability, and alterations in microbiome. These factors activate local reflexes to alter intestinal motility or secretion, and stimulate sensation and pain (reviewed in *ref. 4*).

We propose that these QUANTITATIVE traits, linked to underlying genetic mechanisms or to alterations in tissue expression of pivotal mechanisms, provide the basis for understanding IBS-D *and optimizing treatment*.

Figure 1. Schema of 3 proposed aims of DK92179 application examining germ-line DNA, mucosal expression, and effect of BA sequestrant, Colesevelam, including numbers of participants in each aim.



This protocol addresses the third specific aim of a program on IBS-D with increased fecal BA excretion *A third aim addresses the potential effects of bile acid sequestration on bowel function, transit, permeability and expression of barrier proteins in rectosigmoid mucosa and will thus confirm the relevance of mucosal alterations demonstrated in aim 2.* To enhance the power of studies in IBS-D exploring gene quantitative trait loci (QTLs) and mucosal expression, *it is essential to measure key quantitative traits*. The relevance of the quantitative traits to subtypes of IBS and its symptoms are discussed elsewhere (14).

A.2. Mucosal Expression of Factors Impacting Pathophysiology of IBS. IBS has been associated with changes in the rectosigmoid mucosal expression of immune mechanisms and non-immune protective factors.

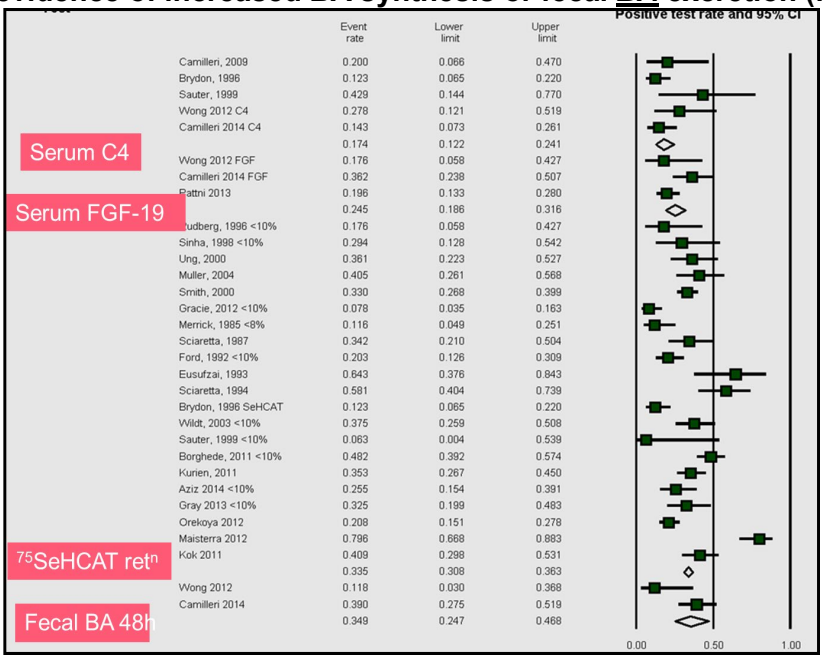
Table 1. Changes in *Colonic* Mucosal Expression in IBS in the Published Literature

Function/ phenotype	Expression rectosigmoid biopsies	Ref.
Cellular defense functions, non-immune-mediated	↑ of mucin gene (MUC20), dual oxidase 2; ↓ caspase-1 and lysozyme	30
Mucosal immune activation: pro-inflammatory IL-8, IL-1 β	↑TLR2 and TLR4 mRNA in epithelial cells; ↑ of CCL-11, CCL-13	30 23
Serotonergic functions	↓ SERT-P mRNA expression	32,33
Immune activation	↓ TNFSF15 mRNA in PI-IBS, ↑ in healthy; ↑ IL-13 mRNA	6, 22
Immune recognition, TJ protein	Not tested	34
TJ protein mRNA expression	↓ mRNA ZO-1 in IBS-D; ↓ ZO-1 and occludin protein; no Δ in mRNA	35 18

There are only a few examples of the inherited genotype that is associated with altered mucosal gene expression, e.g., differential expression of *TNFSF15* genes (which are associated with IBS), has been linked with functional alterations of mucosal immune and protective functions (6,22).

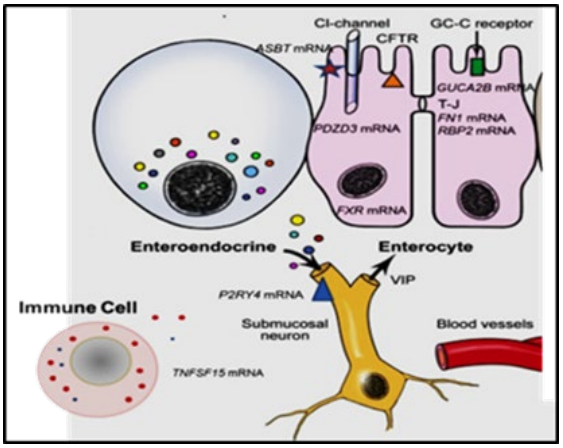
PRELIMINARY DATA

1. **A systematic review and meta-analysis** (39) shows an average **28% of IBS-D patients have evidence of increased BA synthesis or fecal *BA* excretion (figure 2)**



2. **Pilot Study of RNA-Seq of Rectosigmoid Mucosa:**
We examined RNA expression of rectosigmoid mucosa from 9 female IBS-D patients (36) by RNA-Seq (Illumina methods)(Fig. 3), and analyzed using the *edgeR software*. We identified [FDR corrected p<0.05] 21 genes (Fig. 4) with altered **mRNA expression in** that may be biologically relevant in IBS-D: ***neurotransmitters*** [P2RY4 (p=0.001), VIP [p=0.02)], ***cytokines*** [CCL20 (p=0.019)], ***immune function*** (C4BPA complement cascade [p=0.0187]; TNFSF15 [p= 0.0098] and IFIT3 which determines expression of interferon-induced genes [p= 0.016), ***mucosal repair and cell adhesion*** (TFF1 gastrointestinal trefoil protein, [p= 0.012]; RBP2 retinol binding protein [p=0.017]; FN1 fibronectin [p=0.009]), and ***ion transport functions*** with increased expression in: (i) GUC2AB (p= 0.017); guanylate cyclase activator 2B, which encodes uroguanylin which causes enterocyte chloride secretion through guanylate cycles C receptor [GC-C]); (ii).PDZD3 (p=0.029); PDZ protein associates with GC-C and regulates cGMP production, leading to chloride secretion (36). After validating **RNA-Seq** data with RT-PCR of same samples, we proposed in **a model of barrier, ion secretory and immune dysfunctions,** based on mucosal transcriptome changes in IBS-D.

***Figure 3: Summary model (based on RNA-Seq) of* increased mucosal expression of ion secretory mechanisms (GUCA2B, PDZD3), barrier dysfunction (FN1, RBP2), transmitters (P2RY4, VIP), and immune regulation (TNF) in IBS-D.**



3. **Replication of abnormal mucosal mRNA expression in IBS-D and novel observations in IBS-C** (38). The initial observations *by RNA-Seq* were replicated using RT-PCR in rectosigmoid mucosal biopsies from a cohort of 47 IBS-D patients and contrasted findings in 10 IBS-C and 17 healthy controls.

Figure 4. Expression of mRNA (vs. healthy) in IBS-D, *IBS-C (blue histograms)* showing the specificity of the increased expression of guanylin and PDZD3 ion transport and immune mechanisms in IBS-D.

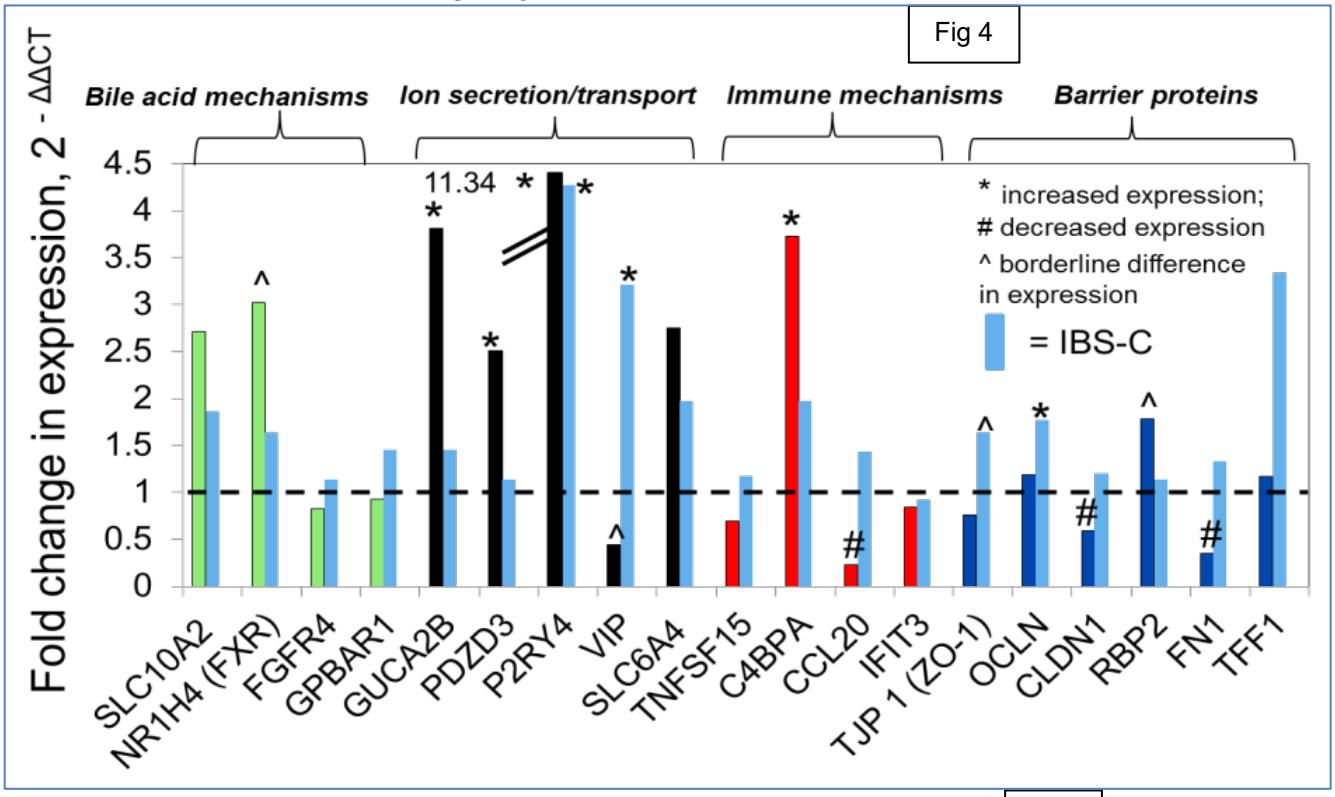
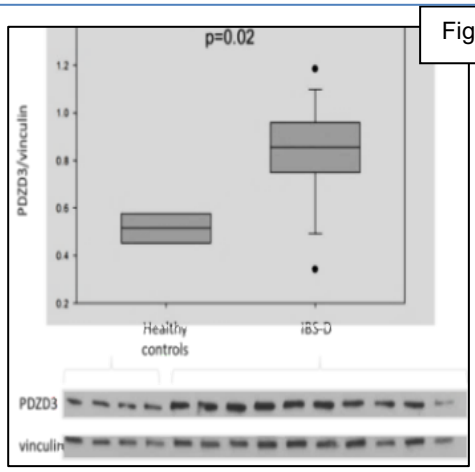
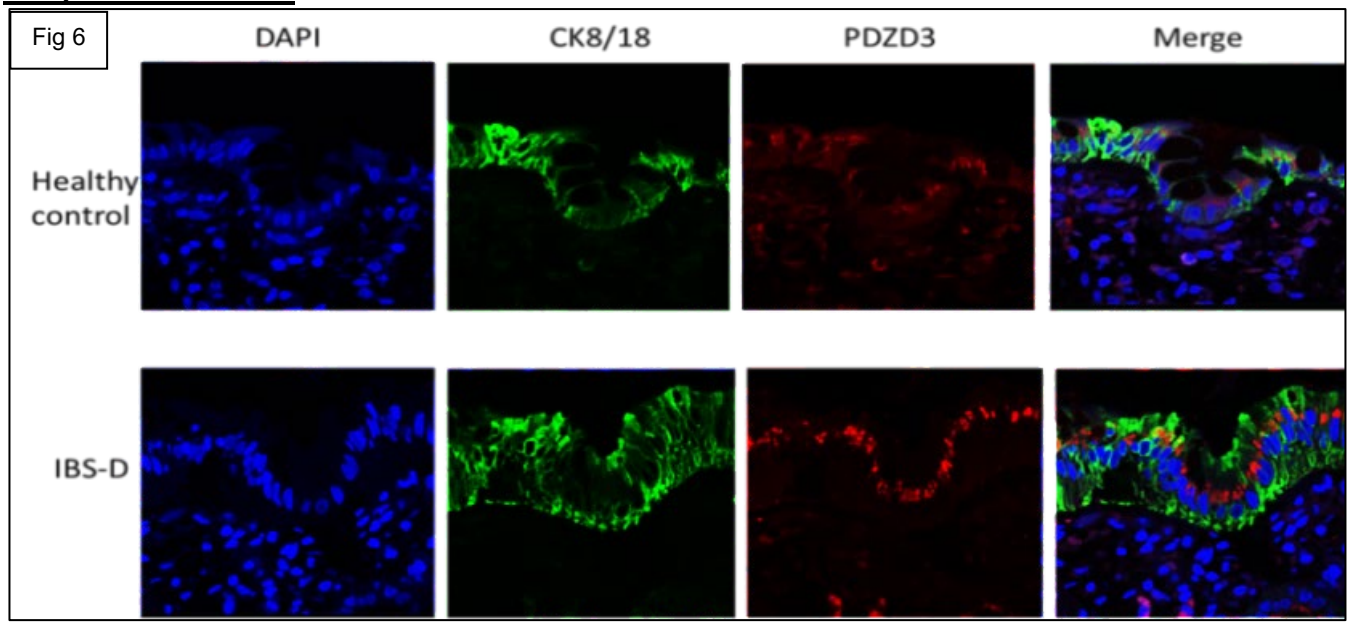


Figure 5. Western blots for protein expression of PDZD3 in colonic mucosa (left) from IBS-D (n=10) *(38),* ***and PPP2R5C (n=7 IBS-D) in small bowel mucosa*** *(43). Note the increased* ***protein*** *expressions relative to vinculin or actin (as control) compared to healthy controls.*

Figure 6. Immunofluorescence of colorectal mucosa showing increased expression of PDZD3 protein in epithelial cells in IBS-D patient compared to control.





Open-label **study of the BA sequestrant, Colesevelam, in 12 patients with IBS-D and high fecal bile acid excretion** demonstrated biological relevance of increased fecal BA excretion (27).

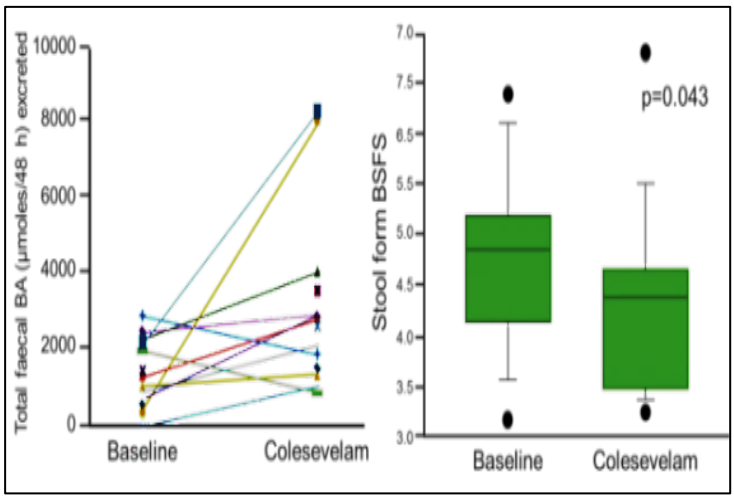


Figure 7. Effects of colesevelam on sequestration of bile acid *(methanol extraction of stool released the bile acids showing that increased amounts were bound)* and *reduced* stool form. Colesevelam improved stool consistency, sequestering and inactivating BA (shown by increased BA in stool after methanol extraction).

METHODS

Participants: Eligibility of 30 patients with IBS-D and evidence of BAM. *Through the application of natural language processing and the electronic medical record, we* have an established database of *~8000* patients with IBS who reside within *~100* miles*, and have been evaluated at* Mayo Clinic in Rochester, Minnesota; **about ~2000 have been identified with IBS-D.** All participants with IBS-D will be selected by computer-based random selection to be invited to participate. The randomly selected 300 patients with IBS-D will complete a validated bowel disease questionnaire, including Somatic Symptom Checklist [BDQ corresponding to Rome III criteria (58)], IBS quality of life [IBS-QOL (59)], and the Hospital Anxiety and Depression Scale [HAD (60)]. Ethnicity and race information is available in >90% of patients at Mayo Clinic.

Consideration of Sex as a Biological Variable*: *Scientific premise for inclusion of both genders *includes: (i) IBS-D affects both genders; (ii) there is no a priori evidence that mechanisms or pathobiology of IBS-D differs between genders.* We shall set up special efforts to recruit males with IBS using REDCAP to identify male patients through the electronic health records (established 17 years ago at Mayo Clinic).

Participants with IBS-D will be screen for BAM 3 ways with either fasting serum alpha C4 or FGF19 taken before 10 am. If C4 ≥ 40 ng/mL or FGF19 ≤ 80 pg/mL either in the past or at the time of screen, the patient will be included in the trial and subsequently randomized. Also IBS-D with a fecal BA >2000µmoles/48h either in the past or at the time of screen will be included in the trial or subsequently randomized.

Quantitative Traits

Colonic permeability is measured by urinary excretion of mannitol and lactulose after oral ingestion (44). We refined a previous HPLC-tandem mass spectrometry technique (45). We validated measurement of colonic permeability after oral delivery by direct instillation of sugars into the colon (46). We have published data showing significantly increased small bowel (P<.001) and colonic (P=0.10) permeability in IBS-D (35); and effects of dietary gluten on small bowel permeability, especially in HLA DQ2/8 +ve (P=0.02) patients with non-celiac IBS-D (35). We validated ^{13}C-mannitol to avoid confounding by dietary ^{12}C-mannitol (47).

Rectosigmoid ***mucosal expression of tight junction (TJ) proteins***, a measure of barrier function: IBS-D patients have lower mRNA expression of TJ proteins [ZO-1 and occludin (both P<.001)], but not claudin proteins (35) relative to healthy controls; values show fold change vs. mean in controls (Table 2).

* P<0.05	Healthy (n=16)	IBS-D (n=25)
ZO-1	1.00 ± 0.024	0.960 ± 0.046*
Claudin	1.00 ± 0.018	1.003 ± 0.020
Occludin	1.00 ± 0.016	0.931 ± 0.025*

Table 2. TJ protein expressions in rectosigmoid mucosa in IBS D and healthy controls (mean ± SD).

In addition, gluten-containing diet was associated with significant decreases in ZO-1 and occludin mRNA expression in rectosigmoid mucosa (48).

Mucosal morphology will assess intra-epithelial lymphocytes, mast cells and immunocytes in lamina propria, and immunohisto-chemistry of TJ proteins (35). GI pathologist will ensure correct orientation.

***Colonic transit*:** Extensive experience over 28 years with validation of colonic transit by radioscintigraphy (49) .

Gallbladder size measurement: Studies have demonstrated that patients with hormones that regulate bile acid synthesis (FGF15 in mice and FGF19 in humans) facilitate gallbladder filling. We want to determine if there is any difference of baseline and post treatment gallbladder measurements. Gallbladder measurement will be conducted fasting both pre and post treatment with a 2-D ultrasound. Patients will be in the supine position with the transducer placed in a sagittal plane in the right upper quadrant of the supine patients and positioned until the greatest length of the gallbladder is obtained. The image will be captured and the length will be measured. The probe will then be rotated 90 degrees to obtain the short axis and obtain the greatest dimension in that window.

BA kinetics*: Assessment of BA synthesis and fecal excretion (uniquely available in USA at Mayo Clinic)*
Serum 7α-hydroxy-4-cholesten-3-one (7α-HCO or C4) measures hepatic cholesterol synthesis, and is a validated method for BA malabsorption [BAM (51,52)]. Serum C4 (ref. 53, assay based on ref. 54) is positively correlated with fecal BA excretion [r_s=0.61, p<0.001 (5)].
Serum fibroblast growth factor 19 (FGF19), a measure of feedback regulation of BA synthesis (54B), will be measured by ELISA (R&D Systems, Minneapolis, MN).
Fecal BA excretion: Using HPLC/tandem mass spectrometry, we adapted a method (55) to measure fecal total and individual BAs. We have previously shown that fecal total BA (5), and **secretory** primary and secondary BAs [chenodeoxycholic (CDCA) and deoxycholic acids (DCA)] are higher in IBS-D, whereas CDCA is decreased and non-secretory lithocholic acid increased in IBS-C (56)].

Short chain fatty acids *(SCFAs) in stool by gas chromatography-mass spectrometry (*Mayo CTSA ICL Lab)
The method is adapted from Tangerman et al. (57) and permits analysis of 8 SCFAs, from C2 to C6, plus an internal standard, 2-ethyl butyric acid using a Thermo Trace GC Ultra Gas Chromatograph (San Jose, CA) fitted with a flame ionization detector (FID). Fecal samples are extracted using acetonitrile and injected without dilution; components were identified according to their retention times compared to standards.

Studies on Sigmoid Mucosal Biopsies: Tight Junction Proteins, Morphology, RNA-Seq. With informed consent, 8 samples will be acquired from the rectum and sigmoid colon and 4 from terminal ileum, for each patient. Biopsies will be immediately preserved in formalin (histopathology), or in a solution of RNAlater (Ambion, Austin, TX), stored at -80°C. RNA extraction will be performed as in the manufacturer's instructions (RNeasy mini kit, Qiagen, Valencia, CA). The quantity and quality of the extracted RNA will be determined by the NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE) and the Bioanalyzer 2100 (Agilent Technologies, Inc., Santa Clara, CA). All RINs will be determined using the Agilent Bioanalyzer. RNA-Seq analysis will be conducted as in section C.1.e and as in Camilleri et al. (36). Ileal mRNA of ASBT (SLC10A2) and FXR (NR1H4) in ileal mucosa will be measured by RT-PCR (see C.2.9.i.).

***Quantitation of Tight Junction (TJ) Proteins in Sigmoid Mucosa Using Real-Time PCR*.**
TJ proteins [zonula occludens 1 (ZO-1), occludin (OCLN), claudin-1 (CLDN-1), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH, control)] will be assayed in rectosigmoid biopsies. cDNA synthesis will be performed using 0.2 µg of total RNA with the High Capacity Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Taqman gene expression assays for ZO-1, OCLN, CLDN-1, and GAPDH will be carried out in triplicate for each gene on an ABI Prism 7300 Real-time PCR System (Applied Biosystems) according to manufacturer's instructions using the comparative $^{\Delta\Delta}$CT method for relative quantification. mRNA expression of each gene (normalized to GAPDH) will be compared by statistical analysis(35,48).

Colonic Mucosal Morphology. The colonic biopsies will be stained with **H&E and tryptase** to assess intraepithelial lymphocytes (IEL) and mast cells. Immuno-histochemical staining of 5µ-thick paraffin sections will be performed using a commercial kit; primary antibodies used will be CD3, CD4, CD8, CD68, CD79, tryptase (Dako, Carpenteria, CA) and ZO-1 (Invitrogen Corporation, Camarillo, CA). Biopsies will be assessed by one experienced GI pathologist (**T. Smyrk, MD**) who will be blinded to IBS-D/C subtype. **Dr. T. Smyrk, co-investigator**, has expertise and experience from our prior collaborative studies (48, DK86182). IEL will be counted on the H&E slides and expressed as IEL/100 epithelial cells. Immune cells stained for CD3 and CD8 will be counted on semi-quantitative scale of 1-4. ZO-1 will be scored as percentage of epithelial cells positive, and semi-quantitative assessment of stain intensity [on scale 0-3 (48)].

In Depth RNA Sequencing of Colonic Mucosa (analysis by **E.W. Klee, Ph.D., Bioinformatics**).
*Assay Protocol: Total RNA will be isolated from mucosal biopsies (*Qiagen RNeasy Mini Kit)*. RNA-Seq will be performed* on the Illumina Hi-Seq 4000, using the RNA-Seq protocol with 101 base, paired-end reads.

Samples will be multiplexed *6* per lane, averaging >*100* million reads-per-sample, with 60.4% of reads mapping to the gene regions. *Bioinformatics tools for pathway analysis will also be utilized (Dr. E. Klee Ph.D.).* Library preparation and *sample sequencing* will be performed by the **Mayo Clinic Advanced Genomic Technology Center Core Facility**. Special techniques for analysis, ensuring uniformity in cross-gene and sample set comparisons and pathway analysis are detailed in the section on laboratory facilities available.

In Depth Proteomics of colonic mucosal biopsies in duplicate by a label-free LC-MS/MS method (see letter of support from Dr. Daniel J. McCormick Ph.D., Proteomics Core, Mayo).

Extraction and separation: Proteins from the biopsy will be extracted in SDS buffer containing protease inhibitors using a bead beater to disrupt tissue. Extracted proteins will be run on a gel, proteins separated by size, segmented, and in-gel trypsin digested to obtain peptides to compare across samples.

Liquid chromatography is used to load peptide sample onto a C8 cartridge, which is then placed in-line with a C18 analytical column. Peptides are sorted by ionic strength using a mobile phase gradient of acidic low to high organic solvent, eluted off the column, captured into mass spectrometer (MS) via electrospray ionization, followed by data dependent ms/ms data for peak identification. D. We also have access to single reaction monitoring (SRM) mass spectrometry on QExactive and triple quad instruments in the Proteomics Core for more accurate quantification of proteins if necessary.

Data analysis from high resolution MS will use MaxQuant, a quantitative proteomics software to compare high resolution MS data. Database searching within MaxQuant assigns amino acid sequence to peptides and assembles peptide information back to proteins. Quantified values of proteins between groups are compared.

Biological Samples Collected

1. **Venous Blood Sample** (fasting) will be collected for **serum FGF19 and C4. Venous blood DNA will be stored for future genetic studies.**
2. **Fecal Sample for Fecal Organic Acids:** Gas chromatography-mass spectrometry for SCFA in stool and HPLC tandem MS for total and individual fecal BAs. Samples will now be a random stool sample without a required dietary change unless they have not had a qualifying diagnosis of BAM as noted in the Research Plan below. Samples will then be processed for total fat (routine van de Kamer method at Mayo Medical Laboratory), **total BAs,** and **primary and secondary BAs** [by LC/MS as previously described from our lab (56)], and **SCFA by GC-MS**
3. Additional **stool** and rectosigmoid **mucosa samples** will be **stored for future microbiome studies**.
4. **Urine Samples to quantitate Excretion of Lactulose and ^{13}C-Mannitol after Oral Ingestion** of lactulose and ^{13}C mannitol (5:1 ratio by mass) (47). The 0-2h urine most closely reflects small intestinal permeability and 8-24h urine reflects colonic permeability (46). HPLC-tandem mass spectrometry will be used for detection of the sugars (Mayo Clinic CTSA Lab). Baseline and post treatment small bowel and colonic permeability will be performed for every patient.

Cumulative (Cum) excretion (0-2h and 8-24h) = Concentration of sugar (µg/mL)] * total urine volume (mL).
Lactulose: mannitol ratio (L:M R) is: 0.2 x (Cum excretion lactulose) / (Cum excretion **^{13}C-**mannitol).

5. **Sigmoidoscopy and Mucosal Biopsies from sigmoid will now be optional.** Participants will have the option to complete sigmoidoscopy at the beginning, end, both time points or refrain from completing this portion of the study. If participants agree to perform the sigmoidoscopy, it will be performed in all fasting participants in the Clinical Research unit, and in the same time of day (7-8am), with the patients' medications stopped 48 hours before the biopsies for the baseline studies. The biopsies will be collected in formalin or RNAlater solution (Ambion, Austin, TX) and stored at -80°C until further analyzed.

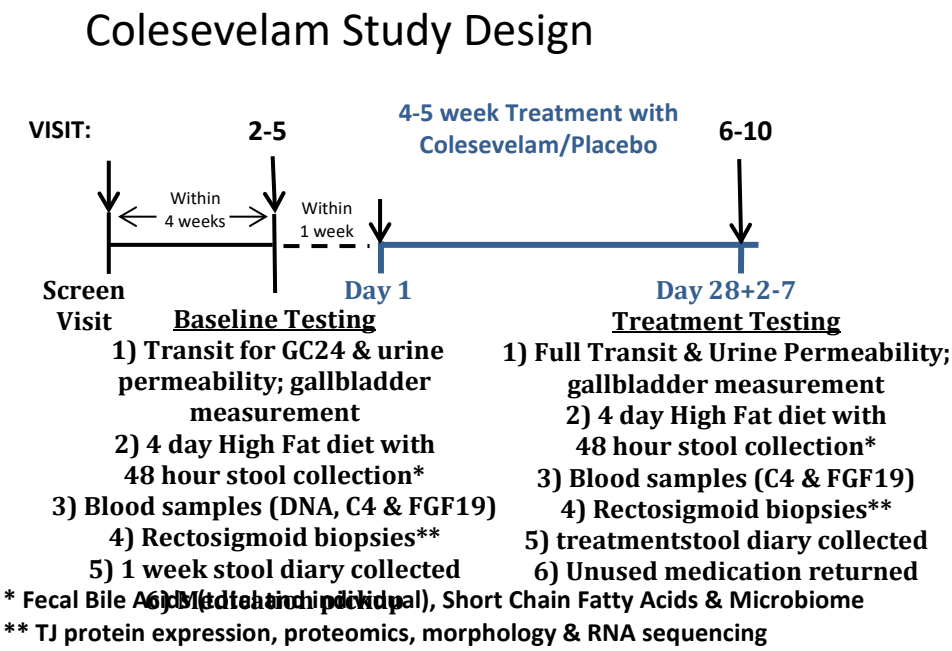
RESEARCH PLAN for Randomized controlled trial of colesevelam in IBS-D with high fecal BA.

Hypotheses: BA sequestration normalizes colonic *functions* in IBS-D with increased fecal BA excretion.

Specific Aim: To compare with a randomized trial *(n=15 per treatment group), effects of* colesevelam 1.875g bid and placebo treatment, on colonic transit, bowel functions*, permeability and tight junction expression in rectosigmoid mucosa* of IBS-D with fecal BA>2000 µmoles/48h (upper limit of normal).

Rationale: BA diarrhea is increasingly recognized as a subtype of IBS-D (39,41). In an open-label study (27), we identified beneficial effects of colesevelam in the treatment of IBS symptoms in patients with high total fecal BA excretion (>2000µmoles/48h), elevated primary bile acids (>4%) with elevated total fecal bile acids (>1,000 µmol/48h), or primary bile acids > 10%, elevated C4 (> 40 ng/mL), and decreased FGF19 (<80 pg/mL) (section C.1.g and figure 7)).

Human subjects and design: 30 IBS-D patients with BAM will be randomized (15/group) in 4-week double-blind, parallel-group trial to Colesevelam, 1.875g b.i.d. or placebo. Patients will be stratified on gender and BMI; we anticipate 90% female and 75% BMI >30kg/m^2.
Experimental procedures: Effects of colesevelam/placebo on symptoms (daily stool diaries), as well as colonic transit, fecal bile acid and fat excretion and fasting serum C4 and FGF-19, *intestinal permeability and TJ expression in rectosigmoid mucosal biopsies at baseline and at end of* 28 days' treatment.
Primary endpoints: 1. Bowel movements/week; 2. Stool consistency based on Bristol Stool Form Scale, BSFS. *Secondary endpoints*: 1. Quantitative total fecal BAs; 2. Proportion of secretory BA (CDCA, DCA) in stool; 3. Fasting serum C4 and FGF-19 levels; 4. Colonic transit, geometric center at 24 and 48 hours; *5. Intestinal and colonic permeability (mannitol, lactulose excretion); 6. TJ expression in rectosigmoid mucosa*.



This study consists of ten visits to Mayo Clinic at the Charlton 7 Clinical Research Trials Unit (CRTU). Some of the visits can be combined.
Visit 1 (Screen visit) Volunteers will be asked to come to the Charlton 7 to review and sign this informed consent form and all have all their questions answered. This visit will require about 90 minutes.

- QUESTIONNAIRES: Volunteers will be asked to complete two questionnaires.
  1. Bowel Disease Questionnaire, which asks questions about their bowel symptoms
  2. Hospital Anxiety and Depression Inventory, which asks about any anxiety or depression they are currently feeling
  3. Quality of Life Questionnaire, which ask you how your bowel symptoms are affecting your life
  4. Symptom Checklist, which ask you about any symptoms you are currently feeling
- VITAL SIGNS, HEIGHT AND WEIGHT: A nurse will take your height, weight and vital signs (pulse, blood pressure, respiration rate and temperature).
- HISTORY AND BRIEF PHYSICAL EXAM: A study physician will review their medical history, current medications and supplements and perform a physical exam.
- POSSIBLE BLOOD DRAW: A small amount of blood may be taken if the study physician determines that blood tests need to be run to see if they qualify for the study. Such as serum alphaC4 or FGF19.

If the study physician finds the volunteer eligible for the study, they will be asked to participate in the rest of the study.

- STOOL COLLECTION: You will be given a stool kit and will be asked to return a stool sample before your Flexible Sigmoidoscopy.
- If the patient has not had previous qualifying FGF19, C4, primary to total fecal bile acids in the past, then fasting serum blood work for FGF19 and C4 will be completed at the Screen visit. A 48 hour stool

test to evaluate for primary and total fecal bile acids will be conducted first. The remaining study examinations, abbreviated colonic transit test, permeability test, random stool sample, flexible sigmoidoscopy, and gallbladder ultrasound will be postponed by at least one week.

Visit 2 (Pretreatment testing) Participants will be asked to report to the desk on Charlton 7 after an 8 hour fast (no food/drink, a small amount of water is OK), and the avoidance of artificial sweeteners 48 hours before and during the permeability testing (Visits 2-3). The visit should take approximately 45-60 minutes.

- PREGNACY TEST, if needed: A urine sample will be collected for a pregnancy test from women who can get pregnant if it was not done within 48 hours of this study day. The results of this test must be negative in order for participants to continue the study.
- FASTING BLOOD DRAW: Participants will be asked to give a small amount of blood for DNA, serum alpha C4 and FGF19 testing if not done previously.

DIARY RETURN: Participants will be asked to return the completed 7-day Pretreatment Diary.

- ABREVIATED COLONIC TRANSIT TEST/Permeability test: : Participants will be asked to swallow a small capsule containing a small amount of a radioactive substance with a liquid containing a small amount of naturally occurring sugars: lactulose and mannitol. The sugars allow the measurement of the permeability of the small intestine and colon. The participants will be instructed to have breakfast one hour later.

VISITS 3-5 MAY BE COMBINED

Visit 3 (24 hour image) Participants will be asked to report to the desk on Charlton 7 (fasting required) approximately 24 hours after they ingested the capsule containing radioisotope during Visit 2. Ultrasound measurement can be performed before or after the 24 hour image is obtained. The ultrasound gallbladder measurement should take ~ 10-15 minutes. This visit should take approximately 25 minutes.

Visit 4 (Flexible sigmoidoscopy) [OPTIONAL] Participants will be asked to report to the desk on Charlton 7 (no fasting required). The visit should take approximately 75 minutes.

- ENEMA: Participants will need to prepare the colon for the sigmoidoscopy by means of a tap water enema at the CRTU. A CRTU nurse will give a tap water enema. Two enemas may be given if needed.
- FLEXIBLE SIGMOIDOSCOPY WITH BIOPSIES: A sigmoidoscopy is a lower endoscopy that uses a flexible endoscope to visually inspect the lining of the sigmoid colon. Several biopsies of the lining of the colon will be removed for examination under a microscope, TJ protein expression, proteomics and RNA sequencing.. No sedation is needed. The nurse will review information regarding post-endoscopy care and the participant will be dismissed.

Visit 5 (Study Medication pickup) Participants will be asked to report to the desk on Charlton 7 (no fasting required).The visit should take approximately 15-30 minutes.

- PREGNACY TEST, if needed: A urine sample will be collected for a pregnancy test from women who can get pregnant if it was not done within 48 hours of this study day. The results of this test must be negative in order to continue the study.
- STUDY MEDICATION: The nurse will dispense a 5 week supply of the Colesevelam/placebo and instruct participants to take 3 tablets orally twice daily with lunch and supper.
- SINGLE RANDOMSTOOL SPECIMENS: Returned as instructed
- DIARIES RETURN: Participants will be asked to return the completed 7-day Pretreatment Bowel diary
-

TREATMENT: Participants will take 1875 mg (3 tablets [625 mg/tablet]) of the medication orally twice daily with lunch and supper for 4-5 weeks. They also will be asked to complete the following visits after 21 days of treatment with the treatment fat diet and stool collections completed after the transit/permeability and flex sig/biopsy tests. A separate stool sample collection kit will be provided on Visit 6 to be returned before the flexible sigmoidoscopy. No special diet is required with this particular stool collection.
Note: Treatment Bowel diary will not be completed by participants during high fat diet.

Visit 6 (Treatment testing) Participants will be asked to report to the desk on Charlton 7 after an 8 hour fast (no food/drink, a small amount of water is OK). And the avoidance of artificial sweeteners 48 hours before and during the permeability testing (Visits 6-7). This visit will require about 9-10 hours total.

- PREGNACY TEST, if needed: A urine sample will be collected for a pregnancy test from women who can get pregnant if it was not done within 48 hours of this study day. The results of this test must be negative in order to continue the study.
- VITAL SIGNS: A nurse will take vital signs (pulse, blood pressure, respiration rate and temperature).
- FASTING BLOOD DRAW: Participants will be asked to give a small amount of blood for serum alpha C4 and FGF19 testing.
- FULL COLONIC TRANSIT/PERMEABILITY TEST: Participants will be asked to swallow a small capsule containing a small amount of a radioactive substance with a liquid containing a small amount of naturally occurring sugars: lactulose and mannitol. The sugars allow the measurement of the permeability of the small intestine and colon. Approximately one hour later, the transit test starts with a scrambled egg breakfast (with toast and a glass of milk). The eggs also contain a small amount of radioactive substance. Participants will eat two additional meals, a lunch of chicken breast (4 hours after the egg breakfast) and a dinner of a roast beef sandwich (8 hours after the egg breakfast). These meals are not radioactive, they are supplied only so all participants in this study will eat the same type and same amount of food this day. They will be asked not to eat or drink anything except for water while undergoing testing today other than what they are given. At the completion of the breakfast test meal they will be instructed to stand in front of a special camera and pictures will be taken immediately after the egg breakfast and then 1, 2, 3, 4, 6 and 8 hours later. Participants will be asked to collect urine samples at scheduled intervals for the next eight hours. After the 8 hour image is taken, they will collect all urine until the next morning. A urine collection kit will be provided to then for this. After they leave the CRTU, they may eat or drink your own foods, but ingestion of alcohol and drinks containing any sweeteners must continue being avoided until after Visit 7 as they interfere with the measurements of the research study.
- STUDY MEDICATION ADMINISTRATION: The CRTU nurse will administer a dose of the study medication with the lunch and snack meals provided during this visit.
- STOOL COLLECTION: You will be given a stool kit and will be asked to return a stool sample before your Flexible Sigmoidoscopy.
-

Visit 7 (24 hour image) Participants will be asked to report to the desk on Charlton 7 (fasting required) for the 24 hour image. Ultrasound measurement can be performed before or after the 24 hour image is obtained. The ultrasound gallbladder measurement should take ~ 10-15 minutes. The visit should take approximately 25 minutes.

VISITS 8 AND 9 MAY BE COMBINED

Visit 8 (48 hour image and urine collection return) Participants will be asked to report to the desk on Charlton 7 (no fasting required) to drop off their 8-24 hour urine collection and have the 48 hour image taken. The visit should take approximately 10 minutes.

Visit 9 (Flexible sigmoidoscopy) [OPTIONAL] Participants will be asked to report to the desk on Charlton 7 (no fasting required). The visit should take approximately 75 minutes.

- ENEMA: same as Visit 4
- FLEXIBLE SIGMOIDOSCOPY WITH BIOPSIES: same as Visit 4

Visit 10: End of Study:

- DIARIES RETURN: Participants will be asked to return the completed Treatment Bowel diary
- MEDICATION RETURN: On the last visit participants will be asked to return any unused medication.

Statistical analyses: Effects of treatment will be compared (using Intention-To-Treat principles) using ANCOVA with BMI, gender, baseline colonic transit and baseline fecal BA excretion as covariates.
November 1 2018: at least 75% of the participants will have completed all study procedures. We plan to complete an interim analysis in order to submit an abstract for a national gastroenterology meeting in May 2019. We anticipate completing all studies in all participants by the time the data need to be presented. At that time, we shall present the data from the ENTIRE patient cohort; that analysis will be used for the one full manuscript emanating from these data.

A senior statistician will review the interim analysis results and analyze the data related to the previously determined primary endpoints. All members of the study team and the investigative team will remain blinded to treatment arm assignment and treatment arm results until the completion of the study. Thus, this would not affect the p value when the study is completed.

Statistical power: Based on our previous open-label study, the proposed sample size (15 per group) has ~80% power (2-sided α=0.05) to detect clinically relevant effect sizes in stool frequency and form (expressed as detectable difference [Δ] in 2 groups: in **Table 3: Detectable differences based on 80% power**.

Response	Mean (SD)	Detectable Δ
#Stools /week	15.1 (6.6)	7.00
Stool Form (BSFS 1-7 scale)	4.4 (1.0)	1.06
Total Fecal BA excretion µmoles/48h	3496 (2456)	2650
Fecal Fat g/24h	6.8 (3.1)	3.30
Serum C4 ng/mL	72.3 (4.3)	45

Anticipated results and significance: Colesevelam will reduce stool frequency, decrease stool consistency and slow colonic transit in IBS-D with high BA excretion.

Precautions and alternative approaches:

1. The RCT has sufficient power based on directly relevant open-label trial data with same drug and disease.
2. Other agents that reduce hepatic BA synthesis and are in development will be alternatives for BA sequestrants, e.g. FXR agonists, FGF-19 analogs.

PLANNED ENROLLMENT REPORT

Comments: 30 patients IBS-D with BAM

Racial Categories	Ethnic Categories				
	Not Hispanic or Latino		Hispanic or Latino		Total
	Female	Male	Female	Male	
American Indian / Alaska Native	1	0	0	0	1
Asian	2	1	0	0	3
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	2	1	0	0	3
White	13	7	2	1	23
More Than One Race	0	0	0	0	0
Total	18	9	2	1	30

Inclusion of Children

Children, 18-21 years of age, and adults 21 years or older will be eligible for participation in the study as active participants or as healthy controls. However, children below 18 years of age will be excluded, since there are differences in the criteria of diagnosis in adults and children, and insufficient quantitative pediatric data on the traits to be analyzed in order to assess the phenotypes of IBS, e.g. transit, permeability, etc.

Inclusion of Women and Minorities

In advertisements for patients and healthy subjects, we shall specifically solicit participation of women and minorities. We anticipate that there will be a majority of whites and representation of blacks and Asians, consistent with the ethnic constitution of communities in southeastern Minnesota, which is approximately 90% Caucasian, 5% Southeast Asian, and 5% other minorities. The prevalence of IBS among whites, blacks and Hispanics in the U.S. is very similar.

Consideration of Sex as a Biological Variable

We plan to recruit approximately 2 females to one male patient for these studies. We shall set up special efforts to recruit males with IBS using REDCAP to identify male patients through the electronic health records

(established 17 years ago at Mayo Clinic. Thus, we have identified through search of electronic medical records that there are:

a. 2000 IBS patients residing within 100 miles of Rochester, MN.
b. 350 patients with IBS-constipation residing within 100 miles of Rochester, MN

Scientific premise for inclusion of both genders includes:
IBS-D affects both genders; there is no a priori evidence that mechanisms or pathobiology of IBS-D differ between genders. Therefore, our study will assess mucosal mRNA and protein expression and response to colesevelam treatment in both genders.

PROTECTION OF HUMAN SUBJECTS

1. Risks to the Subjects

a. Human subject involvement and characteristics

We shall recruit 30 patients with IBS-D, and BAM, residing in an area with a radius of 100 miles from Rochester, MN. We have an established database of ~*2000* patients with IBS-D within *100* miles of Mayo Clinic in MN. Patients will have been evaluated by the investigators in the Division of Gastroenterology at Mayo Clinic, Rochester, MN. Healthy subjects residing in southeastern Minnesota will be recruited without restrictions for gender, race or ethnic group. We anticipate that among healthy participants there will be a majority of whites, and representation of blacks and Asians, consistent with the ethnic constitution of communities in southeastern Minnesota, which is approximately 90% Caucasian, 5% Southeast Asian, and 5% other minorities. Given the ratio of females to males in irritable bowel syndrome in gastroenterology clinics, we **anticipate recruiting 2 females to 1 male.** We shall set up special efforts to recruit males with IBS using REDCAP to identify male patients through the electronic health records (established 17 years ago at Mayo Clinic). *Therefore the proposal addresses the requirement for relevant inclusion of **both genders** in the research proposal.*

To avoid tertiary referral bias, at least 50% of patients selected will reside within 100 miles of Mayo Clinic.

The racial and ethnic characteristics of the study cohort will be characterized in ~90% of participants. This is information that has to be collected on a voluntary basis in the medical record, and patients cannot legally be coerced into providing this information.

Participants: Eligibility of Patients with IBS

We have a database of ~900 patients with functional gastrointestinal diseases who reside within ~150 miles from Mayo Clinic which is located in Olmsted County, MN.

All participants will complete the validated Bowel Disease Questionnaire [BDQ, including questions to correspond to Rome criteria (58)], the Symptom Checklist-90 [SCL-90 (59)] and the Hospital Anxiety and Depression Scale [HAD (60)]. The BDQ also includes a psychosomatic symptom checklist intended to identify somatization disorders. We have used these questionnaires extensively in several studies.

A positive diagnosis of IBS will be based on the Rome III criteria (83): At least 3 months, with onset at least 6 months previously, of recurrent abdominal pain or discomfort associated with 2 or more of the following: a) Improvement with defecation; and/or b) Onset associated with a change in frequency of stool; and/or c) Onset associated with a change in form (appearance) of stool (discomfort means an uncomfortable sensation not described as pain).

Inclusion criteria	IBS patients
Age (yr)	18-75
Gender (F:M)	2:1
BDQ - IBS symptoms	+ve by Rome III criteria
Hospital Anxiety/Depression score	No restrictions
Abdominal surgery (except appy or choly)	None
GI medications	None past 48h

BAM	serum alpha C4 ≥ 40 ng/mL or FGF19 ≤ 80 pg/mL, fecal BA >2000 µmoles/48h, total fecal BA > 1000 µmoles/48h + ≥4% primary BA, or >10% primary BA

Note: If a score of ≥11 for either anxiety or depression is obtained from the Hospital Anxiety Depression Scale, these finding will be documented in their medical record, the volunteer is informed of the findings and their primary care physician is contacted about the score. If the volunteer is experiencing active suicidal/homicidal ideations, they will be sent to the emergency room.

Exclusion criteria

In all patients diagnosed with IBS, we apply rigorous standards to exclude clinically-relevant inflammation. Patients with known bleeding diathesis will be excluded, given the need for sigmoid mucosal biopsies.

PROHIBITED MEDICATIONS

Patients participating will not take any of the following disallowed medications for at least 7 days prior to and during the remainder of the study:

1. Any treatment specifically taken for IBS-D, including loperamide, cholestyramine, alosetron
2. Drugs with a known pharmacological activity at 5-HT4, 5-HT2b or 5-HT3 receptors (e.g, tegaserod, ondansetron, tropisetron, granisetron, dolasetron, mirtazepine, cilansetron);
3. All narcotics (e.g, codeine, morphine, and propoxyphene, either alone or in combination)
4. Anti-cholinergic agents (e.g, dicyclomine, hyoscyamine, propantheline).
5. Tramadol
6. GI preparations:
   - Anti-nausea agents (e.g, trimethobenzamide, promethazine, prochlorperazine, dimenhydrinate, hydroxyzine);
   - Osmotic laxative agents (e.g, lactulose, sorbitol or PEG solutions as Miralax and Glycolax);
   - Prokinetic agents (e.g, cisapride, metoclopramide, prucalopride, domperidone);
   - 5-HT3 antagonists (e.g. alosetron)
7. Oral anticoagulants
8. Antimuscarinics
9. Peppermint oil
10. Systemic antibiotics, as well as antibiotics directed at colonic flora such as rifaximin and metronidazole

In advertisements for patients, we shall specifically solicit participation of women and minorities. The prevalence of IBS among whites, blacks and Hispanics in the U.S. is very similar. Children, 18-21 years of age, will be eligible for participation in the study as patients or as healthy controls. However children below 18 years of age will be excluded since it is unethical to expose healthy children to the potential risks of rectal intubation and radiation, and studies of IBS children in these age groups will be uninterpretable without age and gender matched controls from healthy individuals**.**

Dietary, Fluid and Other Restrictions

During recruitment, informed consent review, and baseline period, the subjects will be informed and reminded of the following restrictions:

- No alcohol for 48 hours before each and during each test.
- Patients must fast for at least 8 hours before visiting the clinic for the colonic permeability test and colonic transit tests.
- No artificial sweeteners for 48 hours [Splenda (sucralose), NutraSweet (aspartame), lactulose or mannitol] before the permeability testing. Examples of foods to avoid are sugar free gums, mints and diet soda
- Avoid taking any additional over-the-counter or prescription medications or herbal supplements that have not been reviewed and approved by the physician or the study coordinator until the study has been completed.

Sites Where Research Will Be Performed

Mayo Clinic Rochester will be the only site where the research is performed; the human studies will be conducted in the CRTU of the Mayo Clinic CTSA; all analyses of biological samples in the Molecular Genotyping core facilities or the Immunochemistry Core Lab (CTSA) at Mayo Clinic and the functional genomics studies in the Department of Physiology and Biomedical Engineering at Mayo Clinic.

b. Sources of research materials

Research material will be the medical records (of those who authorize review of the records for research purposes), prospectively acquired measurements of gastrointestinal and colonic transit with an external gamma camera, observations obtained at flexible sigmoidoscopy, mucosal biopsy mRNA expressions of interest (tight junction proteins and mRNA sequencing), measurements of fecal BAs and short chain fatty acids, and in vivo permeability measurements. There will also be venous blood samples from which DNA will be extracted and stored and subsequently analyzed using whole gene sequencing. Results of studies will be accessed by a secure password available only to study personnel. Data will be merged for studies of associations by the study biostatisticians.
All of the information collected in this study will be for research purposes only.

c. Potential risks

The potential risks associated with the study are radiation exposure from scintigraphy, *sigmoidoscopy,* and rectosigmoid mucosal biopsies. The precautions associated with minimizing these risks are discussed below under 2 b, protection against risk, and under 5, the Data and Safety Monitoring Plan.

2. Adequacy of Protection against Risks

a. Recruitment and Informed Consent

Prior to initiating the study, all subjects will provide written informed consent using forms. The consent form becomes a permanent part of the medical record at Mayo Clinic. All subjects will be given a verbal explanation of the study, provided time to read and study the written consent form approved by Mayo's Institutional Review Board and its information, given opportunities to ask questions and a copy of the consent form. Participants will be informed of their right to withdraw from the study at any time without prejudice to their clinical management now or in the future. Consent will be sought by one of the members of the investigative team (physician or study coordinator) properly trained in the consent process. The consent will be documented by the participant's signature on the consent form. Specific information is provided in the Mayo consent form regarding storage and future use of the DNA sample. The participants will be informed that *since this genetic information is not yet pertinent to clinical practice, the information will not be included in the medical record, but will be maintained in a coded fashion accessible only to study personnel.*

All recruitment or contact information will be approved by Mayo Clinic Institutional Review Board.

b. Protection against risk

Responsible Conduct of Human Research by Study Personnel

Mayo Clinic has established a formal program entitled the Mayo Investigator Training Program or MITP. The MITP is a web based educational course designed to provide all personnel involved in human subject research with training about human subject protection. All Mayo personnel engaged in human subject research are required to complete the course. The primary objectives of the course are to provide the historical framework for current human subject protection regulations and to explore the evolving issues related to human subject research. The course is divided into four sections:

* Course introduction and general overview
* History section with examples of unethical behavior in human subject research
* Review of major human subject protection issues
* Discussion of the various roles and responsibilities of individuals involved in human subject research

At the conclusion of the instruction, individuals are required to complete a thirty-question assessment. All Mayo investigators have completed the Mayo IRB's mandated certification in the responsible conduct of research.

Specific Risks and Precautions Proposed

i. Radiation exposure

Radiation exposure results from ^{99m}Tc (sulfur colloid, SF) and ^{111}In used to measure gut transit. These exposures conform to previously approved levels of radiation exposure approved by the Radiation Control Committee at Mayo Clinic.

The radiation dosimetry and organ exposures (in mrad) are listed below:

GASTRIC, SMALL BOWEL AND COLONIC TRANSIT

Radiopharmaceutical	Activity mCi	Body	Gonads	Breast	Red marrow	Lung	Thyroid	Bone	ULI	Colon	Stomach	Bladder	Liver	Esophagus	Other
$^{111}In\ Cl_3$	0.1	20	140		20			380	740		60	40	10		160
^{99m}Tc S.C.	1.0	20	90		20			420	300		130	20	10		220

(mrad= radiation absorbed dose to organs; S.C. = sulfur colloid))

He or the radiation effective dose to the body summarizes the risk to the whole body as the individual doses to each of the organs; effective dose is used to compare risks among various types of x ray and radionuclide studies: $^{111}InCl_3$ 0.1 mCi: H_e 142 mrem; ^{99m}TcDTPA 1.0 mCi, H_e 90 mrem; (where mrem= radiation equivalent dose).

In view of the radiation exposure, all females of childbearing age will be required to have a negative urine pregnancy test within 48 hours of the radioisotope studies.

ii. Flexible Sigmoidoscopy has very low risk of bowel perforation, estimated at ~1 in 10,000.

To date, these studies have not been associated with any perforation or mucosal damage that resulted in any clinically important issues such as local hemorrhage or abscess formation**.**

iii. Medications:

No unapproved medications are being tested:

Colesevelam will be used for an unapproved indication, but there is no intention to develop plans to acquire approval to market the medication for the indication IBS-diarrhea with increased fecal BA excretion. Mayo Clinic Research Pharmacy will over-encapsulate commercially available medication, produce identical placebo capsules and will be responsible for maintaining the randomization code and study blinding.

Flexible sigmoidoscopy and rectosigmoid biopsies will be performed without conscious sedation.

iv. Genetic information

Results of the studies will be maintained in summaries in computer-secured files protected by a personal password. In the latter files, participants will be identified only by a 7 digit Mayo Clinic registration number rather than by name to ensure confidentiality. *Since genetic information collected is not yet pertinent to clinical practice, the information will not be included in the medical record, but will be maintained in a coded fashion and is accessible only to IRB-approved study personnel.* Many of the principles described by Beskow et al. (84) with respect to the exploratory analysis of gene-disease associations apply, so the consent process for genetic analysis will not require exhaustive genetic counseling.

Plans for ensuring necessary interventions to protect participants are included in the data safety monitoring plan (see under 5 below)

Given the prior track record with use of all of the intubations, measurement devices, secure protection of information on genetic information, there is a very high likelihood that the studies will be conducted safely and the participants will be protected from harm.

3. Potential Benefits of the Proposed Research to the Subjects and Others

The PROPOSED RESEARCH has the potential to develop a new approach to the treatment of gastrointestinal diseases, grouped as irritable bowel syndrome with diarrhea, which is recognized as a spectrum of disorders with significant unmet clinical need. The participants have the potential to benefit from

the information regarding their gastrointestinal and colonic transit rate, serum C4, fecal BA excretion and potentially in future studies, the microbial flora.

The benefits to participants may include recommendations on medications to retard colonic transit or to bind BAs in the colon for those patients with evidence of BA malabsorption. By the time the study is conducted, or possible considerations will be fecal bacteriotherapy, probiotics or non-absorbable antibiotics, such as rifaximin which was recently approved for marketing for IBS-D.

The current state of knowledge about the role of increased mucosal permeability and immune activation is too rudimentary to justify consideration of treatment for those pathophysiological changes. There are, however, medications in development for both potential pathophysiological disorders in association with IBS including the dietary supplement glutamine (85). Therefore, there is potential to propose therapy if such treatments become approved during the "life cycle" of this application.

Given the anticipated mechanistic insights to the participants in this research proposal, and to others with irritable bowel syndrome, and the track record of the investigators in the conduct of such research, the benefits outweigh the risks in the studies proposed**.**

4. Importance of the knowledge to be gained

The PROPOSED RESEARCH has the potential to develop a new approach to the treatment of gastrointestinal diseases, specifically irritable bowel syndrome with diarrhea and increased fecal bile acid excretion, a disorder with unclear relationship to increased mucosal permeability or immune activation or secretory mechanisms. This disorder is associated with significant unmet clinical need. Understanding the potential role of factors leading to motor and sensory dysfunction of the small bowel and colon in patients with IBS will provide mechanistic information that may lead to novel biomarkers that may assist in subtype identification in IBS and therapeutic approaches for IBS.

The risks to the participants are reasonable since many procedures are noninvasive (other than sigmoidoscopy with biopsies), the research team has vast experience in the proposed testing, there is no risk of any significant morbidity or mortality, and precautions are taken to minimize discomfort or adverse effects. Radiation exposure is being kept to the minimum that allows successful completion and data acquisition. There is a high likelihood (e.g., investigator's record and expertise, validated methods, statistical power) of obtaining meaningful, useful information in each specific aim.

5. Data and Safety Monitoring Plan (DSMP)

Name of Principal Investigator (PI) – Michael Camilleri, M.D.

Study Overview

Brief Description of the Purpose of the Study - This *study assesses the effect of bile acid sequestration in patients with IBS-D and bile acid malabsorption in a randomized controlled trial and a study of the patient's DNA* and the transcriptome *and proteome* of the mucosa of the rectosigmoid colon.

This study does not involve administration of any investigational agent and therefore poses minimal risk to participants. Because of this low risk status, the Data Safety Monitoring Plan (DSMP) for this study focuses on close monitoring by the PI in conjunction with an Independent Monitor. Adverse events will be reported to the NIH and to the Mayo Clinic IRB.

Adherence Statement – The Data Safety Monitoring Plan (DSMP) outlined below will adhere to the protocol approved by the Mayo Clinic IRB.

III. Confidentiality

A. Protection of Subject Privacy – During this study, medical history and physical examination will be performed, and questionnaires will be administered. Additional research material will be: review of medical records (of those who authorize review of the records for research purposes), quantitation of gastrointestinal and colonic transit with an external gamma camera, venous blood samples from which DNA will be extracted and stored, venous blood samples for measurement of factors reflecting BA metabolism, urine and stool

collections, colonic mucosal biopsy collections and expression of mRNA *and proteins* in biopsies, and measurements of rectal motility.
Data will be kept in strict confidence. No information will be given to anyone without permission from the subject. This statement guarantees confidentiality. Confidentiality is assured by use of identification codes. All data, whether generated in the laboratory or at the bedside, will be identified with a randomly generated identification code unique to the subject.

B. Database Protection – The database is secured with password protection. The technologist conducting DNA analyses provides the statistician with only coded information, which is entered into the research database using the generated identification code unique to each participant, These precautions permit the objectives of the research to be conducted, specifically to determine the influence of susceptibility genes on the IBS and physiological/quantitative endpoints, and to assess the association between the DNA and the quantitative traits and the expression of relevant mRNA in the sigmoid mucosa. Specifically, it is to be noted that *the DNA and RNA-seq data are not entered into the patients' medical record*, thus protecting the patients participating in the study from any impact of research (clinically unvalidated) information on the clinical management or insurability of the participant. Electronic communication with outside collaborators involves only unidentifiable information.

Sharing of DNA samples will be performed in accordance with NIH guidelines.

Confidentiality during AE Reporting – Adverse Event reports and annual summaries will not include subject-identifiable material. Each will include the identification code only.

IV. Adverse Event Information

Definition - An adverse event (AE) is any untoward medical occurrence in a subject temporally associated with participation in the clinical study. An adverse finding can include a sign, symptom, abnormal assessment (laboratory test value, vital signs, electrocardiogram finding, etc.) or any combination of these.
A Serious Adverse Event (SAE) is any adverse event that results in one or more of the following outcomes:

- Death
- A life-threatening event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly or birth defect
- Important medical event based upon appropriate medical judgment

Classification of AE Severity – AEs will be labeled according to severity which is based on their impact on the patient. An AE will be termed

- 'mild' if it does not have a major impact on the patient,
- 'moderate' if it causes the patient some minor inconvenience and
- 'severe' if it causes a substantial disruption to the patient's well-being.

AE Attribution Scale – AEs will be categorized according to the likelihood that they are related to the study intervention. Specifically, they will be labeled either definitely, probably, possibly or unrelated to the study intervention.

D. Expected Risks –

Risks and/or Anticipated Adverse Events	Assessment measures	Individual doing assessment	Assessment intervals or frequency	Interventions to decrease or respond to risks
Bruising at venipuncture site	Subject symptoms/ vital signs	Study coordinator / RN	Pt instructed to call study coordinator	To be expected rarely; participant information; Decrease with pressure and ice applied by trained personnel
Endoscopy: Sigmoidoscopy	Subject observation	Study coordinator/ Endoscopy technologist,	At time of procedure	Some discomfort expected; instruction to pass gas

		RN and Physician		
Sigmoid colon biopsy	Bleeding at biopsy site	Endoscopy RN and Physician		Exclusion of patients with hemorrhagic disorder *Physician assessment of medical history and current medications/supplements*

E. SAE Reporting

SAEs that are unanticipated, serious, and/or possibly related to the study intervention will be reported to the Independent Monitor, IRB, and Clinical Research Trials Unit in accordance with requirements. Anticipated SAEs or those unrelated to the study intervention will be reported to the same individuals/entities in accordance with requirements."

If abnormal lab values are discovered during the study, the study group shall address this issue and attempt to correct it as early as possible.

V. Data Quality and Safety Review Plan and Monitoring

A. Data Quality and Management

Description of Plan for Data Quality and Management–The PI will review all data collection forms on an ongoing basis for data completeness and accuracy as well as protocol compliance. A statement reflecting the results of the review will be sent to the IRB in the annual report.

Frequency of Review

Data type	Frequency of review	Reviewer
Subject accrual (adherence to protocol regarding demographics, inclusion/exclusion)	Yearly	Principal Investigator, Independent Monitor
Adverse event rates (injuries)	Yearly	Principal Investigator, Independent Monitor
Stopping rules report regarding statistical power implications of drop outs and missing data	Yearly	Principal Investigator, Independent Monitor

Subject Accrual and Compliance

1) Measurement and reporting of subject accrual, adherence to inclusion/exclusion criteria – Review of the rate of subject accrual, adherence to inclusion/exclusion criteria will occur yearly during the 4.5 year recruitment phase. Review will occur at the end of each recruitment wave to assure that participants meet eligibility criteria and ethnic diversity goals outlined in the grant proposal.

2) Measurement and reporting of participant compliance to treatment protocol – compliance will be measured by number of returned tablets at end of treatment phase.

Justification of Sample Size – The application documents the justification of sample size for each specific aim in the proposal.

Stopping Rules – This study will be stopped prior to its completion if adverse effects to the procedures that significantly impact the risk-benefit ratio have been observed

E. **Designation of an Independent Monitor** – Dr. David A. Katzka, M.D. (Mayo Clinic) will serve as independent monitor to perform an independent review of ongoing safety. *In accordance with IRB review, we shall ask Dr. Katzka to constitute an independent DSMB if required. Colesevelam is an approved drug. Since we shall not be applying to FDA to market the drug for a new indication, an IND will not be required for the study.*

Safety Review Plan – Study progress and safety will be reviewed monthly (and more frequently, if needed). Progress reports, including patient recruitment, retention/attrition, and AEs will be provided to the Independent Monitor every year. An annual report will be compiled and will include a list and summarization of adverse events. In addition, the annual report will address:
(1) whether adverse event rates are consistent with pre-study assumptions;
(2) reason for dropouts from the study;
(3) whether all participants met entry criteria;
(4) whether continuation of the study is justified on the basis that additional data are needed to accomplish the stated aims of the study; and
(5) conditions whereby the study might be terminated prematurely.

The annual report will be signed by the Independent Monitor and will be forwarded to the IRB and the CRTU on an annual basis.

VI. Informed Consent

Written informed consent will be obtained from each subject at entry into the study. Informed consent is obtained by the following process:

- The subject will be asked to review the study consent form;
- The PI or Co-Investigator (Co-I) approved as such by the IRB will meet with the subject to review the form, to confirm the subject's understanding of the study, and to answer any questions that the subject might have; and
- Once the subject demonstrates understanding of the study and agrees to participate in the study, the consent will be signed

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