# A Placebo Controlled Study of Colesevelam in Fecal Incontinence

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| **Title of the study:** | A Placebo Controlled Study of Colesevelam in Fecal Incontinence |
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Abstract

Background: Available therapeutic options for idiopathic fecal incontinence (FI) are limited and unsatisfactory. There are no controlled studies documenting the efficacy of medications used to treat FI. We observed that oral clonidine significantly reduced diarrhea in patients with urge FI; while clonidine reduced the frequency of FI, effects were not statistically significant. Small uncontrolled studies suggest that the bile acid binding resin colesevelam also improves functional diarrhea. However, there are no controlled studies that have investigated the effect of colesevelam in FI. In this placebo controlled study, we plan to investigate the effects of combining colesevelam with clonidine on symptoms in women with FI. The primary aim of our study is to investigate the hypotheses that compared to placebo, colesevelam and clonidine will reduce the frequency of FI and rectal urgency. Additionally, we will also investigate the stool and mucosa associated microbiota in these patients at baseline and after treatment. The role of microbiota in pathogenesis of FI is unknown and we hope this study will offer us valuable insight into this question. Sixty women (18-80 y) with symptoms of urge or combined (i.e. urge and passive) FI will be recruited to this study and treated with a combination of colesevelam and clonidine or placebo for 4 weeks. The primary outcome will be the proportion of patients who achieve at least 50% reduction in the frequency of FI (number of FI episodes per Diem) at the end of 4 weeks of treatment.

## A. Specific Aims

Fecal incontinence (FI) is a common symptom that can significantly impair quality of life. [1] The current management of FI, particularly in patients with moderate to severe symptoms, is unsatisfactory. Our previous study showed that clonidine, a α2- agonist significantly decreased the proportion of loose stools in patients with urge or combined FI who also had diarrhea. While clonidine reduced the frequency of FI, effects were not statistically significant. A retrospective analysis of patients with bile acid malabsorption (BAM) observed that colesevelam, a bile acid binding resin improved subjective symptoms of FI in more than 70% patients.[2] A prospective placebo controlled study on the impact of colesevelam in patients with FI is however lacking.

While BAM and alterations in fecal and colonic microbiota have been implicated to contribute to functional diarrhea and IBS respectively[3, 4], the contribution of these disturbances to the pathogenesis or management of FI has not been evaluated. A better understanding of the same is necessary to guide the management of FI. Likewise, while IBS has been associated with gene polymorphisms[5-7], no studies have evaluated the association between gene polymorphisms and FI. Finally, the colonic mucosal microbiota are currently assessed by obtaining mucosal biopsies during a flexible sigmoidoscopy. It is conceivable that the mucosal microbiota can also be sampled with an rectal catheter. However, the characteristics of the colonic mucosal microbiota profile obtained with sigmoidoscopy and a catheter have not been compared.

The **central hypothesis** of this study is that a combination treatment with clonidine and colesevelam is better than placebo in reducing stool frequency and rectal urgency in FI. Additional hypotheses are that FI is associated with (i) BAM, (ii) alterations in the fecal and colonic mucosal microbiota, which are modified by treatment with colesevelam and clonidine and (iii) single nucleotide polymorphisms (SNPs) of selected genes.

In order to investigate these hypotheses we propose five aims as follows:

Aim 1: Compare the effects of combination treatment with colesevelam and clonidine vs. placebo on bowel symptoms in FI

Aim 2: Investigate the prevalence of BAM in patients with FI

Aim 3: Evaluate the fecal and colonic mucosal microbiota in patients with FI and the effects of treatment with colesevelam and clonidine

Aim 4: Compare the colonic mucosal microbiota obtained with mucosal biopsies during a flexible sigmoidoscopy and an rectal catheter in FI

Aim 5: Investigate SNPs associated with FI through microarray analysis of genomic DNA obtained at baseline.

## B. Background and Significance

Fecal incontinence, the involuntary leakage of stool from the anus, is a common symptom not only in nursing home residents, but also in the community. [8] Our observations (2800 women) from Olmsted County demonstrate that one of 10 adult women and one of five women aged $\geq$ 50 years have had one or more episodes of FI, unrelated to an episode of acute diarrhea. [1] Almost one of 15 adult women have moderate to severe FI.

There is very limited, mostly uncontrolled, evidence to support the approaches currently used to manage FI. [9-11] Based on observations of responsiveness to placebo in controlled clinical trials, conservative measures (e.g., dietary modification, behavioral measures to suppress rectal sensation and not including biofeedback therapy) improved symptoms in approximately 25% of patients. [12] Amitriptyline and loperamide improved diarrhea and rectal urgency in uncontrolled studies. [13-15] However, the use of these agents

is often limited by constipation. Biofeedback therapy is better than pelvic floor exercises alone in patients who do not respond to conservative measures.[16] In the only controlled clinical trial of medical therapy for FI, the effects of oral clonidine (0.1 mg twice daily) for 4 weeks on symptoms were not significantly different versus placebo in all comers with FI. However, clonidine significantly decreased the proportion of loose stools in FI patients with diarrhea. Among patients with diarrhea, clonidine decreased the proportion of days with FI; however results were not statistically significant.[17] Uncontrolled studies suggest that the bile acid binding resin colesevelam also increased stool consistency in patients with functional diarrhea.[18] [19] A retrospective chart review and questionnaire suggested that colesevelam improved diarrhea and fecal incontinence in patients with cancer and diarrhea due to suspected bile acid malabsorption.[2] However, among patients with functional diarrhea, the effects of bile acid binding agents on stool consistency were relatively modest. For example, in a study from Mayo Clinic, colesevelam reduced the Bristol score from an average of 4.8 before to 4.4 after therapy. While colesevelam increases stool consistency by binding bile acids, clonidine does so by improving net absorption of fluids and electrolytes.[20] Clonidine can also increase rectal capacity and reduce rectal sensation.[21] Therefore, we propose to compare the effects of a combination of colesevelam and clonidine to placebo on bowel symptoms in patients with urge or combined type of FI.

At present, FI is attributed to bowel disturbances, particularly diarrhea, and anorectal dysfunctions.[17] Among older women with FI, our data strongly suggest that diarrhea and rectal urgency are more important risk factors than obstetric anal sphincter injury.[22, 23] Because many women with FI also have bowel disturbances (i.e., irritable bowel syndrome [IBS]), it is important to understand the contribution of peripheral dysfunctions (e.g., BAM, altered colonic microbiota), which are observed in IBS, to FI. For example, while several small studies observed differences in the microbiota in *feces* between health and irritable bowel syndrome,[24] we recently observed major differences in the *mucosal* microbiota profile between healthy and constipated women.[25] However, no studies have evaluated the fecal and colonic mucosal microbiota in FI. Currently, the mucosal microbiome is evaluated by obtaining mucosal biopsies during flexible sigmoidoscopy. It may be possible to assess the mucosal microbiota with an rectal catheter, which is a noninvasive technique. Hence, we propose to compare the fecal and colonic mucosal microbiota obtained with flexible sigmoidoscopy and an rectal catheter in patients with FI.

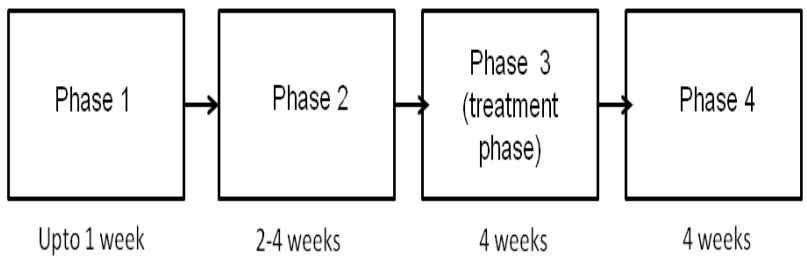
In the community, the prevalence of FI in men is similar to that in women (8.9% and 7.7% respectively).[26] However, in our practice, a majority of patients with FI are women, who have had several vaginal deliveries. By contrast, the 3 main risk factors for FI in men are: proctitis secondary to radiotherapy for prostate cancer, a rectal evacuation disorder, and iatrogenic anal sphincter injury (e.g., due to surgery). Hence, the pathophysiology of FI is differs between men and women. Therefore, and to ensure a more homogenous composition of the patient group, this study is limited to women.

## C. Research Design and Methods

## 1. Overall experimental design.

This study will consist of four phases (Figure 1)

Figure 1. Study design




**1. Phase 1 (Initial screening)**

Patients who consent to participate in the study will visit with the investigator during which a physical examination may be performed. During this visit they will be asked to complete questionnaires designed to determine whether or not they have FI, the type of FI, screen for anxiety or depression and assess quality of life at baseline. **Those who have diarrhea but not FI as assessed by the questionnaires (i.e. Bristol stool type 6 or 7, or >3 bowel movements/day on average) will be excluded from the study.** Eligible patients who give consent will undergo a urine pregnancy test, a 12 lead EKG, anorectal manometry (if not done at Mayo Clinic in the last 6 months), spot stool sample for microbiota analysis, blood draw for genotyping, assessment of the a rectal mucosal microbiota with a rectal catheter, and an optional flexible sigmoidoscopy. Patients will be asked to record their dietary intake for 72 hours prior to collecting the stool sample.

Of these tests in phase 1, only the pregnancy test and EKG *will* determine whether patients proceed to phase 2. The remaining tests will be used to assess specific aims 2-5. Anorectal manometry will help clarify whether these patients have FI because of weakness of the anal sphincter or rectal hypersensitivity. The spot stool sample and rectal mucosal catheter will be used to assess the microbial diversity in FI. Among patients who have a flexible sigmoidoscopy, the mucosal microbiota will be compared on mucosal samples and a rectal catheter. In an attempt at (i) avoiding situations where we recognize subjects are not eligible to participate when they present at the screening visit and (ii) reduce the time taken for screening visits, we propose, when feasible, to ask participants to complete the questionnaires and a 2 week bowel diary before they come for their screening visit. Subjects will return these questionnaires to us by regular or electronic mail. If subjects are not subsequently consented/enrolled into the study, these items will be destroyed. [This bowel diary is identical to that approved for use later in the study.]

**2. Phase 2 (Completion of bowel diaries)**

Patients, who have FI, as documented by the questionnaire during phase 1, are not pregnant, and do not have EKG exclusion criteria documented below will enter phase 2. During this phase, patients will fill out daily bowel diaries to ascertain if they have FI symptoms of sufficient severity. The duration of this phase is variable and ranges from 2-4 weeks as clarified below. Only those patients who meet *all* the following criteria at the earliest weekly review of the bowel diaries starting at week 2 i.e., (i) completion of at least 5 out of 7 days of the diary in the preceding week and 10 out of 14 in the preceding 2 weeks, (ii) experience at least 1 episode of FI per week averaged over 2 weeks, (iii) have a Bristol stool form score ≥ 3, and (iii) an average stool frequency of ≥1/day will enter the treatment phase. Patients who have not filled out the minimum number of days of bowel symptoms will be asked to fill out bowel diaries for an additional 2 weeks. Those who satisfy criteria at the end

of the two additional weeks will proceed to Phase 3. Otherwise their participation in the trial will be terminated and they will exit the study.

**3. Phase 3 (Treatment Phase)**

Patients who satisfy symptom criteria in Phase 2 will be randomized in a 1:1 ratio to receive either a combination of colesevelam (1.875 gm twice daily) and clonidine (0.1 mg oral twice daily) or an identical placebo for 4 weeks. The randomization, to be prepared by Dr. Zinsmeister, will be balanced on age (<50 vs. ≥ 50 y) and BMI (<30 vs. ≥ 30 kg/m2). Patients will fill *daily* bowel diaries for the entire duration of the treatment phase and mail them in every week. Patients will be contacted periodically (by phone or email) to assess compliance with medications, with filling bowel diaries and for any adverse events. At the end of the treatment phase, patients will be asked to fill questionnaires pertaining to their quality of life and satisfaction with treatment. During the treatment phase, patients will be allowed to use rescue medication in the form of loperamide to circumvent the potential for withdrawal from the study due to overwhelming symptoms. Patients are allowed to take loperamide (2mg/dose) up to 3 unit doses in a 24 hour period, 5 unit doses in a 48 hour period or 10 unit doses in any 7-day period. Patients will need to record their loperamide usage in the bowel diary.

**4. Phase 4 (Post treatment phase)**

This phase, which lasts 4 weeks, will begin after patients take their last dose of medication. All patients who complete at least 1 week of treatment will enter this phase. During this phase, study staff will contact patients periodically by phone or email to enquire how they are doing. Patients will fill out daily bowel diaries for 4 weeks and return them weekly for review. At the end of 4 weeks, they will be asked to complete post-treatment questionnaires to assess quality of life, anxiety and depression. They will also be asked to collect a single spot stool sample and mail them in. The questionnaires will be used to investigate if patients had worsening of symptoms after stopping therapy. The spot stool sample will be used to analyze the effect of treatment on the fecal microbiota. Participants will be asked record their dietary intake for 72 hours prior to collecting the stool sample and mail in the food record.

2. Human subjects.

Eligibility criteria for each phase of the study are tabulated below.

A. **Phase 1**

Inclusion Criteria:

i) Females aged 18-80 years with urge predominant or combined (i.e. urge plus passive) FI, as defined by a validated questionnaire, for ≥ 1 year duration will be eligible to participate.[27]

Exclusion Criteria:

(i) History of clinically serious cardiovascular or pulmonary disease or EKG showing 2nd degree atrioventricular block or higher.
(ii) Current or past history of colon or rectal cancer, scleroderma, inflammatory bowel disease, ischemic colitis, small bowel obstruction, congenital anorectal abnormalities, ≥ Grade 2 rectal prolapse, history of rectal resection or pelvic irradiation

(iii) Neurological disorders – Spinal cord injuries, dementia (Mini-Mental status score <21), multiple sclerosis, Parkinson's disease, peripheral neuropathy
(iv) Conditions precluding safe use of clonidine, i.e., symptomatic hypotension, or systolic blood pressure of <100 mm Hg on initial visit in Phase 1 of study
(v) Colon resection greater than limited resection (e.g. sigmoid colectomy for diverticulitis is eligible but right hemicolectomy is not)
(vi) Patients who are currently on or anytime in the past have been on colesevelam for FI
(vii) Malabsorptive bariatric procedures (i.e., Biliopancreatic diversion with or without duodenal switch and Jejunoileal bypass), gastric bypass surgery (i.e., Roux en Y gastric bypass) will be excluded. Those patients who have undergone restrictive procedures (e.g. vertical banded gastroplasty, laparoscopic adjustable gastric banding) however will be eligible to participate
(viii) Currently pregnant or nursing women Prior history of intolerance to clonidine or colesevelam
(ix) History of sphincteroplasty, sacral nerve stimulator, previous injection of Solesta for treatment of FI
(x) Medications

- .
- Relative – opioid analgesics , other antihypertensive agents (i.e. if there is concern about synergistic effects and hypotension). Patients using drugs with anticholinergic effects at high doses (e.g. nortriptyline greater than 50 mg/day or amitriptyline greater than 25 mg/day) will be excluded at the discretion of the physician. Patients who use lower doses will be eligible to participate provided the dose will be stable during the study

**B. Phase 2**

Inclusion Criteria:

i) Females aged 18-80 years with urge predominant or combined (i.e. urge plus passive) FI for $\geq$ 1 year, as defined by questionnaire

Exclusion criteria:

(i) Positive urine pregnancy screen

**C. Phase 3**

Inclusion criteria:

(i) Completion of at least 5 out of 7 days of the diary in the preceding week and 10 out of 14 in the preceding 2 weeks
(ii) At least 1 episode of FI per week averaged over 2 weeks
(iii) Average Bristol stool score of 3 or higher
(iv) Average stool frequency of ≥1/day

Exclusion criteria (if at least one is satisfied):

(i) Missing data in bowel diaries, i.e. if patient did not record bowel symptoms data for more than 2 days in 1 week or 4 days over 2 weeks
(ii) Greater than 6 liquid [Bristol 6 or 7]) stools daily
(iii) Average of less than 1 bowel movement daily
(iv) Average Bristol stool score <3 as assessed from analysis of bowel diaries

**D. Phase 4**

Inclusion criteria:

(i) All patients who complete at least 1 week of treatment with study drugs or placebo

Exclusion criteria:

(i) Patients who completed less than 1 week of treatment with study drugs or placebo

**3.** Study procedures

**a)** History and physical examination a physical examination will not be performed, at the discretion of the investigator, when this has been performed in the past 3 months at Mayo Clinic. The MMSE test will be administered as described previously. [28] The maximum score is 30 and a score of less than 21 is suggestive of dementia.

b) Anorectal manometry will only be performed when this has not been performed in the past 6 months at Mayo Clinic. After rectal cleansing with 1-2 magnesium citrate (Fleets®,C.B. Fleet, Lynchburg, VA) enemas, anorectal functions will be assessed using standard and established techniques in our laboratory [29]. Anal pressures will be measured at rest, during squeeze, simulated evacuation, and a Valsalva maneuver by high-resolution manometry. Since clinical observations suggest that FI is also associated with a rectal evacuation disorder, rectal balloon expulsion will also be assessed.

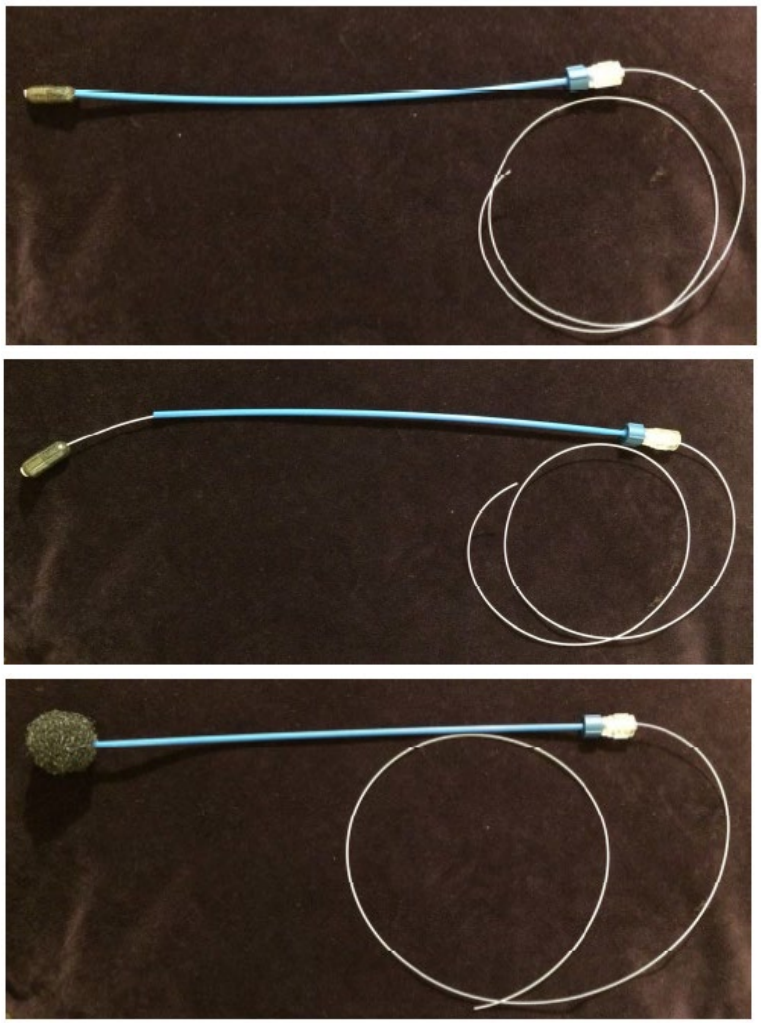


Figure 2. Mega-Brush. A. Undeployed brush with blue sheath and the foam compressed inside a gelatin sheath. B. The foam is pushed out but not yet deployed C. Foam deployed. The foam shown in the figure has 20 pores per inch. We will be using one which is softer with 80 pores per inch.

c) Flexible sigmoidoscopy will be performed in the Clinical Research Unit after 1-2 Fleet's enemas. This is an optional procedure and will be performed in Phase 1 of the study after the rectal catheter and if an anorectal manometry is performed, after that procedure also. Using standard biopsy forceps, 8 mucosal biopsies will be obtained each from the sigmoid colon and the rectum. Eight biopsies will provide adequate tissue for microbial DNA extraction. If any abnormality is seen on sigmoidoscopy, additional biopsies will be obtained for histology. The colonic mucosal

microbiota will be compared to that evaluated with a rectal catheter.

d) Rectal catheter. This test will be done in all patients either just prior to anorectal manometry or during the initial visit in patients who do not have a manometry. The Mega Brush ® (provided by Capnostics LLC., Figure 2) is a mass cytology cell retriever system comprised of a foam sphere made of biocompatible material that is used to obtain specimens from the anorectum. It is FDA cleared (FDA K944614/A) for collection of mucosal specimen with an endoscope. . The foam is enclosed in a flexible outer tube and the tip covered by a gelatin cap. The diameter of the foam is 30 mm when fully deployed and the foam has 80 pores per sq. inch. We propose to introduce the catheter under direct vision during the rectal examination. Once the catheter is in the rectum, the capsule will be pushed out into the rectum and the cap allowed to dissolve over about 5 minutes. This will deploy the foam sphere. The foam sphere will be rotated 1-2 times in the rectum very gently with the help of the sheath. The catheter will then be slowly withdrawn. .

e) Calculating relative abundance of bacterial 16s rRNA to human DNA: Quantitative PCR will be used to assay the quantity of bacterial 16s rRNA in the stool sample. This will be normalized to a single copy human gene (e.g. TNF-alpha) as described in literature [30]. The relative proportion of bacterial to human DNA will be calculated by first generating a standard curve by dilution series using purified plasmids containing a representative target (e.g. *E.coli* 16s rRNA or human TNF-alpha). Purified plasmids will be amplified in parallel with patient samples and a standard curve generated to assess number of copies of the target in each sample.

f) Fecal and colonic mucosal microbiome analysis: Microbial DNA will be extracted from stool and tissue samples using standardized methods [18]. Phylotype profiles of the microbiome from colesevelam and placebo treated FI populations will be generated using deep rDNA hypervariable tag sequencing of the hypervariable V3-V5 region of the SSU rRNA gene, which has been validated for use with human microbiomes and is one of the methods of choice for the HMP. With the longer reads from the MiSeq (250x250 paired end reads), our sequencing will include both the V3-V5 RGTs making for a more optimal phylogenetic analysis [19]. Phylotype profiles of the mucosa-associated microbiome will be generated using rDNA sequencing of the hypervariable V3-V5 region, which has been validated for standard use by the human microbiome project. The 500 base pair reads will ensure a better optimal phylogenetic identification. Barcoding samples prior to sequencing will yield approximately 20,000 reads/sample, ensuring detection of both dominant (core microbiome) and poorly represented taxa (variable microbiome). Identifying the existent taxa, diversity, and ecological relationships within each sample requires that we process large volumes of 16S DNA sequence data. In order to accomplish this task we will follow protocols that we have quantitatively shown to be optimal. Reads will be aligned using our own custom multiple alignment tool (TORNADO v2.0) that has been optimized the production of taxonomic calls and alignments for downstream analysis such as OTU clustering and phylogeny from paired end reads [20]. Sequences will be culled according to initial quality files using QIIME, pre clustered in order to reduce the number of unique reads due to sequencing errors using mother, and aligned using the new version of the TORNADO pipeline for paired end reads. TORNADO v2.0 will then be used to obtain the taxa calls that will be used in later correlation analysis. Second, quantitative PCR assays targeting functional genes mcrA and mrtA (methanogens) dsrA (sulfate-reducing bacteria) and hydA (hydrogen-producing bacteria) will be performed on both stool and mucosal samples following published protocols [21]. Similarly, different sulfate-reducing genera will be quantified with validated 16S primers. The abundance

of functional genes will be correlated to physiological parameters. Subsequent QPCR assays on microbial groups that are significantly distinct in sequencing analyses may also be performed.

(i) Nutritional Assessment: Habitual dietary intake will be assessed at baseline using an electronic graphical food frequency questionnaire (FFQ) (Viocare Technologies, Inc. 145 Witherspoon Street, Princeton, NJ 08542). This questionnaire is based on the Women's Health Initiative FFQ and has been adapted by VioCare in an electronic computer-administered format to evaluate dietary intake over a three month period. It employs the Minnesota NDS nutritional analysis database for nutrient analyses. The FFQ has been validated and compares well with nutrient intake validated against food records and four 24-hr dietary recalls (29, 30). (ii) Participants will keep food records of their actual dietary intake for 72 hours prior to collecting the stool specimens for microbiome analysis. The food records will be analyzed for energy, protein, fat, carbohydrate and fiber content by the CRTU nutrition staff using the ESHA Food Processor software (Version 10.15) developed by ESHA Research, Salem, Oregon 97309-1028.

g) Drug therapy Patients will be treated with colesevelam (1.875 gm) and clonidine (0.1 mg) twice daily or matching placebo for 4 weeks. Patients will need to discontinue any antidiarrheal medications from the start of Phase 1 through end of Phase 3. Loperamide tablets (2 mg) will be provided for rescue when patients experience intolerable diarrhea. Patients can take up to 3 unit doses in a 24 hour period, 5 unit doses in a 48 hour period or 10 unit doses in any 7-day period. They will be required to record their loperamide usage during the treatment phase.

h) Genome wide DNA analysis. We are currently exploring for SNPs associated with FI by genotyping 175 women with FI and 175 age- and gender-matched controls from Olmsted County (IRB 22-01), using a chip with > 500,000 SNPs spaced on average 1 every 5 kb in the genome. Towards our long-term objective of uncovering associations between SNPs and FI, we propose to extract DNA from 20 ml of blood, to be drawn from study participants. The tubes will be spun down in the Clinical Research and Trial Unit immediately and the plasma stored at minus 80 degrees celsius. The buffy coat will be stored at minus 80 degrees Celsius until DNA is extracted. DNA extraction will be conducted at the BAP Lab. Genome-wide analysis will be conducted using Illumina 610 QUAD microarray or comparable approaches. All patients who qualify for the study based on physician assessment in Phase 1 will undergo genotyping. Genotype-phenotype correlations will be examined using these patients and other patients in ongoing studies.

i) Analysis of Differential Gene Expression by RNA Seq analysis. In order to investigate if there is any relationship between the global gene expression in a patient and their response to treatment for FI, we will perform RNA Seq analysis with help of the Gene Expression Core Facility at Mayo Clinic Rochester. We will collect 7.5 ml of blood in PAX gene tubes at the time of the initial visit. RNA will be extracted from whole blood immediately and stored at minus 80 degrees celsius. Further processing of RNA will be done by the Gene Expression core facility at Mayo Clinic, Rochester.

**Summary of Blood Collections.** The total blood collected for this study is 27.5 ml.

| Item | Test ID | Lab | Tube (amount required) |
|---|---|---|---|
| DNA for SNPs | 31669 | CRU Lab | Use buffy coat from EDTA tubes (one tube of 20 ml) |

| | | | |
|---|---|---|---|
| RNA extraction and processing | 31669 | BAP Lab | PAX gene tubes (7.5 ml) |

j) Symptom assessments.

The following instruments will be used to evaluate symptoms, co-morbid conditions, and impact on QOL.

(i) Fecal Incontinence Severity Index (FISI) – which is a validated 4-item scale used to assess the frequency of 3 different types of FI. [31]
(ii) Fecal Incontinence Quality of Life (FI-QOL) – is a 30-item validated questionnaire that assesses the effects of FI on QOL. [32]
(iii) Hospital Anxiety and Depression (HAD) Scale [33]
(iv) Health-status questionnaire (Radosevich DM, Wetzler H, Wilson SM. 1994. Health Status Questionnaire (HSQ) 2.0: Scoring Comparisons and Reference Data. Health Outcomes Institute, Bloomington, MN.) is a self-report inventory designed to measure a patient's overall health and functioning.
(v) Fecal incontinence and Constipation Assessment (FICA) Scale – This validated instrument, developed in our program, has 4-items (frequency, type, amount of leakage, and presence of urgency) used to rate the severity of FI. [34] It is the only instrument that incorporates the amount of leakage, which is an important yardstick of the severity of FI.
(vi) Satisfaction with treatment: In order to determine if patients were satisfied with the treatment received, we will use a previously validated visual analog score**.**

FICA score will be calculated from the bowel questionnaire. FISI, HAD, HSQ and Satisfaction with treatment score will be calculated from the General Questionnaire that will be administered pre and post-treatment (**Table 1**).

**Table 1. Symptom assessment tools and their administration during the study**

| | **Phase 1** | **Phase 2** | **Phase 3** | **Phase 4** |
|---|---|---|---|---|
| Duration | Up to 1 week | 2-4 weeks | 4 weeks | 4 weeks |
| Bowel questionnaire | X | | X | X |
| Quality of life questionnaire | X | | X | X |
| Hospital anxiety and depression questionnaire | X | | | X |
| Daily bowel diaries | | X | X | X |
| Satisfaction with treatment | | | X | |

4. Data and statistical analysis

i. Power Statement:

Based on the observed difference in the frequency of FI (i.e., mean number of FI episodes per subject per diem) between treatment and placebo groups in the clonidine study, we estimate that approximately 33 subjects in each treatment group (i.e. colesevelam and

clonidine vs placebo) will provide 80% power, using a two sided alpha level of 0.05, to detect at least a 50% difference in the frequency of FI between treatment groups.[17]

ii. Data Analysis Plan:

a) Statistical analysis

An Excel (Microsoft, Redmond, WA) spreadsheet of treatment assignments (balanced on age and BMI) will be generated (by Dr. Zinsmeister) and sent to the research pharmacy. Study personnel will be blinded until the study is completed.

Bowel diaries will be analyzed as follows: Stool frequency, form, urgency and use of rescue medications will be averaged first for each day and then over 1-2 weeks (two weeks in Phase 1 and one week in Phase 3) in each subject.

We will compare response to treatment between colesevelam/clonidine and placebo between for the treatment period using the values during Phase 1 as covariates and separately, post-treatment. The analyses will use an α value of 0.05 for each comparison. Treatment groups will compared using analysis of covariance (ANCOVA) with the corresponding baseline (Phase 1) value as the covariate. Data will be analyzed per intent-to treat analysis using all subjects randomized. Missing values will be imputed as described previously and an adjustment in the ANCOVA error degrees of freedom will be made. Separate ANCOVA models will be used to evaluate whether treatment effects were influenced by the presence or absence of diarrhea at baseline. All tests will be two tailed.

Endpoints

The primary and secondary endpoints have been well described in our study of clonidine in FI and will be employed in this study as well[34]

a) Primary endpoint:
Previously, we have shown that in patients whom the frequency of FI (measured as mean number of episodes of FI per person per Diem) decreased by at least 50%, the corresponding FICA severity score exceeded the minimum clinically-important difference (i.e., the smallest change detected by an instrument that is considered clinically significant) in at least 75% of the cases. This means that the majority of people who report at least 50% improvement in FI frequency have a clinically significant improvement. Hence in this study, we will use a 50% reduction in the frequency of FI episodes as our primary end point.

Secondary endpoints:

The following secondary end points will be compared between placebo and colesevelam groups

a) Change in the number of days with FI
b) Volume of FI- this will be graded as small (staining only), moderate (requiring change of underwear) or large (requiring change of all clothes). We will calculate the proportion of FI episodes of each type and compare the proportion before and during treatment.
c) Composition of leaked stools (measured as percentage of all incontinent bowel movements that were type 5-7 on the Bristol stool scale)- average Bristol stool form will be calculated from bowel diaries and will be compared before and during treatment.
d) Number of episodes of passive and urge incontinence per week
e) Rectal urgency (proportion of total and incontinent bowel movements preceded by urgency)
f) Proportion of complete bowel movements

g) Time for which a bowel movement can be deferred after occurrence of urgency
h) Change in Bristol stool form
i) Change in proportion of FI episodes that were diarrhea (Bristol stool score 5-7)
j) Patient reported severity of bowel symptoms as assessed by VAS
k) Severity of FI (FISI score)
l) Impact of FI on quality of life (FI-QOL score)
m) Impact on symptoms of anxiety and depression (HAD score)
n) Satisfaction with treatment measured by the visual analog scale
o) Proportion of days where patient used loperamide

For the above end points, we will obtain information either from weekly bowel diaries or questionnaires administered during each of the phases of this study as outlined in Section 2.

Using standardized approaches established in our laboratory, mucosal microbiota profiles will be compared between rectal catheters and colonic mucosal biopsies obtained at flexible sigmoidoscopy.

iii. Subject Safety. Only subjects who are eligible based on pre-defined criteria, an interview and a physical examination, and screening laboratory tests will be enrolled. The consent form details issues related to privacy, withdrawal from studies, and use of blood for genetic analyses among other items. The eligibility criteria are structured to exclude patients who have a greater risk of side effects with clonidine. Interventions (clonidine, colesevelam and anorectal tests) pose a low risk to participants. In our previous study, clonidine was well tolerated by women with FI; dry mouth was the only side effect that occurred more frequently with clonidine than placebo. [17] There is no known drug interaction between clonidine and colesevelam. Patients who are positive for depression by the HAD score will be offered referral to Psychiatry. If the patient endorses suicidal thoughts then we will first and foremost offer support. If the patient is on Mayo campus and in department, we will then have a co-worker page 911 and ask for Security. The study coordinator will contact the principal investigator immediately to alert them that of the situation. The study staff will stay with the patient until Security arrives at the location. If the patient is not on Mayo campus, then the study staff will keep the patient on the line and have a co-worker page 911. Study procedures are conducted by trained and experienced personnel. Monitoring for side effects will be performed by study personnel. The PI will also review side effects. Each study is reviewed and analyzed; study discussions and communications are generally by e-mail and stored.

iv. Data Integrity. Only subjects who are eligible based on pre-defined criteria, an interview and a physical examination will be enrolled. Transcription of data is accurate and complete. Calculations will be standardized and accurate. The study technician will monitor data after every study. The PI will also review data after every 10 studies.

v. Subject Privacy. Subjects will be consented in a private room. Adequate time for questions will be provided.

vi. Data Confidentiality. All records are maintained in password protected electronic files. Hard copies are filed in private offices.

vii. Product Accountability. Research Pharmacy will obtain and dispense medication. Unused medication will be collected from subjects at the end of the study and destroyed.

viii. Study Documentation. Documentation will be per established guidelines during the study (hard copy and electronic data capture). These files will be sampled annually.

ix. Study Coordination. Quarterly debriefing will be conducted to ensure expectations are clear and if educational needs exist.

## D. Human Subjects

Only subjects who satisfy stringent inclusion and exclusion criteria and provide written informed consent will be included in the protocol. The consent form details issues related to privacy, withdrawal from studies, and use of blood for genetic analyses among other items. Additional details as follows:

1. **Anorectal assessments** are extremely safe; discomfort, if any during rectal distention is mild and tolerable and the risk of rectal perforation in an uninflamed rectum is negligible.
2. **Clonidine** is generally well tolerated at this dose. In a recent study, we observed that the most common side effect with clonidine was dry mouth (about 70% vs. 4.5% in placebo). Other adverse effects included drowsiness (23% vs 14% in placebo), light headedness (27% vs.9% in placebo) and fatigue (36% vs. 23% in placebo) although they did not reach statistical significance. [17]
3. **Colesevelam** is generally well tolerated at this dose. The most commonly reported side effects from Colesevelam are constipation, diarrhea, dyspepsia, hypoglycemia (mild and well tolerated), headache, abdominal cramps, nausea, and flatulence and back pain. Their frequency ranges from 8%-40% in different studies. [35-37]

Patients will be appropriately screened and subjects with an increased risk of complications will be excluded from the study. All side effects will be recorded at the follow-up visit. Since the effects of clonidine and colesevelam in pregnant and nursing women are unknown, women who are pregnant or nursing will be excluded from the study. Women of child-bearing potential will be advised to employ contraceptive measures during the study and a pregnancy test will be checked before beginning clonidine or colesevelam.

Patients who cannot tolerate clonidine 0.1 mg bid will be switched to 0.1 mg once daily (qhs) Clonidine will be discontinued if:-

(i) Subjects have syncope, or lethargy that interferes with safe performance of the volunteer's activities of daily living; or

(ii) if the systolic blood pressure drops to < 90 mmHg for patients with a systolic BP between 90 and 130 mmHg, or if systolic BP falls to < 100 mmHg for subjects with a pre-treatment systolic BP > 130 mmHg; or

(iii) if a subject becomes pregnant during the study.

Patients who cannot tolerate three tablets of 625 mg colesevelam twice daily will have dose reduction by 1 tablet daily until they are able to tolerate the medication. Colesevelam will be stopped if a subject becomes pregnant during the study.

**Data Safety and Monitoring Plan.** The DSMP utilized will adhere to the protocol approved by the Mayo Clinic IRB. We propose the following plan: -

Data quality and management: The principal investigator will review all data collection forms on a three-monthly basis for completeness and accuracy of the data as well as protocol compliance.

Adverse events grading: The common grading scale listed below will be used to grade AEs:

0 No adverse event or within normal limits or not clinical significant
1 Mild AE, did not require treatment
2 Moderate AE, resolved with treatment
3 Severe AE, resulted in inability to carry on normal activities and required professional medical attention

4 Life threatening or disabling AE
5 Fatal AE

Attribution scale: An adverse event includes both, an expected side effect that is of a serious nature, or an unexpected side effect/ event regardless of severity. All events will be graded as to their attribution (unrelated to protocol, or possibly, probably, or definitely related to protocol). Any event that is reported to either the principal investigator or his designated research associates by the subject or medical staff caring for the subject and which meets the criteria will be documented as such.

**Data Monitoring.** The majority of data generated from these protocols will be from analyses performed in our laboratory or the immunochemical core laboratory. Standard quality control procedures are in place for each assay. The frequency of data review for this study differs according to the type of data and can be summarized in the following table:

| **Data type** | **Frequency of review** |
|---|---|
| Subject accrual (adherence to protocol regarding demographics, inclusion/exclusion) | Weekly |
| Adverse event/safety rates (injuries) | Weekly |
| Annual report | Yearly for IRB |

**4. DNA and RNA analyses.** DNA, RNA and plasma samples will be anonymized before being submitted to the genotyping or RNA Seq assays. Specific information is provided in the consent form regarding storage and future use of the biologic sample. These results will not be added to the written record.

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