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Relationships of Microbiome Markers with Extra-intestinal, Psychological Distress and Gastrointestinal Symptoms, and Quality of Life in Women with Irritable Bowel Syndrome

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Abstract

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- Robert J. Shulman, MD. - Co-PI of the study. Co-wrote the grant and contributed to data analytic strategy and interpretation.
- Monica E. Jarrett, PhD. - Co-PI of the study. Directly involved in all aspects of the study from its inception to completion.
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Introduction—Altered microbial diversity has been associated with gastrointestinal (GI) symptoms in persons with irritable bowel syndrome (IBS). Less is known about the relationship of microbiome with extra-intestinal pain and psychological distress symptoms and quality of life (QOL) in persons with IBS. We aimed to evaluate the relationship of fecal microbiota to GI symptoms, stool consistency, psychological distress, extra-intestinal pain, and QOL in participants meeting Rome III criteria for IBS.

Methods—Seventy-six women completed a 28-day diary that included GI, stool consistency, psychological distress, and extra-intestinal pain ratings. Participants completed the IBS-Specific-Quality of Life questionnaire. Stool samples were collected and analyzed by 16S rRNA gene sequencing. Principal component analysis was performed and the first two components (PC1, PC2) were used to test relationships among bacterial families and clinical measures.

Results—Participants were categorized as IBS-constipation (n=22), IBS-diarrhea (n=39), IBS-mixed (n=13), and IBS-unsubtyped (n=2). There was a significant group effect for the *Firmicutes* to *Bacteroidetes* (FB) ratio and PC1. Lower microbial diversity and richness were associated with increased urgency and extra-intestinal pain, worse QOL, and looser stools. Lower extra-intestinal pain was associated with increased *Rikenellaceae*, *Christensenellaceae*, *Dehalobacteriaceae*, *Oscillospiraceae*, *Mogibacteriaceae*, *Ruminococcaceae*, *Sutterellaceae*, *Desulfovibrionaceae*, and *Erysipelotrichaceae* abundances. QOL was positively associated with many of these same bacterial families. Higher FB ratio was positively associated with loose stools. There were no statistically significant relationships between daily psychological distress or abdominal pain and bacterial families.

Conclusion—Stool microbial diversity and composition are linked to daily extra-intestinal symptoms, stool consistency, and QOL in women with IBS.

INTRODUCTION

Approximately 10–20% of adults experience chronic abdominal pain/discomfort symptoms associated with altered bowel habits (constipation and/or diarrhea) compatible with a diagnosis of irritable bowel syndrome (IBS).^{1, 2} The public health impact of IBS in the US is large with direct and indirect costs totaling approximately \$1.35 billion/year.^{3–5} The management of patients with IBS is challenging in that it is a heterogeneous condition with multiple factors (e.g., stress, diet, dysmotility, altered brain-gut axis) likely contributing to its etiology. To date, there are few biomarkers available to help diagnose, identify relevant subgroups and/or provide guidance on the management of IBS symptoms.^{6–8}

Recent studies support the idea that alterations in gut microbiome composition and function (dysbiosis) may play an important role in the pathophysiology and clinical symptoms of IBS.^{9–12} Emerging evidence suggests that IBS symptoms and severity may be linked to microbial signatures. Utilizing retrospective questionnaires regarding IBS symptoms and severity, both Pozuelo et al. (2015) and Tap et al. (2016) identified key microbial taxa associated with IBS characteristics. Pozuelo et al.¹³ found differences in microbial community composition, at various taxonomic levels, between 113 IBS patients and 66 healthy controls. These included lower microbial diversity in patients with IBS as well as higher average counts of phylum *Bacteroidetes* and lower counts of phylum *Firmicutes* in

patients with IBS-D (diarrhea) and IBS-M (mixed) subtypes. Pozuelo et al.¹³ also correlated microbiota composition and subjective IBS symptoms in 44 patients who did not take any medications. Flatulence was positively correlated with family *Lachnospiraceae* from the phylum *Firmicutes*. Abdominal pain was positively correlated with the genera *Bacteroides* (family *Bacteroidaceae*) and genera *Ruminococcus* (family *Lachnospiraceae*) and an unclassified member of the family *Barneisiellaceae*. In contrast, abdominal pain was negatively associated with the genera *Prevotella* (family *Prevotellaceae*) and *Catenibacterium* (family *Erysipelotrichaceae*). Using retrospective questionnaires and a two-week stool diary, Tap et al. found no differences in microbiota richness or composition between healthy controls and IBS participants or among IBS subtypes. However, using a machine-learning approach, Tap et al. identified a microbiota signature for those with severe IBS symptoms (IBS-Symptom Severity Score > 300) that included lower diversity and reduced abundances of genera *Methanobacteriales* (phylum Euryarchaeota) and genera *Prevotella* (family *Prevotellaceae*) and increased abundances of genera *Bacteroides* (family *Bacteroidaceae*).¹⁴

Gut microbiota appear to not only influence immune and inflammatory responses within the GI tract but also systemic inflammatory responses¹⁵ and, in some cases, behavior. A current hypothesis is that dysbiosis activates mucosal innate immune responses which increase epithelial permeability, activate nociceptive sensory pathways and dysregulate the enteric nervous system.^{16–18} Such changes then contribute to visceral hypersensitivity and possibly extra-intestinal or co-morbid symptoms (e.g., fatigue, joint/muscle pain, mood state) that patients with IBS frequently report.¹⁹ Yet the relationship of microbiota to extra-intestinal symptoms of IBS remains unexplored. Studies with rodents show the relationship of the gut microbiota to anxiety-like and depression-like behaviors.^{20–22} When compared to specific pathogen-free mice, germ-free (GF) mice display lower levels of anxiety-like behavior in response to water maze stress testing.²³ In humans, healthy adults show improvement in anxiety after taking probiotics.^{24–29} These pieces of evidence suggest that gut microbiome characteristics may contribute to mood symptoms that are often associated with IBS, such as psychological distress. Assessing memory testing and social behaviors, animal studies provide some support that gut bacteria influence cognition. However, in humans the notion that gut bacteria is linked to quality of life remains unexplored.

Most studies to date relating subjective measures including symptoms and quality of life have relied on retrospective questionnaires, which lack resolution of a daily scale and are often hampered by recall bias³⁰. Prospectively collected daily diaries represent an improvement over this situation.³¹ When used in combination with retrospective measures, the two approaches to symptom capture provide insight regarding the patient's daily experience and overall perception of condition. Understanding the linkages between microbiota and average daily IBS symptoms may offer unique therapeutic strategies. Therefore, we aimed to: 1) test for differences in gut microbiota measures and diversity among IBS subtypes, i.e., IBS-Constipation (IBS-C), IBS-Diarrhea (IBS-D), IBS-Mixed (IBS-M) and IBS-unsubtyped (IBS-U) ; and 2) test the relationships of gut microbiota measures to daily IBS symptoms (e.g., abdominal pain), stool consistency, psychological distress, extra-intestinal pain, and overall quality of life (QOL) measures.

METHODS

Stool samples from a baseline assessment of 76 women enrolled in a behavioral intervention trial were collected and microbiota analyses performed.³² All participants needed to have a diagnosis of IBS given by a health care provider and be between the ages of 18 and 70. The subject's medical history was reviewed by an experienced gastroenterologist. In addition, subjects needed to report current IBS symptoms. Exclusion criteria included had a history of coexisting GI pathology (e.g., IBD, peptic ulcers) or surgery (e.g., open abdominal surgery or midline abdominal incisions), renal or reproductive pathology (e.g., endometriosis). Subjects were excluded if currently taking or had been taking antibiotics in the 2 months prior to study. In addition, we excluded women taking anticholinergics, cholestyramine, narcotics, docusate, enema preparations, iron supplements, or laxatives.³² Although both men and women were recruited into the larger study, microbiome analyses were performed only on the women's samples. Subjects completed a 4-week daily diary recording of GI symptoms, stool consistency, psychological distress, and extra-intestinal pain symptoms. Additional measures included Rome III Questionnaire and IBS Quality of Life (IBS QOL).

Eligibility for the study included the following process. Potential subjects who responded to posted advertisements were screened by telephone. If eligible, questionnaires were mailed to the subject's home and then returned at the first visit. At the visit the subject met with the research nurse, consent was obtained, questionnaires reviewed, and instructions on the use of the daily diary and stool collection given.

Human participants institutional review approval was obtained prior to enrolling participants (February 2008) and renewed thereafter. Written informed consent was obtained from the participants. This study was registered with clinicaltrials.gov through the U.S. National Institutes of Health ([Clinicaltrials.gov](https://clinicaltrials.gov) identifier: NCT00907790).

Microbiota Characterization

Fecal samples were collected at each participant's home and immediately stored at -20°C . Frozen samples were transported to our laboratory and stored thereafter at -80°C . Fecal microbial communities were characterized using 16S rRNA gene sequencing as previously described.³³ Briefly, community DNA was extracted from each sample using the PowerSoil DNA extraction kit (MoBIO, Carlsbad, CA) with modifications to the manufacturer's protocol.³⁴ Community DNA quantity and quality were assessed using fluorometric methods (Qubit, Waltham, MA), and amplicon libraries were prepared using the NEXTflex V4 Amplicon-Seq kit 2.0 (Bioo Scientific, Austin, TX). Sequence libraries were generated using the MiSeq platform (Illumina, San Diego, CA).

Sequence libraries were demultiplexed by barcode, and primer sequence were removed using cutadapt.³⁵ PhiX reads were removed by mapping amplicon reads to the PhiX genome using bowtie2 (version 2.0.6)³⁶ with the fast-local setting and retaining all unmapped read pairs. Reads were then further quality-filtered and clustered into operational taxonomic units (OTUs) using LOTUS (version 1.462).³⁷ The LOTUS platform retained reads with minimum average quality scores >20 and minimum read length of 170 bp. Reads were clustered into OTUs at a 97% similarity threshold using the UPARSE algorithm (version

8.0.1623)³⁸ and potentially chimeric sequences were identified among the OTU representative sequences using the VSEARCH algorithm (version 1.9.7).³⁹ Non-chimeric representative sequences were classified using the following classifier – database pairs: Ribosomal Database Project (RDP) classifier and SILVA (version 123) database, RDP classifier and HitDB database, and UTX classifier and RDP trainset 15 (https://www.drive5.com/utax/data/utax_rdp_16s_trainset15.tar.gz). A consensus taxonomy was derived in which classifications were supported by 2 or more calls at any taxonomic level. In cases of disagreement, the taxonomic call was limited to the finest level of resolution supported by two or more of the classifiers. For example, if a taxonomic call was supported by two or more classifiers at the family level but lacked majority consensus at the genus level, the family-level designation was retained and “unclassified” was used in place of the genus-level designation.

Descriptive Measures

ROME III Questionnaire—The participants completed the ROME III questionnaire for IBS, a diagnostic questionnaire developed by the Rome Foundation to identify patients with IBS. The development and validation of this questionnaire have been previously described.^{2, 40, 41}

Daily Diary Measures

Every day for 4 weeks subjects completed a daily diary which included 26 symptoms rated on a scale of 0 (*not present*), 1 (*mild*), 2 (*moderate*), 3 (*severe*) or 4 (*very severe*).⁴² The score for each individual symptom was summarized as the mean rating over the 28 days. Symptoms analyzed for this report included the GI symptoms of abdominal pain or discomfort, bloating, constipation, diarrhea and urgency. A composite measure of extra-intestinal pain was computed as the average of headache, joint pain, muscle pain, backache, and headache. The psychological distress composite score included anxiety, depression, panic, and stressed.

Participants also rated the consistency of each stool using the following scale of 0 (*no stool*), 1 (*very hard*), 2 (*hard*), 3 (*formed*), 4 (*loose*) or 5 (*watery*) as previously described.⁴³ These daily ratings were summarized as the mean consistency score over 28 days, with higher values indicating looser stools.

Quality of Life for IBS—The IBS-Quality of Life (IBSQOL) questionnaire is a 42-item questionnaire with nine scales: sleep, emotional, mental health beliefs, energy, physical functioning, diet, social role, physical role, and sexual relations.⁴⁴ Example questions are “*How often did your IBS make you feel fed up or frustrated?*” e.g., 1 (*always*), 2 (*often*), 3 (*sometimes*), 4 (*seldom*), to 5 (*never*); and “*My IBS affected my ability to succeed at work/main activity*” e.g., 1 (*strongly agree*) to 5 (*strongly disagree*). The scales were converted to a standard 0 to 100 scale. A total score was computed by averaging all scales. Lower scores indicate worse IBS impact on QOL.

Statistical Analysis

The analysis strategy consisted of two steps. The first step evaluated associations of clinical measures with five microbial summary measures of microbiome composition applying the Benjamini-Hochberg procedure to control for multiple comparisons in these initial analyses. Only those clinical measures that showed significant association with microbiota summary measures in this first step were analyzed in the second step. The purpose of this second step was to understand which families and genera are associated with those clinical measures. No multiple comparisons adjustments were done in the second step, the protection against inflated type 2 error having been accomplished in the first step.

Five microbiome summary measures were computed in the initial analyses: Diversity measures (Shannon and operational taxonomic unit [otu] richness) were computed on the OTU level. FB ratio was computed as the fractional abundance of phylum *Firmicutes* divided by fractional abundance of phylum *Bacteroidetes*. A small constant, 0.02, was added to the denominator so that a fractional abundance of phylum *Bacteroidetes* close to zero would not result in an extremely high ratio.

The remaining two summary measures were defined from principal component analyses (PCA) applied to the logit-transformed family data using only the 35 most abundant bacterial families. The logit transform was applied to the relative abundance of these families: $\log_{10} [(p+.0001) / ((1-p)+.0001)]$, where p is the read count associated with each bacterial family divided by the total read count. The constant 0.0001 was added to accommodate relative abundance being zero or one. This PCA was based on partial correlations controlling for total sequencing read count and subject age. Only the first two PCs were used in subsequent analyses.

Supplemental Figure 1 shows the distributions of these 35 families across subjects. Supplemental Figure 2 shows the correlation of each family to the two PCs, as a description of which families were important components of each of these two PCs.

Analysis of variance (ANOVA) was used to test differences in the five microbiota summary measures by IBS bowel pattern subtypes, controlling for read count and subject age. Partial correlations, controlling for read count and age, were computed between each of the five microbiome summary measures and the clinical measures of diary stool consistency and symptoms, and QOL. The clinical measures which showed significant correlations with one or more of the five summary measures after controlling for multiple comparisons, were included in analyses of individual families and genera. Statistical analyses were performed using with IBM SPSS Statistics for Windows, version 19 (SPSS, Inc., Armonk, NY: IBM Corp, USA).

RESULTS

Demographics

Most of participants were Caucasian (71%) and had a college degree (80%). The mean age was 39.0 years (SD 15.1) (Table 1). Based on Rome III diagnostic criteria, IBS-diarrhea was the predominant bowel subtype (IBS-D, n=39) followed by IBS-constipation (IBS-C, n=22),

IBS-mixed (IBS-M, n=13) and 2 participants who did not meet the criteria for IBS-C, IBS-D, or IBS-M were categorized as IBS-unsubtyped (IBS-U).

Microbiome and IBS Subtypes

Table 2 shows the microbiota summary measures by bowel pattern IBS subgroup, IBS-C, IBS-D, and IBS-M. FB ratio differed significantly by group, being lowest in the IBS-C group and highest in the IBS-D group. PC1 also differed significantly across bowel pattern groups (Table 2).

Correlation of Microbiota Summary Indicators with Gastrointestinal Measures and Quality of Life

Table 3 shows the correlation of the five microbiota summary indicators with stool consistency, GI symptom measures, and IBS-specific QOL. Higher diversity (Shannon and OTU richness) was associated with lower values for stool consistency (i.e., harder stools) and better IBS-specific QOL. The OTU richness, but not Shannon diversity, was associated with less diarrhea and urgency. Increased FB ratio was associated with looser stools, but none of the other individual GI symptom measures. PC1 showed a correlation pattern similar to OTU richness, while PC2 was associated with better QOL. Also, note that none of the microbiome summary indicators were significantly associated with abdominal pain, bloating, or constipation. These correlations are illustrated in Figure 1.

Correlation of Microbiota Summary Indicators with Extra-intestinal Pain and Psychological Distress Measures

The summary measure of extra-intestinal pain includes heartburn, joint pain, muscle pain, backache, and headache. The extra-intestinal pain summary measure was inversely associated with Shannon diversity and number of OTUs (Table 4). PC1 was inversely related to extra-intestinal pain summary measure while no statistical relationships were noted in the individual pain symptoms. Microbiome summary measures were not significantly associated with the psychological distress summary measure (Table 4).

Correlation of Microbiota Families with Daily Clinical Measures and Quality of Life

Supplemental Table 1 shows partial correlations between the logit of relative abundance of phyla and families with mean stool consistency, urgency, psychological distress, extra-intestinal pain, and QOL. As seen in Supplemental Table 1, many correlations were significant at $p < 0.05$. However, focusing on the stronger correlations with $p < 0.01$ the following results were noted. Looser stool consistency was associated with lower abundance of the phylum *Bacteroidetes* and the families *Bacteroidaceae*, *Christensenellaceae*, *Dehalobacteriaceae* and *Oscillospiraceae*, and was associated with higher abundance of the family *Erysipelotrichaceae*. Better QOL was associated with higher abundance of the families *Christensenellaceae*, *Dehalobacteriaceae*, *Mogibacteriaceae*, *Ruminococcaceae*, *Eubacteriaceae* and *Enterococcaceae*. Higher extra-intestinal pain was associated with lower abundance of the families *Christensenellaceae*, *Dehalobacteriaceae*, *Oscillospiraceae*, *Mogibacteriaceae* and *Ruminococcaceae*. There was no significant relationship at $p < 0.01$ between psychological distress and microbial families. Supplemental Table 1 also shows the

bacterial families and their relationship with Shannon diversity. Examples of family relationships with stool consistency are shown in Figure 2.

Correlation of Microbiota Genera with Clinical Measures

Ninety-five genera were identified that met the criteria of being present in at least 10% of the samples. Genus-level data, along with correlations with clinical measures, are shown in Supplemental Table 2. As expected, many of the specific genera from families in which significant relationships were found also demonstrated significant ($p < 0.05$) relationships with clinical measures. For example, genus *Dehalobacterium* from the family *Dehalobacteriaceae* was inversely related ($r = -0.32$) to stool consistency and positively associated with QOL ($r = 0.34$). However, when adjusted for multiple comparisons, none of these relationships remained significant. Supplemental Table 2 also shows the bacterial genera and their relationships with Shannon diversity.

DISCUSSION

In this sample of 76 women with Rome III-diagnosed IBS, we sought to determine if differences in microbial communities existed across IBS predominant bowel pattern groups and the relationships of stool consistency, urgency, psychological distress, extra-intestinal pain, and QOL to specific bacterial taxa. Using Shannon diversity, OTU richness, FB ratio, PC1, and PC2 as microbial summary indicators, we determined that the FB ratio is markedly higher in the IBS-D group and PC1 is higher in the IBS-C group. When the five microbial summary indicators were correlated with clinical measures, greater diversity was associated with harder stools, fewer extra-intestinal pain symptoms, and higher QOL. Further examination of individual families identified a cluster of three families within the phylum *Bacteroidetes* and eight families within the phylum *Firmicutes* that showed positive associations with stool microbial diversity. Bowel pattern group was significantly associated with the abundances of seven families, i.e., *Bacteroidaceae*, *Porphyromonadaceae*, *Christensenellaceae*, *Leuconostocaceae*, *Gemellaceae*, *Methanobacteriaceae*, and *Erysipelotrichaceae*. A summary extra-intestinal pain measure that included daily heartburn, joint pain, muscle pain, backache, and headache was inversely related to microbial summary indicators. We found no statistically significant relationships of microbiota summary indicators with daily abdominal pain, bloating, intestinal gas, and psychological distress ratings.

This is the first study to examine the relationship of bacterial families with both daily GI symptoms as well as extra-intestinal pain, i.e., heartburn, joint pain, muscle pain, backache, and headache. Several *Firmicutes* families (*Dehalobacteriaceae*, *Oscillospiraceae*, *Mogibacteriaceae*, *Ruminococcaceae*) were associated with lower extra-intestinal pain severity. The lack of association with diary abdominal pain symptoms with bacterial families was likely related to the fact that all participants reported abdominal pain on the diary. However, not all women reported extra-intestinal pain symptoms on the daily diary. Such variability in extra-intestinal pain measures may have contributed to the stronger associations with bacterial families. Without healthy controls in this study, we do not know

if these relationships are unique to women with IBS, but they provide intriguing targets for future study.

While not statistically significant, we did observe a trend towards decreased microbial diversity for IBS-D compared to IBS-C patients. This could indicate that an altered gut microbiome may be a differentiating feature and potential underlying mechanism for the development of IBS-D versus IBS-C symptoms.

Somewhat similar to our results, other investigators have demonstrated reduced diversity is associated with diarrhea or loose stools^{45–47} as well as taxonomic differences between controls and persons with constipation. Comparing mucosal microbiota from women with constipation to healthy women using a single taxon approach, Parthasarathy et al.⁴⁸ found the phylum Bacteroides and the genus *Feacalibacterium* were higher in constipated women. Several other genera were associated with reduced colonic transit measured with scintigraphy. Roager⁴⁷ also studied colonic transit time in 98 healthy individuals using radio-opaque markers. Fecal Shannon index and OTU richness were positively associated with slower colonic transit time. Slower colon passage time may contribute to a greater abundance of methanogens (seen more predominantly in harder stools).⁴⁶ While another group has shown no IBS subgroup differences in methane breadth testing⁴⁹, our IBS-C group had a greater abundance of family *Methanobacteriaceae* relative to IBS-D and their abundance showed an inverse trend (Supplemental Table 1) with stool consistency. The role of this bacterial family in symptoms in individuals with IBS remains to be determined. Our observation of an inverse relationship of stool consistency with family *Christensenellaceae* ($r=-0.37$) is consistent with Parthasarathy's association findings related to this family and methane production. However, they found no significant association of this family with colonic transit. To date, little is known about the physiological significance of family *Christensenellaceae* in persons with IBS-C. Whether the presence of increased fecal diversity combined with higher levels of specific families such as family *Christensenella* influence motility could be an impetus for further studies. It is unlikely that variations in the water content from stool samples (i.e., less water in harder stools, more water in looser stools) affect microbial diversity measures.⁵⁰

Although the utility of the FB ratio has been questioned in the context of obesity⁵¹, it appears to be meaningful in the context of IBS. Jeffery et al.¹⁸ found that a high FB ratio was characteristic of IBS and was closely associated with predominant stool form. Likewise, our data support that a relationship between the FB ratio and predominant stool type exists in IBS. The IBS-C group had a lower FB ratio as compared to the IBS-D group (Table 1). Parthasarathy et al. (2016) in a study of mucosal microbiota found that women with chronic constipation and/or IBS-constipation and -mixed had elevated levels of genera from the phylum *Bacteroidetes*.⁴⁸ At this time, it is not known whether a higher ratio of FB contributes to diarrhea and/or is a consequence of rapid transit and diarrhea.

In the current study, the family *Erysipelotrichaceae*, which belongs to the phylum *Firmicutes*, was positively associated with loose stools. There is a growing literature about family *Erysipelotrichaceae*, its taxa, and their potential roles in infection/immune activation.^{52–54} Dietary factors, specifically the dietary non-fermentable fiber hydroxypropyl

methylcellulose, increased the abundance of family *Erysipelotrichaceae* in a murine model.
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This study is the first to address the relationship among prospectively collected daily extra-intestinal pain symptoms and fecal microbiota composition in the context of IBS. A summary measure of daily heartburn, joint pain, muscle pain, backache, and headache was inversely associated with microbial diversity measures. The literature on musculoskeletal pain and microbiome is scarce. Whether these pain-related symptoms are linked to overall heightened somatic pain sensitivity due to central pain pathway activation, a product of immune system activation, and/or a behavioral tendency to report multiple symptoms cannot be discerned.

In the current study only fecal microbiota were examined. Multiple studies in the published literature indicate that the mucosal and fecal microbiota differ from one another. However, they are not completely distinct and bacteria with host-modulating properties can be detected in both locations. For example, this is the case with *Fusobacterium nucleatum* in colorectal cancer.⁵⁶ Despite the imperfect (mucosal) resolution, fecal microbial community profiles do have the potential to provide insight into pathophysiology. This is substantiated further by the number of studies which show relationships between fecal microbial composition and clinical symptoms (e.g., stooling characteristics).⁴⁵

This study has several limitations. A single fecal sample was collected from each woman enrolled in an intervention trial for IBS and as such, there was no control group. We excluded women who had used antibiotics for two months prior to study entry, whereas other microbiome studies, including the Human Microbiome Project, have required longer antibiotic-free periods (e.g., 6 months) prior to microbiome testing.³⁴ All women in the study were between ages 18 and 70 with approximately two-thirds of those under age 45 receiving some form of hormonal contraception. No effort was made to control for menstrual cycle phase or menopausal status, which may be important when describing the microbiota composition.⁵⁷ In addition, we did not have dietary intake information. Because the date of stool collection was not available on a third of our sample, we chose to use the 28-day average for stool consistency and reports of diarrhea and constipation. On the other hand, this study has several strengths including the use of a daily diary to collect symptoms and stool consistency markers and the measurement of quality of life.

CONCLUSION

Stool microbial diversity and composition are linked to greater extra-intestinal pain symptoms and reduced QOL.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Clinical measures and their correlations with the first two principal components (PC1, PC2) defined based on family-level microbial community composition in the stool communities of women with irritable bowel syndrome.

Figure 2.

Mean stool consistency from daily diary versus (A) *Firmicutes/Bacteroides* (FB) ratio, (B) log of family *Christensenellaceae*, and (C) log of family *Erysipelotrichaceae*. IBS-C = IBS-constipation; IBS-D = IBS-diarrhea; IBS-M = IBS-mixed.

Figure 3.

(A) Extra-intestinal pain rating from daily dairy versus Shannon diversity, (B) Extra-intestinal pain summary measure versus logit of family *Mogibacteriaceae*, and (C) IBS-Specific Quality of life with logit of family *Mogibacteriaceae*. IBS-C = IBS-constipation; IBS-D = IBS-diarrhea; IBS-M = IBS-mixed.

Table 1

Descriptive Characteristics Based on Rome III Irritable Bowel Syndrome (IBS) Bowel Pattern Predominance in Women with IBS

Variable	IBS-D diarrhea (n = 39)	IBS-C constipation (n=22)	IBS-M mixed (n=13)	IBS-U unsubtyped (n=2)
Demographics				
Age (mean [SD], yr)	37.8 (14.3)	41.9 (15.6)	33.4 (13.1)	67.5 (0.7)
Body mass index (kg/m ² , mean [SD])	25.8 (6.0)	25.4 (6.8)	24.6 (6.4)	22.8 (0.7)
Race (White, % [n])	69% (25)	91% (20)	67% (8)	100% (2)
Married or partnered (% [n])	49% (19)	55% (12)	30% (4)	0
Education (Bachelors or above, % [n])	69% (27)	76% (16)	62% (8)	100% (2)
Income (> \$60 000/yr, % [n])	44% (14)	47% (9)	46% (6)	50% (1)
Professional job (% [n])	49% (18)	10% (2)	31% (4)	50% (1)
Daily Symptoms (mean [SD])				
Abdominal pain	1.39 (0.66)	1.30 (0.52)	1.49 (0.57)	1.11 (0.15)
Bloating	1.10 (0.83)	1.13 (0.61)	1.48 (0.83)	1.11 (0.05)
Constipation *	0.48 (0.54)	1.14 (0.63)	0.70 (0.58)	0.57 (0.25)
Diarrhea *	0.91 (0.60)	0.17 (0.19)	0.55 (0.81)	0
Urgency *	0.91 (0.70)	0.30 (0.31)	0.90 (1.07)	0
Stool consistency *	3.30 (0.56)	2.44 (0.35)	3.18 (0.43)	2.75 (0.11)
Psychological distress ^a	0.59 (0.49)	0.77 (0.51)	0.59 (0.31)	0.35 (0.28)
Extra-intestinal pain ^b	0.42 (0.42)	0.60 (0.34)	0.43 (0.48)	0.25 (0.33)
IBS quality of life	66.9 (15.8)	68.1 (12.6)	66.0 (18.5)	68.4 (14.7)

^aPsychological distress is a composite of daily reporting of anxiety, depression, panic, and stressed;

^bExtra-intestinal pain is a composite of daily reporting of heartburn, joint pain, muscle pain, backache, and headache.

*p<0.05 by ANOVA

Table 2

Comparison of Microbiota Summary Measures Based on Irritable Bowel Syndrome (IBS) Rome III Bowel Pattern Subgroups

Microbiome summary measures (mean, (SD))	IBS-C (n = 22)	IBS-D (n = 39)	IBS-M (n = 13)	p value ^a
Number of OTUs	324 (106)	290 (84)	256 (86)	NS
Shannon diversity	3.33 (0.58)	3.22 (0.56)	3.00 (0.45)	NS
FB ratio	9.92 (9.68)	21.04 (13.9)	19.04 (16.7)	0.018 *
PC1	0.44 (0.86)	-0.21 (0.93)	-0.26 (1.17)	0.02 *
PC2	-0.20 (1.07)	0.28 (0.96)	-0.39 (0.81)	0.08

^aUnadjusted p-value from analysis of covariance controlling for read count and age.

* Significant under Benjamini-Hochberg procedure to control false discovery rate at 0.05, across the 5 hypothesis tests in this table.

IBS-C = IBS-constipation; IBS-D = IBS-diarrhea; IBS-M = IBS-mixed; OTUs = operational taxonomic units; FB = *Firmicutes/Bacteroidetes* ratio; PC1 = principal component 1; PC2 = principal component 2.

Table 3

Correlations of Microbiota Summary Measures with Stool Consistency, Individual Gastrointestinal Symptoms, Urgency, Psychological Distress and Extra-intestinal Summary Measures and Irritable Bowel Syndrome (IBS)-Specific Quality of Life in Women with IBS^a

	Stool consistency	Abdominal pain	Bloating	Constipation	Diarrhea	Urgency	Psychological distress ^b	Extra-intestinal pain ^c	Urgency	IBS-Specific Quality of life
Shannon diversity	-0.24 *	0.04	-0.07	0.14	-0.06	-0.15	-0.19	-0.36 *	-0.15	0.31 **
Number of OTUs	-0.29 **	-0.12	-0.15	0.11	-0.25 *	-0.29 **	-0.24 *	-0.33 *	-0.29 **	0.43 **
FB ratio	0.38 **	-0.17	-0.12	-0.21	0.21	0.23	0.15	0.06	0.23	-0.05
PC1	-0.33 **	0.05	0.01	0.18	-0.19	-0.31 **	-0.18	-0.25 *	-0.31 **	0.25 *
PC2	0.04	-0.17	-0.17	-0.01	-0.02	-0.04	-0.15	-0.23	-0.04	0.30 **

PC = principal component; OTUs = operational taxonomic units; FB ratio = *Firmicutes/Bacteroidetes* ratio

^aPartial correlations controlled for read count and age;

^bPsychological distress summary measure includes daily anxiety, depression, panic and stressed;

^cExtra-intestinal pain summary measure includes daily heartburn, joint pain, muscle pain, backache, and headache

* unadjusted p<0.05;

** significant under Benjamini-Hochberg procedure to control false discovery rate at 0.05, across the 35 correlations in this table.

Table 4Correlations of Microbiota Summary Measures with Individual Daily Extra-Intestinal Symptoms in Women with Irritable Bowel Syndrome^a

	Heartburn	Joint pain	Muscle pain	Backache	Headache
Shannon diversity	-0.28 [*]	-0.39 ^{*,†}	-0.38 ^{*,†}	-0.20	-0.26 [*]
Number of OTUs	-0.32 [*]	-0.25 [*]	-0.33 ^{*,†}	-0.23	-0.29 [*]
FB ratio	0.07	0.07	0.11	-0.02	0.17
PC1	-0.18	-0.21	-0.22	-0.18	-0.18
PC2	-0.18	-0.17	-0.22	-0.15	-0.15

PC = principal component; OTUs = operational taxonomic units; FB ratio = *Firmicutes/Bacteroidetes* ratio^aPartial correlations controlled for read count and age;^{*} unadjusted p<0.05;[†] significant under Benjamini-Hochberg procedure to control false discovery rate at 0.05, across the 25 correlations in this table.