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TITLE PAGE

Title: No Significant Association Between the Fecal Microbiome and the Presence of Irritable Bowel Syndrome-type Symptoms in Patients with Quiescent Inflammatory Bowel Disease.

Short Title: IBS Symptoms and the Microbiome in IBD

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Abbreviations:

5-ASA	5-aminosalicylate
CD	Crohn's disease
FC	fecal calprotectin
GI	gastrointestinal
IBD	inflammatory bowel disease
IBS	irritable bowel syndrome
PPI	proton pump inhibitor

TNF	tumor necrosis foctor
UC	ulcerative colitis

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ABSTRACT AND KEY WORDS

Background: The microbiome is implicated in the pathogenesis of inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS). Whether a distinct microbiome profile is associated with the reporting of IBS-type symptoms in IBD patients is uncertain. We aimed to resolve this issue using a cross-sectional study design.

Methods: Using clinical disease activity indices, the Rome III criteria for IBS and fecal calprotectin levels, we divided IBD patients into four groups: IBS-type symptoms, quiescent disease, occult inflammation, and active disease. 16S rRNA microbiome analysis was performed to determine whether any taxa were differentially abundant, and whether there were any differences in alpha or beta diversity, in patients reporting IBS-type symptoms compared with those in the other three groups.

Results: Of 270 patients included, 70 (25.9%) had IBS-type symptoms, 81 (30.0%) quiescent IBD, 66 (24.4%) occult inflammation and 53 (19.6%) active IBD. At phylum level, there was a non-significant increase in the abundance of Actinobacteria in patients reporting IBS-type symptoms, but no other differences at any taxonomic level. When compared with patients reporting IBS-type symptoms, mean alpha diversity was greater in patients with quiescent disease, although this was non-significant (28.6 vs. 31.7, P = 0.33), and similar to those with occult inflammation and active disease. Beta diversity variation between the four groups was significant for unweighted (P = 0.002) but not weighted (P = 0.21) UniFrac analysis.

Conclusions: Reporting IBS-type symptoms was not associated with distinct microbiome alterations. Unmeasured confounding could have impacted the significance of our findings.

Keywords: Microbiome; irritable bowel syndrome; inflammatory bowel disease

INTRODUCTION

The inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are chronic disorders of the gastrointestinal (GI) tract. Their etiology remains uncertain, but is likely to be multi-factorial, and may be consequent upon factors including host genetics, exposure to environmental risk factors, disordered intestinal immunity and perturbations in the intestinal microbiome. The majority of available pharmacological therapies for IBD exert their effects by targeting disordered immunity as their mechanism of action. However, in addition to this, other therapies aiming to improve disease outcomes via manipulation of the intestinal microbiome including antibiotics,(1) probiotics,(2) and fecal microbial transfer,(3) are also the subject of research efforts, albeit with mixed results.

Patients with IBD typically report diarrhea, fecal urgency, rectal bleeding, and abdominal pain. However, symptom-reporting in IBD may not be related exclusively to ongoing intestinal inflammation.(4, 5) Indeed, the prevalence of symptoms meeting criteria for irritable bowel syndrome (IBS) in patients with IBD is estimated to be as high as 35% in those with clinically quiescent disease,(6) and appears to be higher in patients with CD than UC.(7-9) Although IBS and IBD are considered dichotomous disorders falling either side of a functional-organic divide, they have a number of etiological factors in common including, but not limited to, alterations in the intestinal microbiome.(10)

The distinction between symptoms related to co-existent IBS and those attributable to genuine inflammatory disease activity is important to make for several reasons. Firstly, the efficacy of traditional pharmacological therapy is poor in patients with a limited inflammatory burden.(11) Secondly, the concept that this group of patients genuinely exist is becoming more widely accepted, due to the advent of non-invasive biomarkers to detect intestinal inflammation. Finally, in recent years, this group of patients has often been

excluded from trials of novel investigational agents for IBD. Despite this, there is a lack of evidence-based therapeutic options available for the management of such patients, who experience a reduced quality of life equivalent to patients with overt inflammatory disease activity.(8) At the present time, there is therefore an increased interest in research investigating potential management strategies for these patients,(12) including those targeting the intestinal microbiome. On the basis of our previous findings,(8) we hypothesized that the presence of genuine IBS-type symptoms in patients with quiescent IBD would be associated with alterations in the microbiome. If proven, this would support the need for randomized controlled trials (RCTs) of therapies targeting the microbiome as potential management strategies for this hard-to-treat emerging cohort of patients.

MATERIALS AND METHODS

Participants and Setting

All individuals who participated had an established radiological, histological or endoscopic diagnosis of CD or UC. Unselected consecutive patients aged ≥16 years attending the IBD clinic at St. James's University Hospital, Leeds, United Kingdom, which serves a local population of 800,000 people, were approached about the study. Exclusion criteria were an inability to understand written English, a diagnosis of IBD-unclassified, isolated fistulizing peri-anal CD, or anyone with an end ileostomy or colostomy, due to the difficulties in assessing disease activity indices in these patients. At the clinic attendance, prior to consultation with a gastroenterologist, individuals were presented with an information sheet explaining the nature of the study. Those who agreed to take part provided written informed consent at this visit. Data collection started in November 2012, and continued until June 2015.

Data Collection and Synthesis

Once informed consent was obtained, demographic data including gender, age, ethnicity, marital status, educational level, tobacco and alcohol use, weight (in kilograms) and height (in meters), which were used to calculate body mass index (BMI), were collected. Medication history, including current use of 5-aminosalicylates (5-ASAs), glucocorticosteroids, immunosuppressants, anti-tumor necrosis factor (TNF)-α therapies, proton pump inhibitors (PPIs), or antibiotics, disease location and behavior for CD, or distribution for UC, as defined by the Montreal classification,(13) and any previous intestinal resection related to CD were also recorded. Details concerning the methods used to judge disease activity, assess for the presence or absence of IBS-type symptoms, anxiety,

depression, somatization, and measure quality of life are as they were described in our previous publications,(4, 8) and are provided in the Supplemental Digital Content.

At their clinic visit participants were asked to provide stool for quantitative fecal calprotectin (FC) analysis (Immundiagnostik, Bensheim, Germany), as an objective marker of mucosal inflammation, within 7 days of study entry. We used a cut-off value of <250µg/g of stool to define no evidence of mucosal inflammation, in line with expert opinion,(14) as other investigators have employed.(4, 5, 8, 15, 16) Stool samples were stored at -20ºC prior to FC ELISA and DNA extraction for microbiome analysis.

Assessment of Microbiome

Isolation of prokaryotic DNA from stool samples was based on methods outlined by Yu et al.(17) All DNA samples were quality controlled by spectrophotometer before being amplified by polymerase chain reaction of the V4 hypervariable region of the 16S rRNA gene, a reliable indicator of bacterial taxonomy.(18) Successfully amplified samples were further processed into libraries using the New England Biolabs NEBNext Ultra DNA Library Prep Kit, with which primers specific to the flow cell of the Illumina MiSeq were ligated onto amplicons with a unique barcode. Samples were sequenced over two runs of an Illumina MiSeq. Samples were pooled and pair-end sequenced (2X250bp). Reads were trimmed of adapters using cutadapt (version 1.2.1).(19) Pairs were merged and a consensus sequence based on best quality was produced using fastq-join (http://code.google.com/p/ea-utils/). As previously described,(20, 21) merged sequences were removed if they contained ambiguous base calls, where more than 2bp different from the expected 240bp PCR length, or contained more than two contiguous bases with a PHRED quality score of less than 33. Further analysis took place using the QIIME software package (version 1.8.0).(22) Operational taxonomic units were picked using USEARCH (version 5.2.236),(23) and aligned to the Greengenes

database (13_8 release 97% similarity version) (24) using PyNAST (version 1.2.2).(25) Genus-level taxonomy was assigned using the Ribosomal Database Project classifier 2.2.(26) Sequence files were deposited in the European Nucleotide Archive (http://www.ebi.ac.uk/ena/data/view/PRJEB23261).

Statistical Analysis

The statistical methods used to compare demographic and disease related characteristics, medication use and psychological variables were as previously described,(8) and are discussed in brief in the Supplemental Digital Content.

A Kruskal-Wallis test, with 1000 Monte Carlo permutations was performed to determine whether any taxa were significantly differentially abundant across clinical groupings. In line with the advice of the producers of QIIME, taxa present at a proportional abundance of less than 0.01 were excluded from this analysis.(22) Alpha diversity (within sample diversity), as measured by the Faith's phylogenetic diversity metric,(27) was computed at a maximum rarefaction depth of 4174 (the minimum number of sequence reads in a sample). A non-parametric two-sample t-test, with 999 Monte Carlo permutations was performed to compare alpha diversity between clinical groupings. Beta diversity (between sample diversity), at a rarefaction depth of 4174, was calculated using the unweighted and weighted UniFrac metrics.(28) Computed beta diversity values were used to construct dissimilarity matrices, and principle coordinates analysis (PCoA) was then used to allow visualisation of beta diversity variation in two dimensions. R-squared values were calculated to determine the proportion of beta diversity variation attributable to clinical groupings. R's 'adonis' function, which is similar to permutational multivariate analysis of variance, with 999 Monte Carlo permutations, was used to determine whether clinical groupings were associated with significant beta diversity variation.

ETHICAL CONSIDERATIONS

The study was approved by the local research ethics committee (REC reference: 12/YH/0443).

RESULTS

In total, 270 patients with IBD provided informed consent, completed questionnaire data and returned a stool sample for FC and subsequent microbiome analysis. Of these 150 (55.6%) had CD and 120 (44.4%) had UC. Demographic, disease related and psychological characteristics of CD and UC patients are displayed in Table 1.

Characteristics of IBD Patients with and without IBS-type Symptoms

Of the 270 IBD patients included, 70 (25.9%) were classified as having IBS-type symptoms (4 (5.7%) fulfilled the criteria for IBS with constipation predominance and 66 (94.3%) fulfilled the criteria for IBS with diarrhea predominance or a mixed stool pattern), 81 (30.0%) as quiescent IBD, 66 (24.4%) as IBD with occult inflammation, and 53 (19.6%) as active IBD (Figure 1).

Differences in demographic characteristics, disease-related characteristics, medication use and psychological data were as previously described,(8) and are displayed in Supplemental Digital Content (Supplementary Tables 1, 2 and 3). Antibiotic and PPI use was no different across the four study groups in CD or UC.

Microbiome Analysis

There was significant variation in the composition of all 270 fecal microbiomes at the genus level. Marked inter-personal variation was apparent, although a handful of genera were present at similar abundances.

Comparison of Microbiome Composition between IBD Patients Divided into those with CD and UC

There was no difference observed between CD and UC at phylum level, where bacteria overwhelmingly belonged to the phyla Firmicutes and Actinobacteria. However, differences were apparent at the family level, with an increase in abundance of Ruminococcaceae, (mean proportional abundance 0.329 vs. 0.235, P = 0.005) and a decrease in abundance of Lachnospiraceae, in UC compared with CD patients. Differences between CD and UC were also apparent at the genus level. There was a significantly greater abundance of Fecalibacterium, in UC relative to CD (mean proportional abundance 0.070 vs. 0.035, P <0.001), and a greater abundance of Ruminococcus in CD relative to UC (mean proportional abundance 0.036 vs. 0.013, P <0.001). The mean alpha diversity value was greater in UC, when compared with CD (33.1 vs. 27.0, P <0.001). Differences in beta diversity between CD and UC patients were not marked, although they reached significance for the unweighted and weighted UniFrac metrics (both P <0.001). Whether patients had CD or UC accounted for 1.64% and 2.34% of variation with the unweighted and weighted metrics, respectively. Inspection of PCoA plots suggested a trend for greater beta diversity variation in CD patients compared with UC patients.

Microbiome Composition Between IBD Patients Divided into those with IBS-type symptoms, Quiescent Disease, Occult Inflammation, and Active Disease

When compared with patients reporting IBS-type symptoms, the median number of read sequences per sample was higher in patients with quiescent disease (10886 (range 5099 to 21170) vs. 8258 (range 5010 to 22065); P = 0.02), and occult inflammation (9937 (range 4174 to 21769) vs. 8258 (range 5010 to 22065); P = 0.01) but lower in patients with active disease (7885 (range 5372 to 11058) vs. 8258 (range 5010 to 22065); P = 0.02).

Slight phylum-level differences existed across the four IBD disease status groups. There was an increased relative abundance of Actinobacteria in patients reporting IBS-type symptoms and an increased abundance of Proteobacteria in patients with active IBD, but these differences were not statistically significant (Figure 2). No differences were observed at other taxonomic levels. No differences were observed when patients were dichotomized into those with CD and UC.

Mean alpha diversity was greater in those with quiescent disease when compared with patients reporting IBS-type symptoms, although this difference failed to reach statistical significance (31.7 vs. 28.6, P = 0.33). No difference in mean alpha diversity was observed between patients reporting IBS-type symptoms and those with occult inflammation (28.6 vs. 29.3, P = 1.00) and active disease (26.6 vs. 28.6, P = 1.00). In CD, there was no difference in mean alpha diversity between patients reporting IBS-type symptoms and those with quiescent disease (26.3 vs. 28.6, P = 1.00), occult inflammation (26.3 vs. 27.4, P = 1.00), or active disease (26.3 vs. 24.9, P = 1.00). Similarly in UC, there was no difference in mean alpha diversity between patients reporting IBS-type symptoms and those with quiescent disease (32.0 vs. 35.0, P = 1.00), occult inflammation (32.0 vs. 32.2, P = 1.00), or active disease (32.0 vs. 32.3, P = 1.00).

Overall, 1.54% of variation in beta diversity was attributable to disease status (P = 0.002). This variation is likely to be complex, as it could not be seen on the unweighted UniFrac PCoA plot (Figure 3). Separating CD from UC showed that 2.55% of variation in CD patients (P = 0.019) and 2.92% of variation in UC patients (P = 0.021) could be accounted for by disease status. Again, this variation was not clearly visible in the PCoA plots, suggesting it is complex in nature. Colouring the weighted UniFrac PCoA plot by disease status also demonstrated no trends (Figure 4). Overall, 1.33% of variation in weighted UniFrac was attributable to disease status, but this was not statistically significant (P =

0.213). Separation of CD from UC patients showed that 2.07% of variation in CD patients, and 2.14% of variation in UC patients was attributable to disease status, but this was not significant ($P = 0.39$ and $P = 0.661$, respectively). The fact that disease status was not significantly associated with variation in beta diversity, as measured by weighted UniFrac, but was for unweighted UniFrac, suggests that the presence or absence of rarer taxa, rather than the relative abundances of commoner taxa, was the cause of the observed variation.

DISCUSSION

The findings of this cross-sectional study suggest that the presence of IBS-type symptoms in IBD is not associated with distinct alterations in the intestinal microbiome. Our results highlight that there was no significant difference in the abundance of bacterial taxa observed between patients reporting IBS-type symptoms and those with quiescent disease, occult inflammation, and active disease in UC or CD, at any taxonomic level tested. There was no difference in alpha diversity across the four disease sub-groups, and the observed variation in beta diversity was not consistent.

Strengths of this study include the large number of patients included and the well-characterized study population, including complete demographic, disease-related, medication, and psychological data. Patients recruited were from a secondary care population and the results are therefore likely to be generalizable to the wider IBD population. To the best of our knowledge, this is the first study to investigate the possibility of the microbiome contributing to the development of IBS-type symptoms in patients with quiescent IBD. Our collection of psychological, as well as disease-related and patient characteristics, is relevant because alterations in the fecal microbiome have been implicated in the development of mental health problems.(29, 30) Furthermore, our collection of all available medication data, particularly those known to affect intestinal microbial diversity, including antibiotics and PPIs, is another strength.

Limitations of this study include its cross-sectional design, meaning that we can only comment on a possible association between the presence of IBS-type symptoms and the intestinal bacterial composition. Therefore, no comment on any causal link between a particular microbiome profile and the development of IBS-type symptoms can be made. Our study is limited to the fecal microbiome, thus no assertion on the association between the mucosa associated microbiome and the development of IBS-type symptoms in IBD can be

determined, despite its potential value. Although our use of FC as an objective marker of intestinal inflammation is a strength, gold standard investigations for the assessment of inflammatory disease activity were not performed. Patients with IBD are, by their nature, a complex and heterogeneous population. Although we provide data on the prevalence of potential confounding variables including previous surgery, disease distribution and phenotype, age, BMI, and medications, including 5-aminosalicylic acids, immunomodulators, biologics, antibiotics, and PPIs, which may independently or collectively affect the intestinal bacterial composition, these confounders are not accounted for in our analyses. It is possible that our inability to account for this is responsible for the lack of variability in the microbiome composition and bacterial diversity observed across the four sub-groups in our study population. We describe a significant difference in the prevalence and severity of anxiety, depression and somatization between patients reporting IBS-type symptoms and those with quiescent disease and occult inflammation (Supplementary Table 1). These findings highlight a potential role for central processing in the generation of IBS-type symptoms in IBD, as has been described in IBS,(31) and that the presence of psychological co-morbidity, and its potential impact on the microbiome,(32) may have affected our findings. To address this issue directly, sensitivity analysis was performed to compare the microbiome in IBD patients with and without anxiety, depression, or somatization. Here, there was no difference in the relative abundance of bacterial taxa, or bacterial diversity when patients with anxiety or depression were compared with patients without these complaints. Mean alpha diversity was lower (29.2 vs. 34.6, $P = 0.02$), and beta diversity, as measured by the unweighted UniFrac metric, was lower ($P = 0.04$) in patients with high somatization severity, when compared with those with mild severity. However, the lack of any consistent association between psychological co-morbidity and microbiome composition suggests its impact on our primary analysis is likely to be negligible. Finally, the lack of a healthy control

group, or a comparator group of patients without IBD, but with established IBS is a further limitation, as we are unable to comment on the association between brain-gut activity and the microbiome in patients without IBD.

The relationship between the microbiome and the host enteric immune system is thought to be central to the development of IBD. However, whether disordered immunity in the presence of a 'normal' microbiome, or an appropriate immune response to an 'abnormal' microbiome is what leads to the development of chronic GI inflammation in these patients is uncertain. Previous studies investigating the microbiome in IBD have suggested that disease activity is associated with an increased abundance of pro-inflammatory bacterial species, and a reduction in anti-inflammatory bacterial species such as Fecalibacterium prausnitzii, as well as a reduction in overall alpha diversity, when compared with healthy controls.(33) In IBS, interest in the relationship between the microbiome and the presence of GI symptoms has arisen in part due to the high prevalence of these symptoms in patients with a post-infectious etiology.(34) Furthermore, evidence suggesting that disordered enteric immunity is common in IBS patients has highlighted the potential for microbiome-host interactions to contribute to the development of GI symptoms in these patients.(35)

The recognition of shared etiological factors contributing to the development of both IBS and IBD, and a recognition that GI symptoms in IBD may persist in the absence of objectively quantifiable inflammatory disease activity,(7, 8, 36, 37) has led to support for a change in the management of these patients by some. Indeed, the results of clinical trials of anti-TNFα therapy in such individuals have been disappointing.(11) Targeting manipulation of the intestinal microbiome in these patients may be of interest, and our results have implications for future trial design. To date, the majority of clinical trials of therapies directed at manipulation of the intestinal microbiome in IBD have been concerned with the management of symptomatic disease activity, rather than objective indices of inflammatory

disease activity, and none have addressed patients with co-existent IBS. Furthermore, only a minority of these studies have attempted to identify any demonstrable difference in the microbiome pre-and post-intervention, and fewer still have sought to address the potential effects of such therapies on the metabolome or proteome. The results of RCTs of probiotics,(2) antibiotics(1) and fecal microbial transfer(3) in IBD have been mixed, and none have focussed their attention on the treatment of patients reporting IBS-type symptoms specifically. Despite this, trials of therapies such as probiotics and a low fermentable oligosaccharides disaccharides monosaccharides and polyols (FODMAP) diet in IBS have shown more promising results,(38, 39) potentially acting via manipulation of the intestinal microbiome.(40) Although we did not detect any difference in the microbiome composition in patients reporting IBS-type symptoms, we are unable to confirm or refute whether therapies that modulate the microbiota will be beneficial in these patients, therefore RCTs of these interventions may still be worthwhile in this hard-to-treat cohort of patients.

In summary, the results of this cross-sectional study investigating the relationship between the intestinal microbiome and the presence of genuine IBS-type symptoms in patients with quiescent IBD has demonstrated that, bar a non-significant reduction in alpha diversity, a characteristic microbiome footprint is not apparent in these patients when compared with those with quiescent disease, occult inflammation, or active disease. The implications of these findings need to be taken into context when considering trials of future management strategies. Despite there being a variable effect of interventions aiming to modulate the intestinal microbiome in IBD, these therapies have only been trialled as a treatment for active symptomatic disease. The heterogeneous patient population included here, and the effect of unmeasured confounding that may have affected our results, limits the validity of these findings. Therapies targeting the intestinal microbiome are evidence-based in

IBS,(38) and may still provide much-needed novel therapeutic strategies in IBD patients with symptoms that persist in the absence of inflammatory disease activity.

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REFERENCES

1. Khan KJ, Ullman TA, Ford AC, et al. Antibiotic therapy in inflammatory bowel disease: a systematic review and meta-analysis. Am J Gastroenterol. 2011;106:661-673

2. Derwa Y, Gracie DJ, Hamlin PJ, et al. Systematic review with meta-analysis: the efficacy of probiotics in inflammatory bowel disease. Aliment Pharmacol Ther. 2017;46:389-400

3. Vermeire S, Joossens M, Verbeke K, et al. Donor Species Richness Determines Faecal Microbiota Transplantation Success in Inflammatory Bowel Disease. J Crohns Colitis. 2016;10:387-394

4. Gracie DJ, Williams CJ, Sood R, et al. Poor Correlation Between Clinical Disease Activity and Mucosal Inflammation, and the Role of Psychological Comorbidity, in Inflammatory Bowel Disease. Am J Gastroenterol. 2016;111:541-551

5. Targownik LE, Sexton KA, Bernstein MT, et al. The Relationship Among Perceived Stress, Symptoms, and Inflammation in Persons With Inflammatory Bowel Disease. Am J Gastroenterol. 2015;110:1001-1012

6. Halpin SJ, Ford AC. Prevalence of symptoms meeting criteria for irritable bowel syndrome in inflammatory bowel disease: systematic review and meta-analysis. Am J Gastroenterol. 2012;107:1474-1482

7. Jonefjall B, Ohman L, Simren M, et al. IBS-like Symptoms in Patients with Ulcerative Colitis in Deep Remission Are Associated with Increased Levels of Serum Cytokines and Poor Psychological Well-being. Inflamm Bowel Dis. 2016;22:2630-2640

8. Gracie DJ, Williams CJ, Sood R, et al. Negative Effects on Psychological Health and Quality of Life of Genuine Irritable Bowel Syndrome-type Symptoms in Patients With Inflammatory Bowel Disease. Clin Gastroenterol Hepatol. 2017;15:376-384 e375

9. Lovell RM, Ford AC. Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. Clin Gastroenterol Hepatol. 2012;10:712-721 e714

10. Kassinen A, Krogius-Kurikka L, Makivuokko H, et al. The fecal microbiota of irritable bowel syndrome patients differs significantly from that of healthy subjects. Gastroenterology. 2007;133:24-33

11. Colombel JF, Sandborn WJ, Reinisch W, et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. N Engl J Med. 2010;362:1383-1395

12. http://www.nihr.ac.uk/funding-and-support/funding-opportunities/1733-management-of-diarrhoea-in-patients-with-stable-ulcerative-colitis/5984. (accessed online 10/10/2017)

13. Silverberg MS, Satsangi J, Ahmad T, et al. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. Can J Gastroenterol. 2005;19 Suppl A:5A-36A

14. Rogler G, Aldeguer X, Kruis W, et al. Concept for a rapid point-of-care calprotectin diagnostic test for diagnosis and disease activity monitoring in patients with inflammatory bowel disease: expert clinical opinion. J Crohns Colitis. 2013;7:670-677

15. D'Haens G, Ferrante M, Vermeire S, et al. Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. Inflamm Bowel Dis. 2012;18:2218-2224

16. Lin JF, Chen JM, Zuo JH, et al. Meta-analysis: fecal calprotectin for assessment of inflammatory bowel disease activity. Inflamm Bowel Dis. 2014;20:1407-1415

17. Yu Z, Morrison M. Improved extraction of PCR-quality community DNA from digesta and fecal samples. BioTechniques. 2004;36:808-812

18. Claesson MJ, Wang Q, O'Sullivan O, et al. Comparison of two next-generation sequencing technologies for resolving highly complex microbiota composition using tandem variable 16S rRNA gene regions. Nucleic acids research. 2010;38:e200

19. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnetjournal. 2011;17:10-12

20. Watson H, Mitra S, Croden FC, et al. A randomised trial of the effect of omega-3 polyunsaturated fatty acid supplements on the human intestinal microbiota. Gut. 2017

21. Taylor M, Wood HM, Halloran SP, et al. Examining the potential use and long-term stability of guaiac faecal occult blood test cards for microbial DNA 16S rRNA sequencing. Journal of clinical pathology. 2017;70:600-606

22. Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7:335-336

23. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 2010;26:2460-2461

24. DeSantis TZ, Hugenholtz P, Larsen N, et al. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol. 2006;72:5069-5072

25. Caporaso JG, Bittinger K, Bushman FD, et al. PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics. 2010;26:266-267

26. Wang Q, Garrity GM, Tiedje JM, et al. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol. 2007;73:5261-5267

27. Faith DP. Conservation evaluation and phylogenetic diversity. Biological Conservation. 1992;61:1-10

28. Lozupone C, Knight R. UniFrac: a new phylogenetic method for comparing microbial communities. Appl Environ Microbiol. 2005;71:8228-8235

29. Burokas A, Arboleya S, Moloney RD, et al. Targeting the Microbiota-Gut-Brain Axis: Prebiotics Have Anxiolytic and Antidepressant-like Effects and Reverse the Impact of Chronic Stress in Mice. Biological psychiatry. 2017

30. Hoban AE, Stilling RM, Ryan FJ, et al. Regulation of prefrontal cortex myelination by the microbiota. Translational psychiatry. 2016;6:e774

31. Ford AC, Lacy BE, Talley NJ. Irritable Bowel Syndrome. N Engl J Med. 2017;376:2566-2578

32. Dinan TG, Cryan JF. The impact of gut microbiota on brain and behaviour: implications for psychiatry. Current opinion in clinical nutrition and metabolic care. 2015;18:552-558

33. Gevers D, Kugathasan S, Denson LA, et al. The treatment-naive microbiome in new-onset Crohn's disease. Cell host & microbe. 2014;15:382-392

34. Klem F, Wadhwa A, Prokop LJ, et al. Prevalence, Risk Factors, and Outcomes of Irritable Bowel Syndrome After Infectious Enteritis: A Systematic Review and Meta-analysis. Gastroenterology. 2017;152:1042-1054.e1041

35. Ford AC, Talley NJ. Mucosal inflammation as a potential etiological factor in irritable bowel syndrome: a systematic review. J Gastroenterol. 2011;46:421-431

36. Berrill JW, Green JT, Hood K, et al. Symptoms of irritable bowel syndrome in patients with inflammatory bowel disease: examining the role of sub-clinical inflammation and the impact on clinical assessment of disease activity. Aliment Pharmacol Ther. 2013;38:44-51

37. Hoekman DR, Zeevenhooven J, D'Haens GR, et al. The prevalence of irritable bowel syndrome-type symptoms in inflammatory bowel disease patients in remission. Eur J Gastroenterol Hepatol. 2017;29:1086-1090

38. Ford AC, Quigley EM, Lacy BE, et al. Efficacy of prebiotics, probiotics, and synbiotics in irritable bowel syndrome and chronic idiopathic constipation: systematic review and meta-analysis. Am J Gastroenterol. 2014;109:1547-1561; quiz 1546, 1562

39. Staudacher HM, Lomer MCE, Farquharson FM, et al. Diet Low in FODMAPs Reduces Symptoms in Patients with Irritable Bowel Syndrome and Probiotic Restores Bifidobacterium Species: a Randomized Controlled Trial. Gastroenterology. 2017; 10.1053/j.gastro.2017.06.010

40. Staudacher HM, Whelan K. The low FODMAP diet: recent advances in understanding its mechanisms and efficacy in IBS. Gut. 2017;66:1517-1527

Table 1: Demographic and Disease-related Characteristics of CD and UC patients.

	CD (n = 150)	**UC (n = 120)**	**P value***
Mean age in years (SD)	47.5 (16.9)	53.3 (16.2)	0.005
Female gender (%)	90 (60.0)	61 (50.8)	0.13
Married or co-habiting (%)	90 (60.0)	82 (68.3)	0.16
University graduate/professional (%)	34 (22.8)	34 (28.8)	0.26
Mean BMI (SD)	26.5 (5.7)	27.2 (5.8)	0.29
Tobacco user (%)	32 (21.3)	7 (5.9)	<0.001
Alcohol user (%)	92 (61.7)	80 (66.7)	0.40
5-ASA use (%)	45 (30.0)	95 (79.2)	<0.001
Immunomodulator use (%)	67 (44.7)	30 (25.0)	0.001
Anti-TNFα use (%)	40 (26.7)	2 (1.7)	<0.001

Glucocorticosteroid use (%)	16 (10.7)	12 (10.0)	0.86
PPI use (%)	46 (32.4)	32 (27.8)	0.43
Antibiotic use (%)			
None	43 (38.4)	39 (41.5)	
≤30 days from inclusion	8 (7.1)	4 (4.3)	
31-100 days from inclusion	8 (7.1)	9 (9.6)	
>100 days from inclusion	53 (47.3)	42 (44.7)	0.73
Previous intestinal resection (%)	52 (34.7)	N/A	N/A
Rome III IBS criteria fulfilled (%)	74 (49.3)	47 (39.2)	0.10
In remission on HBI or SCCAI (%)	91 (60.7)	70 (58.3)	0.70
FCP <250µg/g (%)	83 (55.3)	68 (56.7)	0.83
Mean HADS anxiety score (SD)	7.8 (4.4)	7.4 (4.8)	0.47
Anxiety categories (%)			
Normal	75 (50.0)	66 (55.0)	
Borderline abnormal	33 (22.0)	25 (20.8)	
Abnormal	42 (28.0)	29 (24.2)	0.69
Mean HADS depression score (SD)	5.4 (4.1)	4.8 (4.2)	0.24
Depression categories (%)			
Normal	107 (71.3)	99 (82.5)	
Borderline abnormal	25 (16.7)	8 (6.7)	
Abnormal	18 (12.0)	13 (10.8)	0.04
Mean PHQ-15 score (SD)	10.8 (4.8)	9.1 (5.1)	0.009
PHQ-15 somatization categories (%)			
Mild	11 (7.8)	22 (19.2)	
Low	45 (31.9)	36 (31.6)	
Medium	56 (39.7)	32 (28.1)	
High	29 (20.6)	24 (21.1)	0.03

Mean SF-36 score (SD)			
Physical functioning	70.6 (30.0)	78.7 (24.7)	0.02
Role limitations physical health	48.9 (43.2)	60.1 (43.2)	0.04
Role limitations emotional problems	66.4 (43.8)	67.8 (41.7)	0.80
Energy/fatigue	41.0 (23.9)	48.0 (23.0)	0.02
Emotional well-being	66.5 (19.2)	68.6 (20.7)	0.40
Social functioning	63.8 (29.7)	70.6 (28.7)	0.06
Pain	57.6 (26.2)	67.0 (25.4)	0.004
General health	41.6 (22.0)	54.3 (24.5)	<0.001

*Independent samples t-test for continuous data, and χ^2 for comparison of categorical data.

Figure 1: Disease Activity and IBS Symptom Status for IBD Patients Using a Cut Off <250µg/g.

Figure 2: Phylum Level Differences in Bacterial Abundance in IBD Patients Sub-divided into IBS-type Symptoms, Quiescent Disease, Occult Inflammation and Active Disease.

Figure 3: Unweighted UniFrac PCoA Plot Demonstrating Beta Diversity in IBD Patients Sub-divided into IBS-type Symptoms, Quiescent Disease, Occult Inflammation and Active Disease.

Figure 4: Weighted UniFrac PCoA Plot Demonstrating Beta Diversity in IBD Patients Sub-divided into IBS-type Symptoms, Quiescent Disease, Occult Inflammation and Active Disease.