Safety, Clinical Response, and Microbiome Findings Following Fecal Microbiota Transplant in Children With Inflammatory Bowel Disease

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Background: The role of fecal microbiota transplant (FMT) in the treatment of pediatric inflammatory bowel disease (IBD) is unknown. The aims of this study were to assess safety, clinical response, and gut microbiome alterations in children with Crohn's disease (CD), ulcerative colitis (UC), or indeterminate colitis (IC).

Methods: In this open-label, single-center prospective trial, patients with IBD refractory to medical therapy underwent a single FMT by upper and lower endoscopy. Adverse events, clinical response, gut microbiome, and biomarkers were assessed at baseline, 1 week, 1 month, and 6 months following FMT.

Results: Twenty-one subjects were analyzed, with a median age of 12 years, of whom 57% and 28% demonstrated clinical response at 1 and 6 months post-FMT, respectively. Two CD patients were in remission at 6 months. Adverse events attributable to FMT were mild to moderate and self-limited. Patients prior to FMT showed decreased species diversity and significant microbiome compositional differences characterized by increased *Enterobacteriaceae*, *Enterococcus*, *Haemophilus*, and *Fusobacterium* compared with donors and demonstrated increased species diversity at 30 days post-FMT. At 6 months, these changes shifted toward baseline. Clinical responders had a higher relative abundance of *Fusobacterium* and a lower diversity at baseline, as well as a greater shift toward donor-like microbiome after FMT compared with nonresponders.

Conclusions: A single FMT is relatively safe and can result in a short-term response in young patients with active IBD. Responders possessed increased *Fusobacterium* prior to FMT and demonstrated more significant microbiome changes compared with nonresponders after FMT. Microbiome characteristics may help in predicting response.

Key Words: children, fecal microbiota transplantation, inflammatory bowel disease, microbiome

Compelling research in the past decade has detailed how the gut microbiota makes important contributions to human health and disease. These can be local effects with relevance to the gastrointestinal tract or systemic effects impacting metabolism and immune function. Numerous recent studies in a wide range of clinical settings have described perturbations in the gut microbiome (dysbiosis) in affected individuals relative to controls.[1] As a result, many investigators have set out to determine whether disease pathogenesis and phenotypes can be mitigated or altered by modifying the gut microbiota. Fecal microbiota transplantation (FMT) in the last decade has come to be regarded as a rational and effective approach to "resetting" the gut microbiota. It has been best studied as a treatment for refractory or recurrent *Clostridium difficile* infection, with mean cure rates in the range of 87%–90%.[2–5]

The role of FMT in treating patients with inflammatory bowel disease (IBD) remains unclear. Both Crohn's disease (CD) and ulcerative colitis (UC) are believed to involve alteration of the host-microbe relationship although the perplexity of this relationship is still evolving.[6, 7] An important recent meta-analysis evaluated the outcomes of 122 IBD patients who received FMT in 18 separate studies.[8] The overall short-term clinical remission rate was 45%. Among the 9 cohort studies included in this analysis, the proportion of patients who achieved clinical remission was 36.2%. In a subgroup analysis, the remission rate was 22% for UC and 65% for CD. Results were better in children with CD, but the studies in this meta-analysis were limited by patient and protocol heterogeneity. Other meta-analyses

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have shown similar promising results of FMT in UC patients.[9, 10] Recently, 3 randomized placebo-controlled trials evaluated the efficacy of FMT in adults with UC. The first study administered weekly enemas for 6 weeks and reported remission rates of 24% and 5% in the FMT and placebo arms, respectively, at week 7 evaluation.[11] The response rate was skewed toward a single donor who was associated with most of the responders. The second study administered FMT by 2 naso-duodenal infusions given 3 weeks apart; however, this reported no significant difference in response between FMT and placebo groups at 12 weeks.[12] The third study administered FMT as a fecal enema for 5 days a week for 8 weeks and found a remission rate of 27% compared with 8% in the placebo group.[13] Taken together, evidence suggests continued guarded optimism for FMT as a treatment option for IBD. Furthermore, it has been speculated that pediatric IBD patients may have better outcomes with FMT compared with adults as their immune systems and microbiota are still developing.[14]

Unlike earlier investigations of FMT, more recent studies have not only analyzed clinical outcomes after FMT but have also performed a detailed analysis of the microbiota in fecal samples from donors and recipients.[11–13, 15–27] These have clearly shown that donor microbiota can be successfully engrafted and sustained for a variable period of time. Remarkably, many trials in *both* CDI and IBD patients have concluded that clinical response to FMT is associated with a post-FMT increase in bacterial diversity, including increased abundance of *Lachnospiraceae*, and a decrease in Proteobacteria.[11, 19, 20, 28] Importantly however, very little is known about whether changes in the fecal microbiome after FMT are associated with clinical response or are even sustainable over time. This problem is academic in the setting of CDI, in which assessment of clinical response is relatively straightforward. However, assessing clinical response in IBD patients is complex and multifactorial, making it critically important to understand whether clinical gains made by FMT in IBD patients can be durable and associated with mucosal healing. With this in mind, at least 2 studies have pursued the strategy of serial or step-up FMT treatments.[11, 22, 23]

In this paper, we describe results from a prospective study of a single FMT performed in children with medically refractory IBD. All patients received FMT by duodenal/jejunal and colonoscopic route and were serially followed for adverse effects and clinical response following the procedure for 6 months. In addition, microbiome analyses were performed on stool samples from the donor, and pre- and post-transplant in the FMT recipients, and microbiome data were examined for correlation with clinical outcomes.

MATERIALS AND METHODS

Study Design

This single center, open-label prospective trial was conducted in children with the primary objective of observing safety, and the secondary objective was to examine and correlate clinical response with microbiome changes before and after FMT. This study enrolled subjects with clinically active IBD despite standard medical therapy and was conducted at the Children's Hospital of Pittsburgh of the University of Pittsburgh School of Medicine from October 2014 to October 2016. Subjects were mostly recruited from the institution's outpatient gastroenterology clinic or enrolled from across the country by self-referral or at the recommendation of their gastroenterologist. This institutional review board–approved study was conducted under IND 015758 and was registered at clinicaltrials.gov (NCT02108821).

Eligibility Criteria and Study

Subjects eligible for screening were between the ages of 2 and 22 years with IBD (CD, UC, or indeterminate colitis [IC]) diagnosed based on the Porto criteria by their treating gastroenterologists.[29] Due to their small numbers, subjects with IC were enrolled and assessed similarly to UC patients. Subjects were defined as being medically refractory if they had clinically active disease despite an adequate trial of standard therapy dictated by their treating gastroenterologist.

Inclusion criteria

Inclusion criteria included: (i) those undergoing a medically indicated colonoscopy for clinically active mild to moderate disease; (ii) subjects on infliximab, eligible only if they failed to respond after a full induction dose; (iii) subjects with no changes in medications or their dosage for at least 4 weeks prior to transplantation; (iv) subjects with mild to moderate disease, defined by the Pediatric Crohn's Disease Activity Index (PCDAI) or the Pediatric Ulcerative Colitis Activity Index (PUCAI) in the range of 10–40 or 10–64, respectively; (v) subjects in whom the biomarkers of disease activity (calprotectin or lactoferrin) were at least more than 2 times the upper limit of normal value (when employed as sole enrollment criteria).[30]

Exclusion criteria

Exclusions criteria included: (i) subjects with active infections including *Clostridium difficile*; (ii) subjects who had severe disease, defined by PUCAI ≥65 or PCDAI >40; (iii) subjects who were receiving immunosuppression with high-dose steroids (1 mg/kg or 30 mg/d or equivalent) in combination with a biological agent; (iv) subjects who had a central venous catheter in place; (v) subjects who were critically ill with life support such as vasopressors, assisted ventilation, etc.; (vi) subjects who had CD with disease limited to the small bowel (at diagnosis) or presence of a stricture or bowel obstruction, phlegmon, an abscess, perforation, or active fistulizing disease (at screening); (vii) subjects in whom medical therapy was changed within last 4 weeks.

Donors were healthy family members, first-degree relatives, or trusted friends. Donor inclusion criteria included not

being on prescription drugs, no antibiotic exposure in the previous 3 months, body mass index <30, being free of any current or past history of malignancy, chemotherapy, chronic systemic or gastrointestinal disease, or functional disorders including chronic fatigue, irritable bowel syndrome, and fibromyalgia. Informed consent and assent (where applicable) were obtained prior to performing screening procedures.

Subjects and donors were tested for infections within 35 days prior to FMT and were re-evaluated if there was any change in symptoms. Blood work included screening for hepatitis A, B, C, syphilis, and HIV. Stool tests included routine culture (*Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, *Escherichia coli* O157), *Clostridium difficile* DNA testing, and microscopic examination for ova, parasites, Giardia, Cryptosporidium, Cyclospora, and Isospora. The donor stool was additionally tested for *Helicobacter pylori* antigen. Female patients and donors were screened for pregnancy at the time of enrollment and before the procedure. All donors were additionally screened for risk of communicable diseases by the American Association of Blood Banks donor questionnaire (http://www.aabb.org/tm/questionnaires/Documents/dhq/v2/DHQ%20v2.0.pdf).

Fecal Microbiota Transplantation

Pretransplant preparation

Stool was collected from all donors and subjects for microbiome analysis before starting pretransplant medications. All subjects were given antibiotics for 5 days starting 7 days prior to procedure with either metronidazole or vancomycin (10 mg/kg/dose with maximum dose of 500 mg/dose 3 times daily for either drug). Subjects also took omeprazole (1 mg/kg/dose up to 20 mg twice daily) or equivalent starting 5 days before the procedure for 7 days. The donors were allowed to use over-the-counter laxatives prior to FMT if needed. All subjects received 2 to 4 mg of loperamide 2 hours prior to the procedure.

FMT procedure

Fresh donor stool was collected on the day of the procedure up to 3–4 hours prior to FMT. Approximately 150 g of stool was blended using 250–300 mL of nonbacteriostatic normal saline in a new blender at high speed for 2–3 minutes. Stool slurry was then sieved through 2 layers of gauze to remove large particles. Stool suspension was drawn into multiple 60-mL syringes and labeled for use.

All patients received general anesthesia and an endotracheal intubation for airway protection. A pediatric colonoscope (PCF-140) was used for the upper endoscopy to facilitate jejunal intubation. Biopsies were first obtained from the esophagus, stomach, and duodenum, followed by infusion of 20–30 mL of the fecal suspension into the distal duodenum or proximal jejunum followed by a 15-mL flush of normal saline. The subjects then underwent a colonoscopy, and biopsies were obtained from all segments of the colon as the scope was being advanced into the terminal ileum. Approximately 200–250 mL of the fecal suspension was then delivered into the terminal ileum and right colon. The subjects were transferred to the postoperative recovery unit and discharged after 1–2 hours of observation. Subsequently, they were advised to continue omeprazole for 2 additional days and use loperamide at a dose of 1–2 mg every 6 hours for 24 hours after FMT.

Post-FMT Follow-up

Adverse events and clinical response were assessed at 1 week, 1 month, and 6 months following FMT. Adverse events were recorded using the National Institutes of Health common terminology criteria as mild, moderate, severe, and life threatening. Each event was then evaluated for being related, possibly related, or unrelated to the study procedure. All subjects documented adverse events in a diary for 1 week following transplantation, and all adverse events were recorded until 6 months after fecal transplantation. Disease activity was assessed by PCDAI or PUCAI depending on the underlying diagnosis. All subjects underwent routine blood counts, erythrocyte sedimentation rate, and C-reactive protein testing at 1 and 6 months. Fecal biomarkers were determined locally at the discretion of treating gastroenterologists at 1 and 6 months after FMT. Stool was evaluated for infection only in subjects having diarrhea or bloody stools at follow-up. Subjects from out-of-region were allowed to follow up with their local gastroenterologists.

Response criteria

The clinical response was assessed at 1 month and 6 months after FMT. Response was defined as a decrease of 15 points in PUCAI or 12.5 points in PCDAI at 1 month, as used in previous studies[30–37] Remission was defined as normalization of previously elevated fecal biomarkers and a PCDAI/PUCAI of 0 points. If subjects required escalation of medical therapy prior to 1-month evaluation, they were considered to be nonresponders. Subsequently, any escalation of medical therapy was considered a loss of response.

Clinical Data and Statistical Analysis

Descriptive statistics are reported as means and standard deviation or median with interquartile range for continuous variables and frequency with percentage for categorical variables. Differences in outcome between responders and nonresponders at 30 days were assessed using the independent *t* test or Wilcoxon rank-sum test for continuous variables and the chi-square or Fisher exact test for categorical variables, as appropriate based on cell size. Subgroup statistical analysis within disease types of CD and IC/UC between responders and nonresponders was not feasible because of small sample sizes (n = 7 and n = 14, respectively).

All statistical tests were 2-sided and conducted at the alpha = 0.05 level. Statistical analysis was done using SAS software v9.4 (SAS Institute Inc., Cary, NC).

Microbiome Analysis

Stool samples were collected for microbiome analysis from subjects and donors prior to the transplant procedure and from subjects at 1 week, 1 month, and 6 months after FMT.

Bacterial DNA Extraction and Sequencing

Microbial DNA was extracted from stool samples using the PowerSoil DNA Isolation kit (MO BIO Laboratories, Carlsbad, CA). Bacterial 16S rRNA gene sequences were amplified and sequenced on the Illumina MiSeq platform. 16S amplicons were produced using fusion primers adapted for the Illumina MiSeq that target the V4 region (515F and 806R primers). Samples were sequenced with blank extraction and no-template-added PCR controls at the University of Illinois Roy J. Carver Biotechnology Center, Urbana, Illinois.

Sequence Processing and Analysis

Sequence data were analyzed using Quantitative Insights Into Microbial Ecology (QIIME) with default parameters and normalized numbers of sequencing reads. Samples were rarefied to 1500 sequences. Rarefaction curves of all samples show that at 1500 reads the curves are in the linear segment of the curve (Supplemental Fig. I). Rarefaction to 15,000 sequences showed no significant differences compared with 1500 sequences (Supplemental Figs. II, III, IV). Alpha diversity (observed operational taxonomic unit [OTU] metric) and beta diversity were calculated using QIIME.[31] Significant differences were assessed using analysis of variance (ANOVA) and post hoc Tukey HSD test where appropriate to account for multiple hypothesis testing. Variations in beta diversity were assessed with the PERMANOVA and PERMDISP algorithms in QIIME. Linear discriminant analysis effect size (LEfSe) was used to determine differentially abundant taxa across groups of samples.[33] Only taxa with an average relative abundance >1% in at least 1 group of samples were considered for this analysis. A P value of 0.05 was used to determine significance in all statistical tests.

Accession Numbers

All 16S rRNA gene sequences have been deposited at the National Center for Biotechnology Information under the BioProject ID PRJNA380944.

RESULTS

Study Enrollment and Patient Characteristics

Of the 34 subjects who were screened, 23 were found eligible and underwent study procedures, and results from 21 subjects with adequate follow-up are reported here. Reasons for screen failure included *Clostridium difficile* infection, small bowel stricture, severe disease, withdrawal of consent, or low PCDAI <10 in 4, 2, 2, 2, and 1 subject(s), respectively.

Individual patient and disease characteristics are summarized in Supplemental Table 1.[33] The median age at the time of FMT was 12 years (range, 8–21 years). Twelve patients were males, and 9 were females. Their diagnoses were CD, UC, and IC in 7 (34%), 12 (57%), and 2 (9%) patients, respectively. Median time elapsed since diagnosis was 3 years (range, 0.6–10 years).

At the time of FMT, the PCDAI disease severity was mild in 5 patients, moderate in 1 patient, and inactive in 1 patient who had moderately elevated calprotectin levels. In patients with UC/IC, the PUCAI disease severity was mild in 6 and moderate in 8 subjects. The maximal histological severity was mild, moderate, or severe in 19%, 62%, and 19% of the subjects, respectively. One subject with UC had normal biopsies despite having a PUCAI of 45, anemia, and elevated sedimentation rate at the time of screening and prior to FMT. The clinical disease activity index correlated well with histological severity in 3 out of 7 subjects with CD and 4 out of 14 subjects with UC/IC. Five out of the 7 CD subjects had pan-colitis and ileitis, 1 had ileitis and right colonic inflammation, and 1 had mildly active disease limited to the terminal ileum. Eight of the 14 subjects with UC/IC had pan-colitis, while 4 had left-sided colitis, 1 had inactive pan-colitis, and 1 subject had normal biopsies. Seven patients had gastritis, and 4 subjects also had terminal ileum inflammation. The endoscopic severity was also not concordant with clinical disease activity or histological scores consistently, possibly because of patchy disease, obtaining limited biopsies, and limited visualization secondary to conservative use of normal saline flushes during colonoscopy to avoid fluid accumulation in the colon that could result in post-FMT diarrhea and loss of implanted stool.

Seventeen subjects were on mesalamine at the time of FMT. Eighteen subjects had been exposed to steroids since their initial diagnosis, while 3 were steroid dependent and on a stable dose of steroid prior to transplant. Of the 18 subjects who were previously on immunomodulators, 12 were still receiving those at the time of FMT. Twelve subjects were on treatment with anti–tumor necrosis factor (anti-TNF) antibodies, 4 had previously failed infliximab, of whom 1 was also not responding to vedolizumab (for at least 6 months) at the time of FMT. One subject with CD was on a drug holiday after failing steroids, mesalamine, mercaptopurine, and infliximab (Supplemental Table 1).

Clinical Response to FMT

Patient outcomes are summarized in Tables 1 and 2. At the 1-month assessment, 5 of 7 with CD (71%) and 7 out of 14 patients with UC/IC (50%) were judged to be responders. At the 6-month assessment, 3 of 7 patients (43%) with CD and 3 of 14 patients (21.4%) with UC/IC had maintained their clinical response (Table 1). While none of the UC or IC patients achieved clinical remission, 2 patients with CD were in clinical remission at 6 months (Supplemental Table 1). There was no

TABLE 1: Disease Activity and Medication Profile Comparison Between Responders and Nonresponders

		UC / IC			CD		
		Non-responders	1-Mo Responders	6-Mo Responders	Nonresponders	1-Mo Responders	6-Mo Responders
No. patients		7	4	3	2	2	3
PUCAI or PCDAI	Baseline	39.2	30	40	17.5	28.75	11.6
	1 mo	37.8	8.75	11.6	17.5	13.75	1.6
	6 mo	25.8	28.7	21.6	15	2.5	0
Median age, y		14	10		10	11	
No. patients on drug at FMT, %	Steroids	3 (42)	3 (42)		1 (50)	2 (40)	
	Immuno-modulators	4 (57)	4 (57)		2 (100)	0 (0)	
	Mesalamine	4 (57)	5 (71)		2 (100)	2 (40)	
	Biologic agents	4 (57)	3 (42)		1 (50)	3 (60)	

Nonresponder: no response at 1-month assessment; 1-month responder: response lasted >1 month but less than 6 months; 6-mo responder: response >6 months.
CD, Crohn's disease; FMT, fecal microbiota transplant; IC, Indeterminate colitis; PCDAI, Pediatric Crohn's Disease Activity Index; PUCAI, Pediatric Ulcerative Colitis Activity Index; UC, ulcerative colitis.

TABLE 2: Biomarkers and Disease Activity Index in Long-term Responders

	Diagnosis			
Patient ID	UC	Pre-FMT	1 Mo Post-FMT	6 Mo Post-FMT
28	Calprotectin	1366	NA	NA
	PUCAI	30	15	10
23	Calprotectin	1670	NA	336
	PUCAI	45	5	30
36	Calprotectin	177	171	144
	PUCAI	45	15	25
	CD			
11	Calprotectin	783	29	31
	PCDAI	0	0	0
42	Calprotectin	293	NA	147
	PCDAI	10	0	0
34	Lactoferrin	52.5	<30	NA
	PCDAI	25	5	0

Calprotectin normal range is 0 to 162 μg/g, lactoferrin normal range is <30 μg/mL.
CD, Crohn's disease; FMT, fecal microbiota transplant; NA, not available; PCDAI, Pediatric Crohn's Disease Activity Index; PUCAI, Pediatric Ulcerative Colitis Activity Index; UC, ulcerative colitis.

significant difference in age, disease duration, location, severity, or pretransplant medications between responders and nonresponders (Supplemental Table 2). All 3 of the steroid-dependent subjects were initially able to wean from steroids, but 1 relapsed 5 weeks later while 2 subjects relapsed 5 months after FMT. The fecal calprotectin and lactoferrin values either dropped significantly or normalized at 1 and 6 months in the long-term responders (Table 2). The subject with a normal-appearing colon had improvement in her symptoms, along with normalization of her hemoglobin and sedimentation rate at 1- and 6-month follow-up. It is possible that she had patchy histological disease that was missed on biopsies.

Adverse Events

Follow-up data were available on all 21 patients. There were no serious adverse events directly due to FMT, and all related occurrences were managed with outpatient supportive care. Reported adverse events related to FMT were seen in 12 patients (57%). These included mild to moderate abdominal pain (11, 52%), diarrhea (5, 24%), flatulence and bloating (5,

24%), emesis (3, 14%), bloody stools (2, 10%), nausea (2, 10%), and fever (1, 5%). None of the patients had any infectious complication due to biopsies obtained during colonoscopy for FMT. One patient was admitted within 72 hours of the procedure due to a flare. The patient's gastroenterologist attributed it to an abrupt steroid withdrawal just prior to FMT without medical advice due to negligence. This subject had a prompt response to intravenous steroids. One subject who was unresponsive to FMT and had been refractory to steroids, antibiotics, immunomodulators, and infliximab prior to FMT additionally failed a subsequent trial of tacrolimus also and required colectomy 5 months later. Another similarly refractory patient who had also failed vedolizumab was being evaluated for colectomy at 6-month follow-up. Two patients were found to have *Clostridium difficile* at 5 weeks and 6 months after FMT.

Microbiome Analysis

A total of 102 fecal samples were collected, 24 samples from CD patients, 55 samples from UC patients, and 23 samples from donors. The mean number of 16S sequences per sample was 39,158, with a sequence read length of 250 bp. After quality filtering, there were 46,880 unique OTUs (with a mean of 1550 OTUs per sample) included in the analysis. For each FMT study subject, we analyzed microbial diversity within fecal samples collected prior to FMT and 1 week, 1 month, and 6 months post-FMT. A pretransplant fecal sample from each donor was also analyzed.

Microbial diversity and composition in donors were similar despite age differences

Donors were selected from healthy first-degree relatives and close family friends. The ages of the donors ranged from 8 to 60 years. Donor ages broadly fell into 2 cohorts: younger than age 20 years (n = 9) and older than age 30 years (n = 12). We found no significant differences in alpha diversity (Supplemental Fig. V), beta diversity (Supplemental Fig. VI), or phylogenetic distance to their respective pre-FMT recipients (Supplemental Fig. VII). Furthermore, clinical response to FMT was not correlated to donor age (*t* test, $P = 0.96$).

Microbial Diversity Differs in Healthy Control and Pre-FMT Samples From Refractory IBD Patients

We found that alpha diversity within pre-FMT IBD samples was markedly reduced relative to donor samples, as observed by the OTU metric (Student *t* test, $P << 0.00001$) (Fig. 1A). To examine beta diversity, we constructed principal coordinate analysis (PCoA) plots of taxonomic distances between samples in each group (Jaccard index in Fig. 1B, weighted UniFrac in Supplemental Fig. VIII). As shown, most donor samples clustered closely together in PCoA space. Although some IBD pre-FMT samples overlapped with donor samples, most were more widely dispersed in PCoA space (PERMANOVA, $P = 0.001$).

Using LEfSE, we found that donor samples were sharply enriched with sequences from the families *Lachnospiraceae* ($P = 0.02$), *Rikenellaceae* ($P = 0.0004$), *Porphyromonadaceae* ($P = 0.02$), and *Verrucomicrobiaceae* ($P = 0.002$) and from the genera *Blautia* ($P = 0.005$), *Coprococcus* ($P = 0.001$), *Ruminococcus* ($P = 0.0001$), *Akkermansia* ($P = 0.0002$), *Parabacteroides* ($P = 0.04$), and the *Clostridiales* order ($P = 0.0002$) (families in Fig. 1C, genera in Supplemental Fig. IX). In contrast, pre-FMT IBD fecal samples were enriched with sequences from the *Enterobacteriaceae* ($P = 0.001$) and *Enterococcaceae* families ($P = 0.04$) and the genera *Haemophilus* ($P << 0.00001$). Interestingly, these observed taxonomic features of pre-FMT IBD samples, namely the abundance of *Enterobacteriaceae* and depletion of *Lachnospiraceae*, are very similar to findings in other recent studies of IBD patients and controls.[28]

Post-FMT Samples Adopt Donor-Like Configuration

Phylum-level taxonomic profiles of all samples are shown in Fig. 2 (genus level taxonomic profiles in Supplemental Fig. X). As shown, post-FMT samples of several study subjects rapidly adopted a donor-like configuration. Importantly, as shown in Fig. 3A, alpha diversity of fecal samples at 1 week and 1 month after FMT was significantly increased compared with the pre-FMT IBD samples, approaching the level of the donor samples (ANOVA, $P << 0.00001$). However, alpha diversity within 6-month samples was closer to pre-FMT alpha diversity, suggesting that initial post-FMT responses are not permanent.

This dynamic was also observed in the analysis of beta diversity (Fig. 3b). We calculated phylogenetic distances (Jaccard index) between donor and recipient samples. As shown, the distance between donor and pre-FMT IBD samples was substantially higher than the distances between the donor 1-week and donor 1-month pairs (ANOVA, $P = 0.001$). This demonstrates that the study subjects rapidly adopted a donor-like configuration after FMT. At 6 months, the distance between subject and donor samples was highly variable, but generally matched the distances seen prior to FMT. This indicates that the effect of FMT on beta diversity in the gut lasted less than 6 months for many study subjects.

The abundances of the taxa *Enterobacteriaceae* and *Lachnospiraceae* were also seen to be dynamic and inversely related (Fig. 4). The relative abundances of each of these families changed rapidly after FMT, but drifted toward baseline at 6 months post-FMT. Fecal samples from patients with CD have been previously reported to contain a high relative abundance of *Fusobacterium*.[34, 35] Interestingly, although most samples did not have a high relative abundance of *Fusobacterium*, the only 3 samples in our data set with *Fusobacterium* at a relative

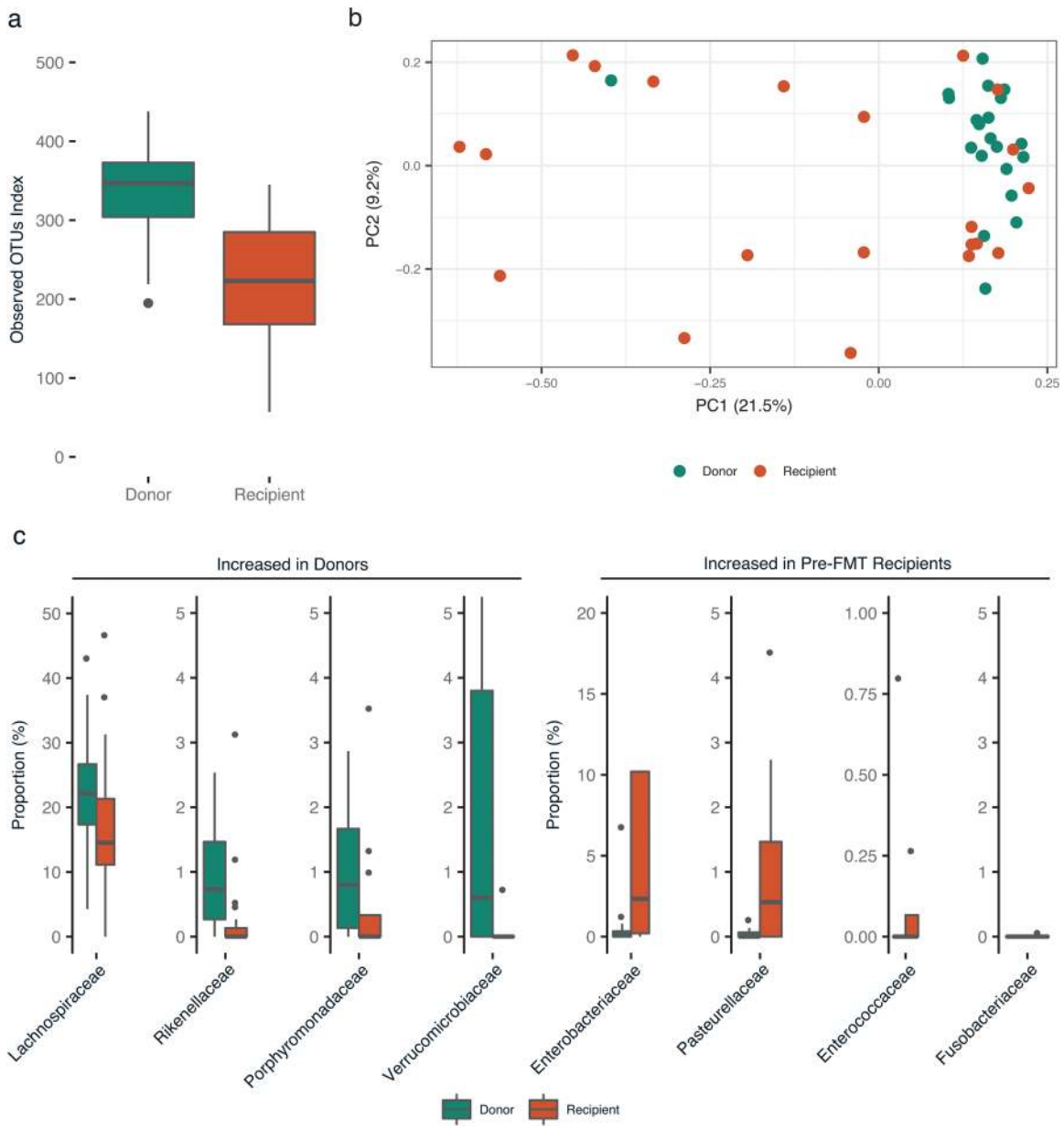

FIGURE 1. A, Alpha diversity comparisons of microbial communities between pre-FMT recipients and donors. Shown is a boxplot of observed OTUs for samples from pre-FMT recipients (red) and donors (green). There is a significant reduction in alpha diversity in pre-FMT recipients compared with donors (Student *t* test, $P << 0.00001$). B, Beta diversity comparisons of microbial communities between pre-FMT recipients and donors. Shown is the principal coordinate analysis of abundance Jaccard distances between pre-FMT recipients (red) and donors (green). Axis labels indicate the proportion of variance explained by each principal coordinate. Donor samples cluster significantly closer together than pre-FMT samples (PERMANOVA, $P = 0.001$). C, Sample abundance comparisons of bacterial families between pre-FMT recipients and donors. Shown are boxplots of bacterial families identified on LEfSe to be statistically significant between donors and pre-FMT recipients ($P < 0.05$).

abundance of >5% were pre-FMT IBD samples in 2 patients with UC and 1 patient with CD.

Responders and Nonresponders

We assessed pre-FMT differences in alpha and beta diversity for the 12 responders and 9 nonresponders (using the 1-month time point to define clinical response). While alpha diversity was lower in responders than nonresponders, this difference did not reach significance (Fig. 5A). Similarly, we compared the phylogenetic distance of pre-FMT recipient samples from responders and nonresponders with their respective donor samples. We again found an increased pre-FMT phylogenetic distance in responders relative to the nonresponders that did not reach significance (Fig. 5B). LEfSe analysis of pre-FMT

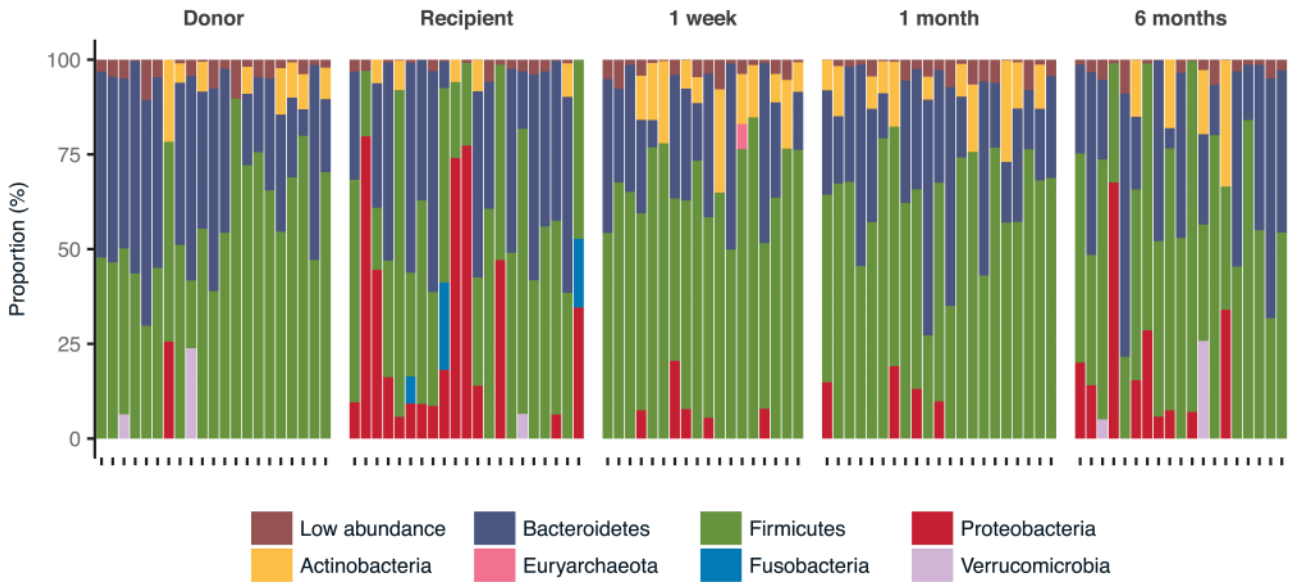

FIGURE 2. Phylum-level taxon summary of stool samples collected from the donors and recipients (pre-FMT and 1 week, 1 month, and 6 months post-FMT).

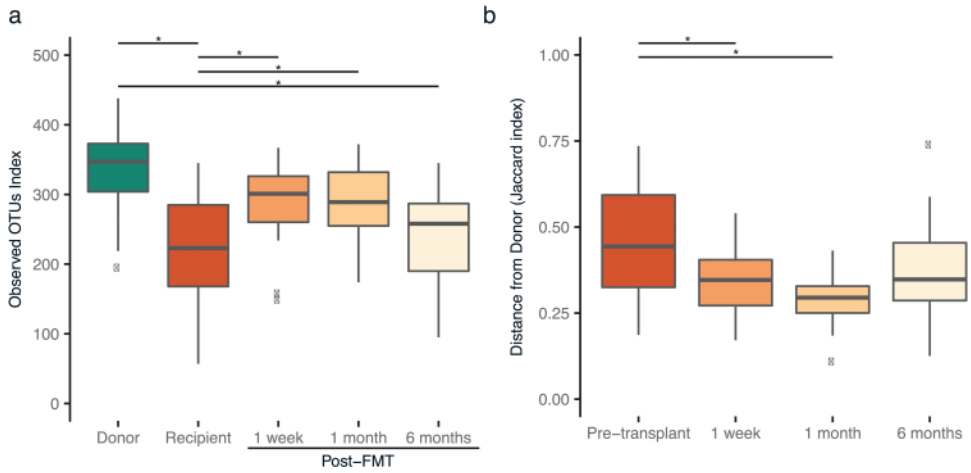

FIGURE 3. A, Alpha diversity comparisons of microbial communities of donors and recipients pre- and post-FMT. Shown is a boxplot of observed OTUs for donors and recipients' pre-FMT and 1 week, 1 month, and 6 months post-FMT. There is a significant increase in alpha diversity at 1 month post-FMT compared with pre-FMT, but this effect disappears at 6 months (ANOVA, $P << 0.00001$). B, Beta diversity distance comparisons of recipient samples with their respective donor samples. Shown is a boxplot of abundance Jaccard distance indices of recipient samples pre-FMT and 1 week, 1 month, and 6 months post-FMT compared with their respective donors. There is a significant decrease in distance to the donor samples at 1 week and 1 month post-FMT compared with pre-FMT (ANOVA, $P = 0.001$).

samples demonstrated that the responders contained a higher abundance of *Fusobacterium* (0.5% vs 0.0%, $P = 0.03$), and the abundance of *Enterobacteriaceae* was higher in responders, but this did not reach significance (19.7% vs 12.8%, $P = 0.34$). There were no significant differences in species diversity or composition in the donors of responders to those of nonresponders.

Interestingly, after FMT, alpha diversity in nonresponders did not significantly increase from their pretransplant sample to the 1-month post-FMT sample. This was in contrast to the responders that showed a significant increase in alpha diversity from the pretransplant sample to the 1-month post-FMT sample (ANOVA $P = 0.001$, post hoc Tukey HSD test, $P = 0.03$) (Fig. 5A). At 6 months, both the responders and nonresponders similarly showed no statistically significant differences in alpha diversity relative to pre-FMT samples.

Significant differences were also seen in beta diversity after FMT between responders and nonresponders. In the responder group, there was a statistically significant decrease in phylogenetic distance between the 1-month post-FMT sample and the corresponding donor sample (ANOVA, $P = 0.01$; post

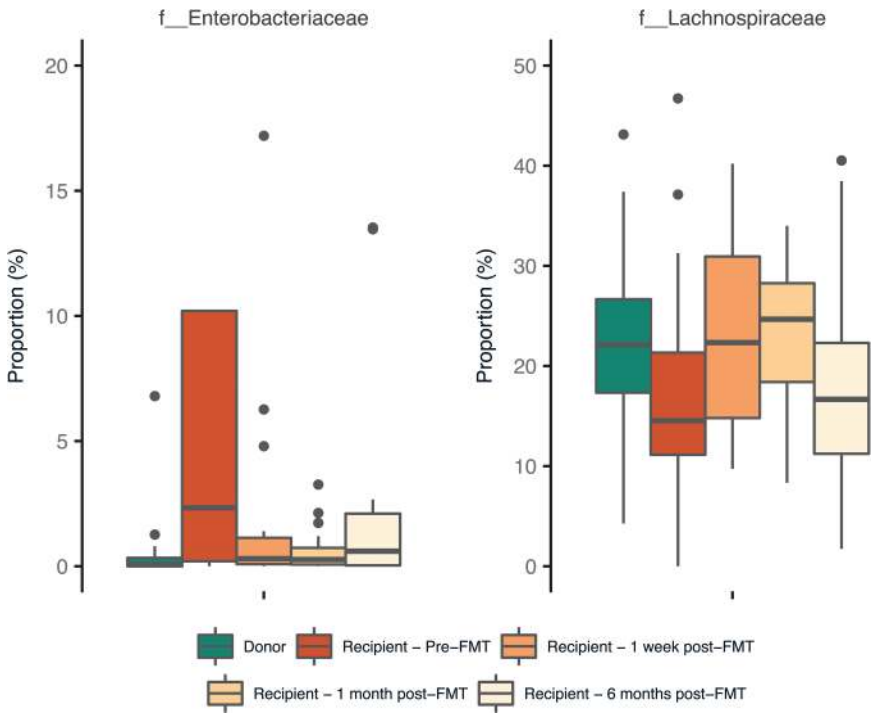

FIGURE 4. Boxplot showing the relative abundance of the bacterial families *Enterobacteriaceae* and *Lachnospiraceae* between donor and recipient samples. *Enterobacteriaceae* is elevated in pre-FMT recipient samples but decreases significantly after FMT ($P = 0.001$). *Lachnospiraceae* is decreased in pre-FMT recipient samples but increases significantly after FMT ($P = 0.02$).

hoc Tukey HSD test, $P = 0.005$). This was in contrast to the nonresponders who did not show this decrease. This suggests that the microbiome of responders became compositionally more similar to donors at 1 month than the nonresponders.

In a subgroup analysis, no significant differences were observed between short- and long-term responders in alpha diversity or beta diversity. This may have been affected by a small sample size.

DISCUSSION

To our knowledge, this study represents the largest open-label trial of FMT as an adjunct therapy for medically refractory IBD in pediatric patients. In this prospective trial, we used a combined duodenal/jejunal and colonoscopic method for FMT and found that the procedure was safe and well tolerated. A majority of patients (57%) experienced short-term improvement in their disease activity. Patients prior to FMT showed decreased species diversity and significant microbiome compositional differences compared with their donors. Clinical responders to FMT compared with nonresponders developed significantly higher species diversity closer to donor-like microbiome configuration after FMT, but these changes were not sustained at 6-month evaluation.

Other published studies on the use of FMT in UC patients have shown a wide range of response rates, from 0% to 67%.[11, 20, 36, 37] There could be a number of potential reasons to account for such variability in response to FMT. These include cohort size and selection criteria, method of delivery, and frequency of FMT. Studies varied drastically in whether the FMT was given via the upper or lower GI tract, how often and how many doses are given, and the manner in which donors are selected and the time when the efficacy is assessed. In our study, donor fecal microbiota was delivered into both the upper and lower GI tract. Studies involving UC patients where FMT was delivered by multiple enemas demonstrated significant efficacy.[11, 13, 37] However, the results of FMT in UC were disappointing when fecal suspension was delivered only by a naso-gastric or duodenal tube in an open-label study or a placebo-controlled trial.[12, 26] In our study, where a single FMT was delivered by upper and lower routes, the response rate for UC were mostly short lived, lasting from 1 to 5 months. In CD, there is a relative paucity of published cohort studies on the use of FMT, but response rates of 86.7% and 66.7% (at 1 and 6 months, respectively), 57.9% (11 of 19 patients), and 77.8% (7 of 9 patients) have been reported.[21, 26, 38] Our study similarly showed a 71% response rate for CD at 1 month, which dwindled to 42% at 6 months, though in a small number of patients. There were no serious adverse events directly related to FMT. One patient, however, had a flare following sudden steroid withdrawal 2 days prior to FMT. We did not experience any infection despite obtaining biopsies during colonoscopy for FMT. There have been no major safety concerns due to FMT in meta-analyses

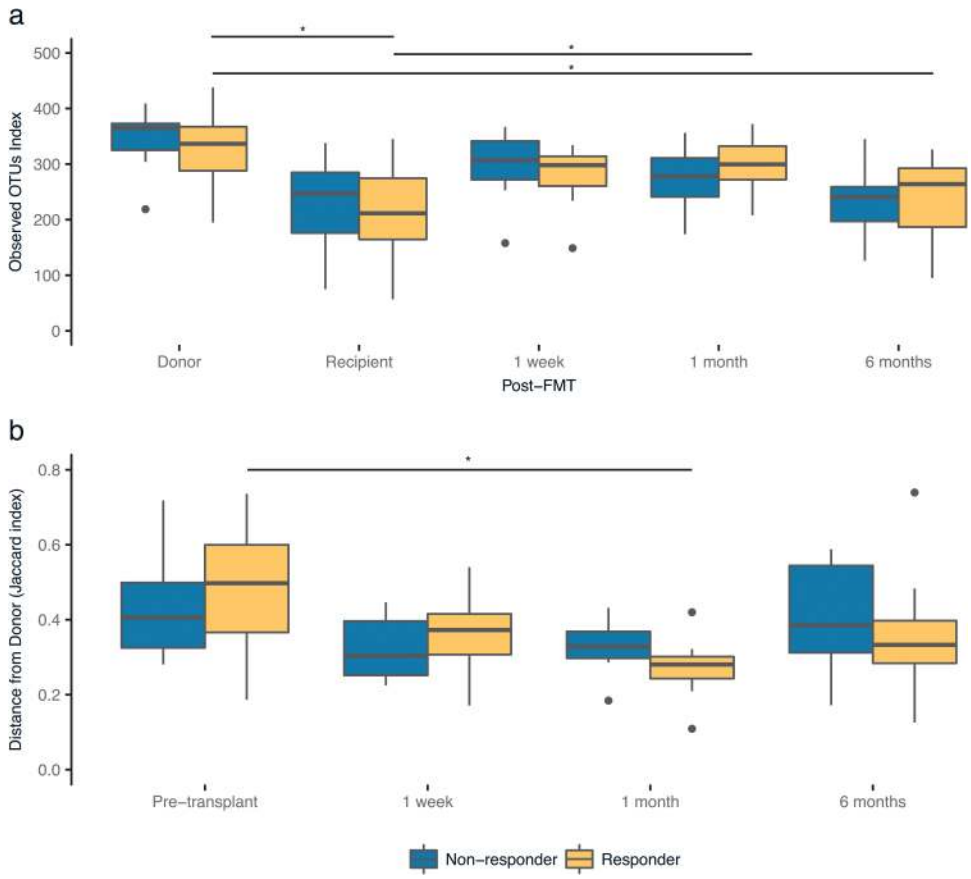

FIGURE 5. A, Alpha diversity comparisons of microbial communities of responders and nonresponders. Shown is a boxplot of observed OTUs for donors and recipients' pre-FMT and 1 week, 1 month, and 6 months post-FMT, divided into responders and nonresponders. In the responder group, there was a significant increase in alpha diversity at 1 month compared with pre-FMT (ANOVA, $P = 0.001$). This was in contrast to the nonresponder group, which showed no significance. B, Beta diversity distance comparisons of responder and nonresponder recipient samples with their respective donor samples. Shown is a boxplot of abundance Jaccard distance indices of recipient samples pre-FMT and 1 week, 1 month, and 6 months post-FMT compared with their respective donors, divided into responders and nonresponders. In the responder group, there was a decrease in distance to donor samples at 1 month post-FMT (ANOVA, $P = 0.01$). This was in contrast to the nonresponder group, which showed no significant differences.

involving multiple studies on IBD patients, though there have been case reports of serious events including aspiration, infection, and IBD flare.[5, 8, 38]

Our data showed that the clinical and microbiome responses to a single FMT are short lived. This may suggest a potential advantage in using a protocol with multiple serial transplantations. Due to a small cohort size and discrepant endoscopic findings, we were unable to highlight significant differences among UC or CD patients.

In analyzing the microbiome, we found that pre-FMT fecal samples from CD and UC patients harbored bacterial communities very different from healthy controls. Importantly, these communities were similar to those seen in published IBD data sets.[28, 39] Reduced alpha diversity and a preponderance of *Enterobacteriaceae* from the phylum Proteobacteria were the most commonly observed and most striking abnormalities prior to FMT. A corollary finding is that fecal samples from IBD patients in our study and others are generally depleted of anaerobic taxa such as from the family *Lachnospiraceae* that could be associated with gut inflammation.[20, 40, 41]

We also observed that that pre-FMT samples differed between responders and nonresponders. Specifically, pre-FMT samples from responders were characterized by significantly increased abundance of *Fusobacterium*. This contrasts with the results of 2 other studies, which detected an association between *Fusobacterium* abundance and lack of response to FMT or medical therapy.[13, 42] Further study will be required to discern whether the abundance of *Fusobacterium*, an organism linked to numerous adverse health outcomes, has prognostic value in the setting of FMT for IBD.[43]

Other differences observed here between responders and nonresponders, for example, decreased alpha diversity and a higher abundance of *Enterobacteriaceae*, may be important but did not reach statistical significance in this study. After FMT, dysbiosis was frequently mitigated for a significant period of time, and the greatest mitigations were seen in responders.

Thus, it could be argued that responders suffered from a more severe and correctable form of dysbiosis than nonresponders. We did not identify features of the donor microbiota that were associated with clinical response after FMT, though some studies have attributed successful response to FMT to donor characteristics.[11]

Our trial used a uniform approach to FMT, patients were followed carefully for an extended period of time, and detailed correlative microbiome analyses were performed. However, this study also has a number of limitations. Given the limited sample size and relative heterogeneity in terms of patient diagnosis, we did not have the statistical power to test clinical variables with responses. Enrollment was based on clinical disease activity; it did not always correlate well with mucosal disease severity, though patchy histological disease could have been missed. Finally, the end point for response did not include an endoscopic evaluation for mucosal healing, and noninvasive biomarkers like calprotectin and lactoferrin were also not performed for all patients.

Overall, the study recapitulates some findings from other groups regarding features of dysbiosis in patients with poorly controlled IBD and a higher short-term response rate after FMT. Additionally, we identified discrete differences in pre-FMT samples between responders and nonresponders. Future studies could integrate these findings in at least 2 specific ways. First, it is conceivable that a future iteration of personalized medicine for IBD patients will include an assessment of whether a patient is likely or unlikely to respond to FMT. Second, for those likely to respond, it is possible that multiple FMT procedures may generate superior clinical outcomes compared with a single procedure. Well-powered future randomized trials should make it possible to discern whether these findings hold true.

In an age of reductionist science and targeted therapeutic interventions, fecal microbiota transplantation seems oddly unsophisticated. Nevertheless, the efficacy of FMT in CDI is undeniable, and the rationale for pursuing FMT in IBD patients is sound. However, much work is still required to prove whether changes in the microbiome following FMT can be durable or associated with clinical response in IBD patients as in other clinical settings.[24, 25] Likewise, further larger randomized trials are required to define which patients with IBD, if any, will benefit most from 1 or more FMT procedures.

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Author contributions: The study was conceived, planned, and conducted by Alka Goyal. Dr. Andrew Yeh did most of the microbiome analysis. Dr. Siebold helped in follow-up of study patients and completing the study. Adam Kufen was the principal research coordinator. Brian Bush, Brian Firek, and Brian Rodgers played an important role in microbiome analysis under the leadership of Dr. Michael Morowitz. Dr. Alka Goyal and Dr. Andrew Yeh drafted the manuscript with equal contributions. The rest of the authors helped in reviewing the manuscript with helpful suggestions.

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