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Higher alpha diversity and Lactobacillus blooms are associated with better engraftment after fecal microbiota transplant in inflammatory bowel disease

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Fecal Microbiota Transplant (FMT) has shown some success in treating inflammatory bowel diseases (IBD). There is emerging evidence that host engraftment of donor taxa is a tenet of successful FMT. We undertook a double-blind, randomized, placebo-controlled pilot study to characterize the response to FMT in children and young adults with mild to moderate active Crohn's disease (CD) and ulcerative colitis (UC). Subjects with CD or UC were randomized to receive antibiotics and weekly FMT or placebo in addition to baseline medications. We enrolled 15 subjects aged 14–29 years. Four subjects had CD, and 11 had UC. Subjects exhibited a wide range of microbial diversity and donor engraftment. Specifically, engraftment ranged from 26 to 90% at week 2 and 3–92% at 2 months. Consistent with the current literature, increases over time of both alpha diversity ($p < 0.05$) and donor engraftment ($p < 0.05$) correlated with improved clinical response. We discovered that the post-antibiotic but pre-FMT time point was rich in microbial correlates of eventual engraftment. Greater residual alpha diversity after antibiotic treatment was positively correlated with engraftment and subsequent clinical response. Interestingly, a transient rise in the relative abundance of Lactobacillus was also positively correlated with engraftment, a finding that we recapitulated with our analysis of another FMT trial.

Abbreviations

CD	Crohn disease
FMT	Fecal microbiota transplant
PUCAI	Pediatric Ulcerative Colitis Activity Index
PCDAI	Pediatric Crohn Disease Activity Index
IBD	Inflammatory Bowel Disease
IBS	Irritable Bowel Syndrome
rCDI	Recurrent *Clostridioides difficile* Infection
UC	Ulcerative colitis

Fecal microbiota transplant (FMT) is the transfer of healthy fecal microbial communities to a patient with an illness associated with gut microbiome perturbances. The best example to date is the use of FMT to treat *Clostridioides difficile* infection, which has proven to be both safe and highly effective for most patients[1–3]. This success has driven an interest in identifying other diseases in which FMT may be beneficial. Perturbations of the microbiome may contribute to the pathogenesis of IBD, and this makes it a promising target for FMT[4].

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Data from several studies, including recent meta-analyses, have demonstrated the efficacy of FMT in some patients with ulcerative colitis (UC)[5–15]. However, the factors that determine responsiveness to FMT are poorly understood. Based on the preliminary data, researchers have proposed several theories. Among the proposed factors are the patient characteristics, patient and donor microbial compositions and diversity, degree of donor engraftment, and timing and duration of engraftment[7,12,16–20]. Deeper analyses into the determinants of engraftment have shown highly variable microbe-specific, disease-specific, and recipient-specific dynamics[7,12,16,19,21,22]. Recently, meta-analyses employing strain-level tracking have identified important generalizable determinants of engraftment. However, more research is needed to further delineate a "rule book" of engraftment in FMT for IBD[18,19,21,23].

Given that the mechanisms and predictors of FMT success remain unknown, there are no standardized pre-conditioning, treatment, or delivery regimens for IBD. Previous studies have employed various pre-conditioning regimens, including no bowel prep, bowel prep with laxatives, dietary changes, proton pump inhibitor use, and both narrow and broad-spectrum antibiotics[16,24,25]. There is some suggestion that antibiotic pre-treatment is favorable for both engraftment and clinical response, though the evidence relies on challenging cross-study comparisons[13,24,26]. Furthermore, other studies have reached conflicting conclusions. Our group recently demonstrated that antibiotic pre-conditioning decreased engraftment after FMT, though in patients with irritable bowel syndrome (IBS) and not IBD[27]. The literature also provides conflicting answers for even simple questions, such as the effect of recipient alpha diversity on donor engraftment.

In this small FMT feasibility study, we present a weekly microbiome time series in adolescents and young adults with IBD. Longitudinal sampling allowed us to analyze changes in alpha diversity associated with donor engraftment and identify critical time points in this FMT protocol. More specifically, we demonstrated that increased diversity and abundance of specific taxa (Lactobacillaceae) post-conditioning and pre-FMT was correlated with increased engraftment and clinical response[12,28–34].

Results

Study enrollment/subject characteristics

We selected individuals with mild to moderate colonic UC or CD without stricturing or penetrating disease. Four subjects with CD and 11 with UC were consented and randomized to a treatment arm (Fig. 1A). The median age at enrollment for those with CD was 20 years (range 18–23), and 24 years (range 14–29) for those with UC (Table 1). All subjects were white, and none were Hispanic or Latino. At baseline, all subjects had mild to moderate disease activity index scores. All 4 CD subjects had mildly active disease (PCDAI of 11–25). With respect to subjects with UC, 3 had mildly active disease (PUCAI scores of 10–24), 3 had mild to moderate disease (PUCAI scores of 25–39), and 3 had moderate disease activity (PUCAI scores of 40–69). All participants had failed one or more standard-of-care therapies; some had failed all FDA-approved therapies for this indication. Despite high levels of interest in this study (200 people prescreened), many individuals were ineligible. Ineligibility was most often due to more severe disease activity, recent dose changes in corticosteroids or biologic therapy, or inability to meet the rigorous study requirements of weekly in-person visits in Boston for 8–16 weeks. The study was

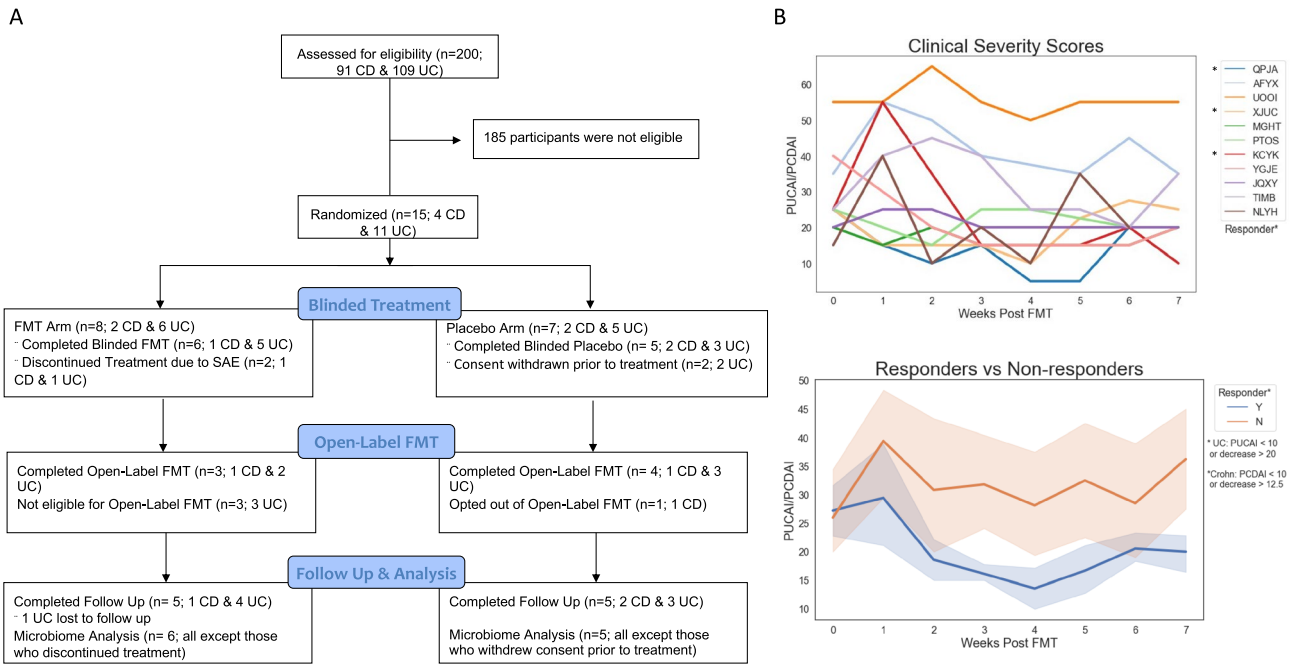

Figure 1. (**A**) Consort diagram. (**B**) Clinical symptom scores (PUCAI for UC and PCDAI for CD) for individual participants (top) and for responders vs non-responders (bottom). Responders were defined as subjects with a drop in disease activity indexes of at least 12.5 (CD) or 20 (UC) or subjects in remission (disease activity indexes of 10 or less at 2–7 weeks after FMT).

	Crohn's disease patients (n = 4)	UC patients (n = 9)
Sex	3 Male	9 Male
Race	4 Caucasian	9 Caucasian
Age at diagnosis (years)	12 (Range: 8–16)	19 (Range: 10–25)
Age at enrollment (years)	20 (Range: 18–23)	24 (Range: 14–29)
Disease index		
Mild	4	3
Mild/moderate	0	3
Moderate	0	3
Concomitant medications*		
Amino-salicylates	1	4
Immunomodulators (6MP, MTX)	0	2
Anti-TNF biologics	3	4
Other biologics	2	0
Corticosteroids	1	5
Antibiotics	1	2
Baseline fecal calprotectin (μg/g)**	1440 (Range: 1430–1450)	2190 (Range: 5–5730)
Normal: < 50	N = 2	
Baseline CRP (mg/L)**	2.45 (Range: 0.3–5.2)	1.9 (Range: 0.3–14.7)
Normal: 0–4.9 mg/L		
Baseline ESR (mm/h)**	14.5 (Range: 2–41)	12 (Range: 2–79)
Normal: 0–32 mm/h		
Prior IBD-related hospitalization		
Yes	2	6
No	0	1
Unknown	2	2
Arm assigned		
Treatment	2	6
Placebo	2	3

Table 1. Demographic and pertinent medical history at baseline. Information is divided based on disease type and includes demographics, age at disease onset, extent of disease, medications for disease management throughout the trial, inflammatory markers from blood and stool samples and trial arm assigned. *Total > n because patients may be on multiple medications. **Median values shown.

closed due to difficulty finding eligible subjects and subjects were unable to commit to the intensive study visit and follow up requirements.

Eleven (3 CD and 8 UC) subjects completed the blinded phase of the study (Fig. 1A). Three (3 UC) subjects were ineligible to continue into the open-label phase because they did not respond to FMT treatment in the blinded phase of the study (Fig. 1A) and 1 CD subject did not want to continue into the open-label portion of the study. Seven (2 CD and 5 UC) subjects completed the open-label phase of the study, and 6 (2 CD and 4 UC) subjects completed long-term follow-up (Fig. 1A).

Clinical response

Overall, three subjects reported a decrease in IBD-related symptoms, 1 CD and 2 UC, and were classified as responders. One CD and 1 UC participant were randomized to the FMT arm, and 1 UC participant was randomized to placebo in the blinded portion of the study. After a period of non-response to the placebo arm, the subject was given open-label FMT treatment, which they responded to. Two of the responders received FMT from one donor, and one responder received FMT from the other donor.

Subjects with CD experienced an average decline in PCDAI score of 5, and subjects with UC had an average decline in PUCAI of 7.5 while receiving the experimental arm or during open-label therapy (Fig. 1B). PUCAI and PCDAI scores range from 0 to 85 (PUCAI) or 100 (PCDAI). A score of 0 indicates clinical remission and increasing scores indicates more severe disease. Although not statistically significant, most subject reported a subjective decrease in disease symptoms and increased general well-being during FMT treatment. The FMT was safe and well-tolerated in a majority of participants. There were two serious adverse events (SAE): one individual with UC had an episode of Grade 3 colitis that was determined to be possibly related to antibiotic treatment (and not FMT). Another individual with UC experienced a hypersensitivity reaction directly after the induction enema that was deemed probably related to FMT. Both subjects were withdrawn from the study. Other reported adverse events were determined to be unrelated to the study intervention (Supplemental Text).

Host microbiome response

On average, alpha diversity decreased after a week of antibiotic therapy and increased throughout the course of FMT. There were large inter-individual differences in the alpha diversity post-FMT, with Shannon Indices ranging from less than 1 to greater than 5 (Fig. 2A). We generated a weekly time series of gut microbial changes using serial stool samples for sequencing. The microbial differences between responders and non-responders diverged early. Starting at 2 weeks post-FMT, we observed higher alpha diversity (Shannon Index) in the clinical responders compared to the non-responders (Mann–Whitney with Bonferroni corrections, p-value < 0.05) (Fig. 2A). Clinical responders also had higher baseline, pre-antibiotic, alpha diversity (Fig. 2A). We observed similar trends using Pielou, Evenness, and Faith's Phylogenetic Diversity as alpha diversity metrics (Supplemental Fig. 1). The measured difference in alpha diversity between responders and non-responders was significant even when we used a broader definition of clinical response (Supplemental Fig. 2).

We next explored the relationship between alpha diversity and clinical symptoms within individuals. In the three clinical responders, we were able to measure a statistically significant relationship (Pearson's adjusted p-values all < 0.05) between the Shannon Index and PUCAI or PCDAI, where higher diversity correlated with lower disease activity (Fig. 2B). We did not observe the same relationship in the non-responders (Fig. 2B). Finally, we sought to determine at what point in the treatment course the degree of microbial diversity was most correlated with symptom severity. There was no correlation between alpha diversity and disease activity index pre- or post-antibiotic treatment despite a large range of alpha diversity in the participants (Fig. 2C, small panels, top). There was a trend towards higher alpha diversity at earlier time points correlated with lower symptom scores after FMT, but this was not statistically significant (Fig. 2C, small panels, bottom). On the other hand, starting at 5 weeks post-FMT, the alpha diversity was significantly correlated with PUCAI or PCDAI (Pearson's correlation, adjusted p-value < 0.01 for weeks 5, 6 and 7). Higher alpha diversity similarly demonstrated a strong association with lower disease activity (Fig. 2C, right panel). We also searched for taxa associated with response at weeks 2, 5 and 7 using ANCOM-BC, but none reached statistical significance.

Donor engraftment

We hypothesized that the relationship between alpha diversity and clinical response was driven largely by donor engraftment. There were two healthy FMT donors. Each recipient was randomly assigned to receive FMT from a single donor. The donor microbiomes were easily distinguishable through 16S sequencing and beta-diversity

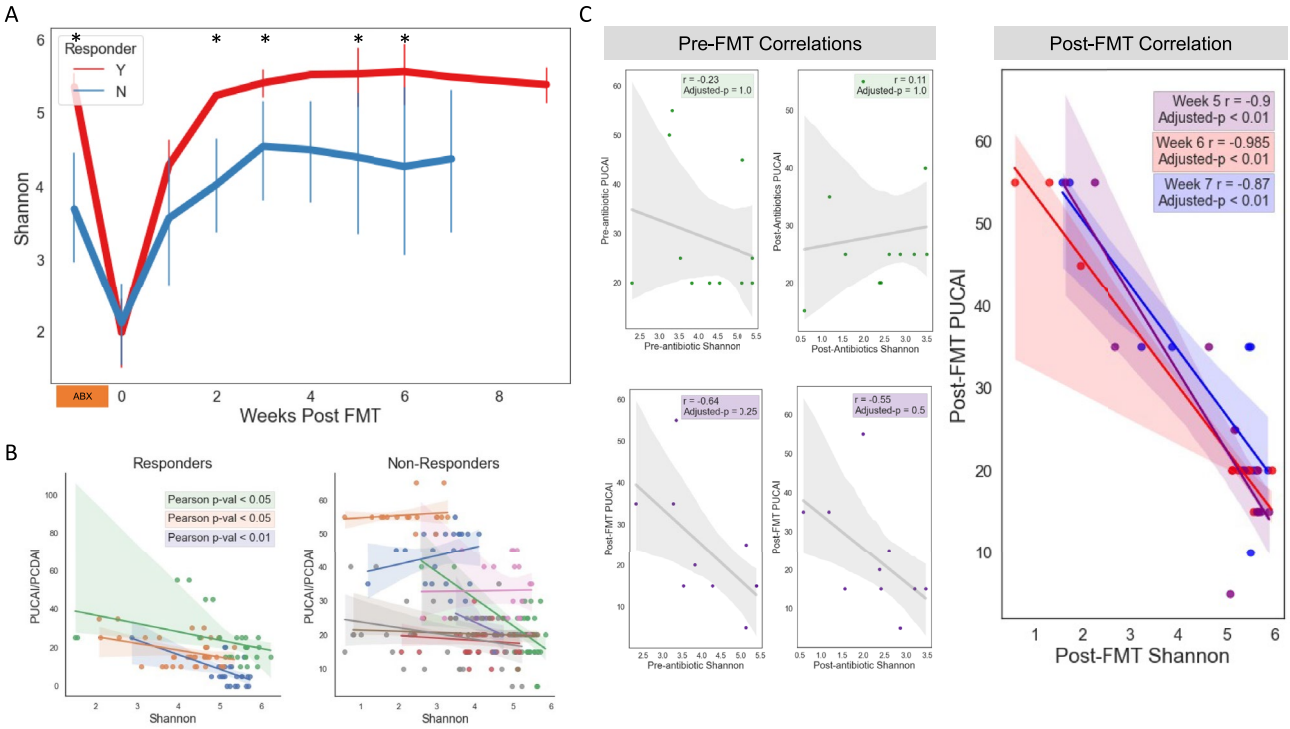

Figure 2. (**A**) Stool alpha diversity (Shannon index) time series for all subjects, clinical non-responders (blue) vs. clinical responders (red), and for individual subjects, each in a unique shade of gray (inset). *Corrected p-value < 0.05, Mann–Whitney with Bonferroni correction. (**B**) Correlation between clinical symptom score (PUCAI for Ulcerative Colitis, PCDAI for Crohn's Disease) and alpha diversity (Shannon index) for clinical responders (top) and non-responders (bottom). (**C**) Correlation between PUCAI and alpha diversity at 5, 6 and 7 weeks post-FMT (large panel). Smaller panels: top panels show a correlation between PUCAI and alpha diversity at the pre-and post-antibiotic time points, and bottom panels show the correlation between the late post-FMT PUCAI and earlier (pre- and post-antibiotic) alpha diversity metrics.

measurements (Supplemental Fig. 3). For engraftment analysis we removed the single participant who did not complete the antibiotic conditioning prior to FMT. We measured donor engraftment using SourceTracker2, a Bayesian algorithm based on Gibbs sampling that ultimately assigned a predicted proportion of a stool microbial community that originated from a set of input sources[28]. For each stool sample post-FMT, we used 16S sequences from the recipient's pre-antibiotic stool sample, the recipient's post-antibiotic stool sample, and the assigned donor's stool as potential sources. (The SourceTracker2 scores for each participant can be found in Supplemental Table 2). Additionally, we added the other donor (the donor not used for FMT in that individual) as a negative control for SourceTracker2. On average, the non-transferred donor was assigned a proportion of 0.016, and there were only 3 of 111 instances where the assigned portion was above 0.05 (Supplemental Fig. 4 and Supplemental Table 2). This confirmed that SourceTracker2 could accurately determine which donor was utilized for FMT and that misassignment of a known non-source was rare.

Starting at 2 weeks post-FMT, responders had higher donor engraftment levels than non-responders (Fig. 3A and Supplemental Table 2). This association was statistically significant at week 2 and week 7 post-FMT (T-test, p-value < 0.05). During weeks 6 through 8, responder microbiome compositions were much closer to their assigned donor than non-responders (Bray–Curtis dissimilarity) (Fig. 3B,C). In 1 case, the pre-FMT microbiome was similar to the donor microbiome in a non-responder. However, after FMT, the participant's microbiome shifted away from the donor's microbial profile. (Fig. 3C, top-right panel). In this instance, perhaps antibiotics played as big a role in the resultant microbiome community as the FMT itself. We defined a high-engraftment state as subjects with greater than 50% engraftment at all timepoints between weeks 2 and 8 after FMT. All three responders were high-engrafters; interestingly, there were also two non-responders who were high-engrafters (Fig. 3A and marked with red boxes in Fig. 3B,C).

Correlates of engraftment

The degree of engraftment correlated with both restoration of gut microbial diversity and clinical response to FMT. At 3–8 weeks post-FMT, there was a wide range of engraftment amongst participants, from less than 0.3–91.8% (Fig. 3A, inset). We searched for clinical, laboratory, and microbial correlates of engraftment by comparing the engraftment at 2 weeks with other features of the participants. These features included symptom

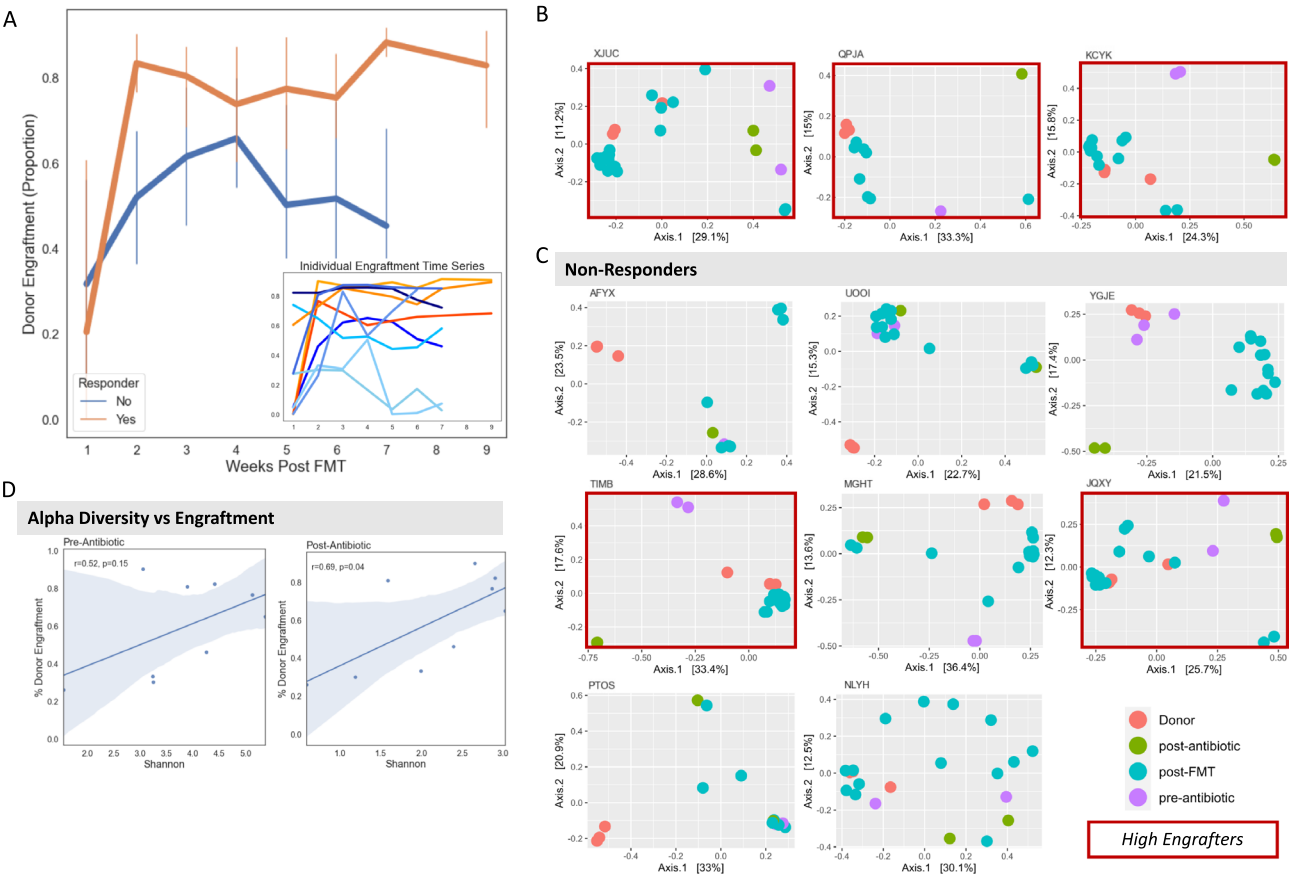

Figure 3. (**A**) Percent Donor Engraftment, as estimated by SourceTracker, in responders vs. non-responders and for each subject, each in a unique shade of orange (responders) or blue (non-responders) (inset) (**B**) Beta diversity plots (Bray–Curtis) for three example responders. (**C**) Beta diversity plots for three example non-responders. High-engrafters are highlighted with red boxes. (**D**) Correlation between alpha diversity (Shannon Index) and engraftment at the pre-antibiotic (Left) and post-antibiotic (Right) time points.

severity scores, inflammatory markers in the stool and blood, as well as the baseline diversity of the gut microbiome before and after antibiotics. The strongest correlate of engraftment at 7 weeks post-FMT was engraftment at 2 weeks post-FMT (Pearson R = 0.69, p-value < 0.05), suggesting that early engraftment—or lack thereof—is predictive of late engraftment (Supplemental Fig. 5). Fecal calprotectin was the only clinical or laboratory measurement significantly correlated with engraftment at 2 weeks. At the post-antibiotic but pre-FMT time point, there was a negative correlation between calprotectin and engraftment (Pearson R = 0.72, p-value < 0.05), suggesting that high levels of gut inflammation may prevent engraftment (Supplemental Fig. 5).

Antibiotic pre-conditioning significantly decreased alpha diversity and changed the ecology of the microbiome (Supplemental Fig. 6A,B). The unanswered question, however, is whether lower diversity (a "cleaner slate") or higher residual diversity after antibiotic conditioning supportive environment for engraftment. In our small study, we found that higher alpha diversity after antibiotic treatment correlated with higher engraftment (Pearson R = 0.69, p-value < 0.05) (Fig. 3D). We found no such correlation between a participant's baseline (pre-antibiotic) alpha diversity and subsequent engraftment.

Members of family *Lactobacillaceae* are associated with high donor engraftment

We next sought to determine if the presence of specific taxa after antibiotic treatment was correlated with engraftment. To that end, we performed an Analysis of Compositions of Microbes (ANCOM) at the genus and family level to compare taxonomic differences at the post-antibiotic time point between subjects with high and low engraftment (Fig. 4A). We found that the genus Lactobacillus (ANCOM W score = 70) and the family Lactobacillaceae (ANCOM W score = 28) were significantly increased in subjects with high engraftment (Fig. 4A and Supplemental Tables 3 and 4). We also found that the genera Pediococcus, Morganella, and Sutterella had at least tenfold higher mean relative abundances in high engrafters. In contrast, low engrafters had tenfold or higher mean relative abundances of the genera Bacteroides and Faecalibacterium. However, none of those differences were significant by ANCOM. At the time point immediately following antibiotic treatment, 4 of the 12 species identified within the family Lactobacillaceae were higher in relative abundance in participants who eventually exhibited high engraftment, including *Lactobacillus zeae* and *Lactobacillus brevis,* as well as unclassified Lactobacillaceae species. (Fig. 4B). We generated individual taxa time series and compared subjects with eventual high versus those with low engraftment. For the genus Lactobacillus, as well as the species *Lactobacillus zeae*, *Lactobacillus brevis,* and an unclassified Lactobacillus species, we observed a spike in the relative abundance of the taxa immediately following antibiotic therapy (Fig. 4C and Supplemental Fig. 7). This spike occurred in all responders (who all had high engraftment) as well those with high engraftment but no clinical response to FMT (Fig. 4C and Supplemental Fig. 8). The spikes occurred prior to FMT, but to ensure no contribution or

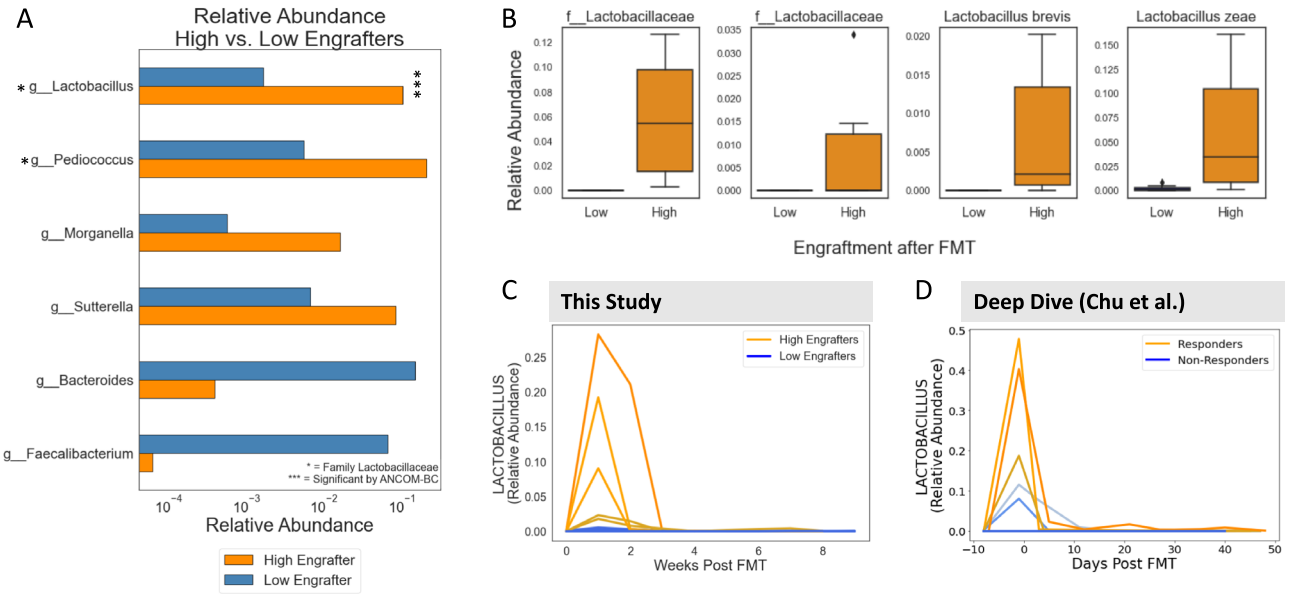

Figure 4. (**A**) Important features (on the genus level) from a random forest classifier using the post-antibiotic microbiome to classify high vs. low engrafters (AUC = 1.00). All taxa are genera, and the lowest identified taxonomic level is labeled. *Asterisks denote members of the Family Lactobacillaceae. (**B**) Boxplot of relative abundance (proportion of total reads) of species in the Family Lactobacillaceae detected in at least three individuals. ***FDR p-value < 0.001; **FDR p-value < 0.01; *FDR p-value < 0.05. (**C**) Time series of relative abundance of genus Lactobacillus (top left) and three species within genus Lactobacillus, comparing high (each participant represented in a unique shade of blue) vs. low (each participant represented in a unique shade of orange) engrafters in this study. (**D**) Time series of relative abundance of the genus Lactobacillus (top) and two species within genus Lactobacillus, comparing responders (each participant represented in a unique shade of blue) vs. non-responders (each participant represented in a unique shade of orange) in an independent (n = 6) FMT study in IBD (Chu et al.).

contamination from the donors, we searched the donor microbiomes for Lactobacillaceae and found none. As the FMT and engraftment progressed, the Lactobacillus taxa decreased in relative abundance. In a separate study of the longitudinal dynamics of the microbiome after FMT therapy, 16S sequencing data from the post-conditioning pre-FMT time point was publicly available[7]. In this study by Chu and colleagues, of six subjectss receiving FMT, three responded clinically, and three did not. Interestingly, we found that the responders had a very similar transient spike in the relative abundance of the Genus Lactobacillus and the 2 Lactobacillus species that were detected in all six subjects in our study (Fig. 4D and Supplemental Fig. 7).

Discussion

The clinical response to FMT in IBD is typically between 20 and 40%, and our study showed similar efficacy[5,8,9,11,13,14]. Three of the 12 participants who completed FMT had a clinical response. In this study, we focused our analysis on what separated eventual success from eventual treatment failure. We found that (1) engraftment of the donor microbiota was higher in the responders vs. the non-responders, (2) higher residual alpha diversity after antibiotic therapy was associated with better engraftment, and (3) a relative rise in the abundance of several Lactobacillaceae taxa after antibiotic therapy correlated with engraftment.

The determinants of engraftment are still being worked out, and several studies have now proposed a few important factors, such as the degree of dysbiosis prior to FMT and the metabolic flexibility of engrafting strains[19,21,23]. Among the many unanswered questions about the rules of engraftment, we focused on two questions related to kinetics. First, how quickly does a donor microbiome take hold in individuals who eventually engraft? Second, at what point prior to FMT are recipient microbial features correlated with eventual engraftment?

In subjects who engraft and clinically respond, the engraftment takes root within 2 weeks. Except for one individual, every participant that achieved engraftment of roughly 75% or greater at the end of the FMT period had greater than 75% engraftment at week 2. The other individual achieved greater than 75% engraftment at week 3. This suggests that features supporting engraftment exist early and perhaps even before the initiation of FMT.

We found no baseline clinical features in our participant pool associated with engraftment. However, we found that elevated fecal calprotectin after antibiotic therapy was negatively correlated with engraftment, and this suggests that decreased gut inflammation supports engraftment. While we could not find any microbial correlates of engraftment at baseline (prior to antibiotics), multiple microbial factors correlated with engraftment after antibiotic treatment. Given the relative importance of this time-limited community to engraftment, we propose that future FMT studies should sample at this point to understand how to best prepare a niche for donor engraftment[5,7].

Pre-FMT antibiotics theoretically help eliminate pathobionts and commensals that may outcompete donor microbes as they establish their niche in the recipient's colon. Recent studies tracking strain dynamics in FMT across various disease indications have shown that both antibiotic pre-treatment and decreased alpha diversity of recipients are associated with improved engraftment[19,21]. Contradictory to this, our study data suggest that maintaining a higher level of diversity after antibiotics predicts better engraftment. Our observation is in keeping with two other studies that assessed the determinants of engraftment. In one study of FMT in people with *Clostridioides difficile* infection, higher recipient alpha diversity was associated with improved donor microbiota engraftment[12]. In a separate study of FMT for people with irritable bowel syndrome (IBS), antibiotic treatment and the resulting drop in diversity seemed to decrease engraftment after FMT[27]. The discrepant effect of antibiotics in IBD versus IBS (supportive of engraftment in IBD but detrimental in IBS) demonstrates that different diseases may require different pre-FMT conditioning regimens. Perhaps higher pathobiont abundance in IBD makes antibiotics more favorable. Even within participants with IBD, Podlesny and colleagues noted a wide variation in how much alpha diversity contributed to engraftment[21,29]. There are likely recipient factors (e.g., degree of intestinal inflammation) and medication factors (i.e., every study used different antibiotics) that account for this variability. Our data show that there is also high inter-individual variability in the effect of antibiotics and that even within the same disease, the antibiotics created a supportive milieu for engraftment in some but not in others. Determining who would benefit from antibiotics (and which antibiotics to use) is an area of much-needed continued research.

The final facet of our analysis aimed to determine the specific taxa associated with engraftment. There were clear differences at the beta-diversity level of the high and low engraftment subjects at the time-point immediately following antibiotics (but before FMT). The main differences were the relative abundance of commensals from the Lactobacillaceae family. However, not every participant exhibited a relative Lactobacillus bloom, consistent with the current literature, demonstrating that the change in Lactobacillus abundance can be highly variable[30,31]. Subjects who eventually had high engraftment tended to have higher relative levels of these taxa. This proved to be true in our re-analysis of another FMT trial in IBD, in which sequencing data was available at the post-antibiotic time-point[7]. One other FMT trial was considered for re-analysis as well. A small minority of subjects (only 2 of 18) compared to more than half in our study and that of Chu et al. had a significant rise in Lactobacillus after antibiotics. This may have been related to the inclusion of an aminoglycoside in the antibiotic conditioning[29]. Further supporting a role for Lactobacillaceae, in an FMT trial for *Clostridioides difficile* infection, Lactobacillaceae had the highest dependency score amongst the taxonomic determinants of engraftment identified[12]. Similarly, in a study of hospitalized individuals, Lactobacillus species were key factors that supported the recovery of commensal organisms and exclusion of multidrug-resistant Enterobacteriaceae after antibiotic treatment[32]. Altogether, these findings suggest that Lactobacillaceae may promote engraftment, perhaps by creating a more favorable niche for the donor microbiome. Multiple species in the genus Lactobacillus have been shown in pre-clinical models to support intestinal regeneration. More specifically, emerging evidence has demonstrated that Lactobacilli can increase goblet cells and mucin production. This could support the engraftment of a new microbiome[33]. At a broader level, these findings suggest that creating an ideal environment for engraftment is key. Improving FMT

engraftment depends on our ability to understand—and maybe one day engineer—the microbial and ecological environment into which FMT microbes enter.

There are several important limitations to our study. First, our study is small and underpowered to fully delineate the determinants of engraftment. This study was primarily focused on safety and efficacy; it did not have adequate power to evaluate clinical response, so findings would need to be replicated in a larger study. Nevertheless, the focus on children and young adults in this study helps extend our knowledge of FMT outcomes and engraftment potential in an important population affected by IBD. Furthermore, the weekly time series contributes a deeper dataset for each subject despite a small number of total subjects. Second, the only available sequencing data was 16S profiling. We were able to estimate engraftment using SourceTracker2, a strategy employed by others in the field[28,34–36]. However, for future studies, tracking engraftment using shotgun metagenomic data is more effective, especially with the development of new computational tools designed for metagenomics[18,19,21,37].

Conclusions

Our study demonstrates the need for more inquiry into recipient characteristics that predict FMT engraftment. By analyzing the large inter-individual differences in engraftment, we propose that higher residual microbial diversity after antibiotics supports engraftment, and that several Lactobacillaceae taxa may mediate that effect. We argue that the effects of antibiotic pre-conditioning are still largely unknown and that methodical characterization (microbial sequencing, deeper clinical phenotyping, multi-omics techniques) of the post-antibiotic (but pre-FMT) state will help determine the factors that make FMT in IBD succeed in some but fail in others.

Methods

Study design

We conducted a single-center, randomized, double-blind, placebo-controlled trial of FMT in subjects with colonic or ileocolonic Crohn's Disease (CD) and ulcerative colitis (UC). Subjects were recruited from the Boston Children's Hospital IBD Center and through referrals from providers across the country. Standard anthropometric data, past medical and surgical history, and medication history were abstracted from participants' medical records.

The primary objective of this study was to assess the safety and tolerability of FMT compared to placebo in pediatrics and young adults (ages 5–30) with IBD who failed first-line maintenance therapy. The secondary objectives were to identify biomarkers in both donors and recipients that correlate with clinical response.

Eligibility

Eligible individuals were aged 5–30 years with mild to moderate disease activity. Mild to moderate CD Disease Activity was defined as Pediatric Crohn's Disease Activity Index (PCDAI) >10 but ≤ 30; mild to moderate UC was defined as Pediatric Ulcerative Colitis Activity Index (PUCAI) >9 but <30. Additional eligibility criteria included the presence of visual or histologic evidence of inflammation no more than 105 days before randomization; negative test results for Hepatitis B (HBV), Hepatitis C (HCV), and Human Immunodeficiency Virus (HIV); negative urine pregnancy test for people of childbearing potential; ability to swallow antibiotic, FMT or placebo capsules; and the absence of any known food allergy.

Exclusion criteria included extensive and severe CD (i.e., fistulizing disease, abscess, small bowel obstruction, fevers); subjects with recent (within 4 weeks) dosage changes of biologics, 5-ASA, steroids or immunomodulators; toxic megacolon; known drug allergy to vancomycin, metronidazole or polymyxin; history of aspiration, gastroparesis, surgery involving the upper gastrointestinal tract (that might affect upper gastrointestinal motility) or unable to swallow pills; esophageal dysmotility or swallowing dysfunction; known food allergies; unable or unwilling to receive a retention enema for purposes of induction therapy; recent (within 6 weeks) systemic antibiotic use; testing consistent with active clostridium difficile; and known prior experience with FMT.

Subjects were maintained on their standard of care medications at the primary provider's discretion. Participants with mild to moderate disease activity were consented and randomized to receive antibiotic treatment followed by FMT or placebo. This study was approved by the Boston Children's Hospital Institutional Review Board and was registered on https://clinicaltrials.gov (Identifier: NCT02330653) registered on 29/12/2014. All study procedures took place at Boston Children's Hospital.

Randomization

Subjects enrolled were randomized in a 1:1 ratio according to a pre-determined block randomization procedure to receive either the treatment or the placebo arm. An unblinded study team member maintained the randomization list, ensured subjects were appropriately randomized, and dispensed the correct treatment. Even though this was a feasibility study, the decision to include a placebo arm was important as prior randomized controlled trials of FMT had shown a response within the placebo group[9,14,38].

Study groups

Subjects in the treatment arm (hereafter referred to as the FMT arm) received seven days of antibiotic pre-treatment beginning on Day 8. Antibiotic selection was determined based on prior research suggesting broad-spectrum gut-specific agents that are poorly absorbed[3,39]. One capsule containing metronidazole (weight-dependent dosing, maximum dose of 500 mg) was administered twice daily. Capsules containing 125 mg of vancomycin and 62.5 mg of polymyxin were administered three times a day. The number of capsules given was based on Body Surface Area parameters.

Approximately 48 h after the discontinuation of antibiotic pre-treatment, on Day 0, subjects were given an induction retention enema of 120 mL of fecal material suspended in saline over the course of 15–30 min. They were encouraged to retain the fecal matter for as long as possible. Subjects were then observed for at least 60 min before discharge. Subjects were subsequently received 30 FMT capsule once weekly, taken on an empty stomach for the next 7 weeks.

FMT material was obtained from OpenBiome in Cambridge, MA, using established protocols[12,40]. OpenBiome is a non-profit stool bank dedicated to treating and researching the microbiome. Their work focuses on providing safe and affordable FMT material, thereby removing logistical barriers for patients and physicians.

Subjects in the placebo arm received seven days of corresponding placebo capsules instead of antibiotic treatment. On Day 0, these subjects received a placebo retention enema. Study staff were blinded to the enema color and contents. Subjects were subsequently treated with a weekly dose of 30 placebo capsules for the next 7 weeks. Placebo capsules and FMT capsules looked identical to avoid accidental unblinding of the assigned arm.

Subjects provided weekly stool samples and PCDAI/PUCAI surveys to monitor disease activity regardless of the assigned arm. At Week 4 and Week 8, routine laboratory assessments were collected to measure clinical response.

Open-label eligibility and treatment

After 8 weeks of treatment and unblinding, subjects in the FMT arm that responded to treatment and all subjects in the placebo arm were eligible to receive an additional 8 weeks of open-label FMT. Subjects in the FMT arm that continued with treatment did not receive a second enema and continued on capsule therapy. Response to treatment was defined as a decrease in disease activity index score of at least 10 points or a score of 10 points or less.

Endpoints/outcomes

Primary outcome measures included any FMT-related adverse events, grade 2 or above, and the proportion of subjects reporting any FMT-related adverse events of grade 2 or above at 8 weeks post-FMT. Subject-related outcomes of abdominal pain and the average daily number of bowel movements were recorded. Clinical response for CD subjects was defined as a decrease in PCDAI of at least 12.5 points at any timepoint post-FMT; for UC subjects, it was defined as a decrease in PUCAI of at least 20 points at any timepoint post-FMT. Clinical response was also defined as a subject entering remission with a PCDAI or PUCAI < 10[41,42]. Response was assessed from weeks 2 to 7 after FMT. To test the robustness of microbiome correlates of clinical response, we also created a more inclusive definition of response, which was defined as the average disease activity index of 20 or less and no single disease activity score of greater than 30 at 2–7 weeks after FMT.

Secondary outcome measures included remission as defined by a disease activity index of < 10, improvement of inflammatory biomarkers such as fecal calprotectin, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), changes in gut microbial composition, subject-reported improvement of disease symptoms, and assessment of engraftment of donor microbes into recipients.

Fecal sample collection

Subjects submitted stool samples during screening, baseline, after antibiotics but before FMT, then weekly during blinded and open-label treatment, and during follow-up. These samples were stored at − 80 °C.

Microbiome analysis

We extracted DNA using a Powersoil DNA extraction kit (Qiagen). 16S rDNA libraries were prepared using primers targeting the V3-V4 region and sequenced by the Broad Institute Genomic Platform, using paired-end 250-bp reads on an Illumina HiSeq. We analyzed 16S data using Qiime2, DADA2, Phyloseq in R, and custom Python scripts[43–45]. We calculated alpha diversity using the Shannon index, Peilou evenness, and the Faith PD score within the Qiime2 environment. Beta diversity was calculated using Bray Curtis dissimilarity scores using Phyloseq. To test differences in alpha diversity at various timepoints, between responders and non-responders, we used the Mann–Whitney test and Bonferroni corrections with a corrected p-value cutoff of 0.05. To test correlations between alpha diversity and clinical disease variables, such as PUCAI, we calculated Pearson correlations, adjusted by Bonferroni corrections when multiple tests were performed. We assigned taxonomic labels to 16S sequences using the SILVA database[46]. We used SourceTracker2 to estimate the sources of various bacteria after FMT using the flag "-p-no-loo"[28]. We used the pre-antibiotic sample, the post-antibiotics sample, and the known donor as sources for each participant. To test correlations between engraftment and clinical disease variables, we calculated Pearson correlations between the engraftment score from SourceTracker2 at week 2 and week 7 (the early and late timepoints where we had sampling from all subjects) and the clinical variables. We also included the other donor, not used in that participant's FMT, as a negative control. To differentiate the participants who achieved high engraftment from those who did not, we used ANCOM-BC within the Qiime2 environment[35,47]. To create abundance time series of specific taxa, we extracted relative abundance tables from Qiime2 at the species and genus levels and graphed time series using custom python scripts.

Ethics approval and consent to participate

The study was conducted in accordance with the protocol, applicable ICH Guidelines, Good Clinical Practice and the World Medical Association (WMA) Declaration of Helsinki and its amendments concerning medical research in humans. In accordance with guidelines and the U.S. Code of Federal Regulations applicable to clinical studies, the protocol and informed consent/assent forms were reviewed and approved by the Boston Children's Hospital Institutional Review Board (IRB). The investigator informed the IRB and FDA of subsequent protocol amendments and reportable events as defined by IRB policy and FDA regulation. These studies were registered

on clinicaltrials.gov as Fecal Microbiota Transplant (FMT) in Active Pediatric Ulcerative Colitis and Active Pediatric Crohn's Colitis under NCT02330653 on 29/12/2014. This study used the CONSORTreporting guidelines[48]. Informed consent was obtained from all participants and/or their guardians to participate in this study.

Data availability

The datasets supporting the conclusions of this article are available in the National Center for Biotechnology Information (NBCI) with the Sequence Read Archive (SRA) bioproject number PRJNA950106 (https://www.ncbi.nlm.nih.gov/sra/PRJNA950106). The full trial protocol can be accessed upon request from the corresponding author.

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Author contributions

YZ performed the microbiome analysis and wrote the manuscript. AB contributed to trial design, performed clinical trial procedures, and critically reviewed the manuscript. EA and TN helped perform the microbiome analysis and edited the manuscript. AK performed clinical data analysis and helped write the manuscript. MD, MW, PR, ML, LZ, BB and GR recruited patients, performed the clinical tasks related to the trial and edited the manuscript. BB was the research manager and supported the clinical trial procedures. GR designed the trial and edited the manuscript. SK was the primary investigator, contributed to clinical trial design, oversaw clinical trial procedures, analyzed clinical data, and was a major contributor in writing the manuscript.

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Competing interests

AB receives research support as a subinvestigator on protocols for Janssen, Abbvie, Takeda, Buhlmann, Arena, Eli Lilly, Bristol Myers Squibb, PROCISE diagnostics. AB receives consulting revenue from Takeda, Best Doctors, Eli Lilly, and Fresenius Kabi. AB received an honorarium from Boston University and Royalties from Up To Date. No other authors had any competing interests.

Additional information

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