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Temporal Bacterial Community Dynamics Vary Among Ulcerative Colitis Patients After Fecal Microbiota Transplantation

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OBJECTIVES: **Fecal microbiota transplantation (FMT) from healthy donors, which is an effective alternative for treatment of *Clostridium difficile*–associated disease, is being considered for several disorders such as inflammatory bowel disease, irritable bowel syndrome, and metabolic syndrome. Disease remission upon FMT is thought to be facilitated by an efficient colonization of healthy donor microbiota, but knowledge of the composition and temporal stability of patient microbiota after FMT is lacking.**

METHODS: **Five patients with moderately to severely active ulcerative colitis (Mayo score ≥6) and refractory to standard therapy received FMT via nasojejunal tube and enema. In addition to clinical activity and adverse events, the patients' fecal bacterial communities were monitored at multiple time points for up to 12 weeks using 16S rRNA gene-targeted pyrosequencing.**

RESULTS: **FMT elicited fever and a temporary increase of C-reactive protein. Abundant bacteria from donors established in recipients, but the efficiency and stability of donor microbiota colonization varied greatly. A positive clinical response was observed after 12 weeks in one patient whose microbiota had been effectively augmented by FMT. This augmentation was marked by successive colonization of donor-derived phylotypes including the anti-inflammatory and/or short-chain fatty acid–producing *Faecalibacterium prausnitzii*, *Rosebura faecis*, and *Bacteroides ovatus*. Disease severity (as measured by the Mayo score) was associated with an overrepresentation of *Enterobacteriaceae* and an underrepresentation of *Lachnospiraceae*.**

CONCLUSIONS: **This study highlights the value of characterizing temporally resolved microbiota dynamics for a better understanding of FMT efficacy and provides potentially useful diagnostic indicators for monitoring FMT success in the treatment of ulcerative colitis.**

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/ajg

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INTRODUCTION

Humans and their trillions of intestinal microorganisms have coevolved to form a largely beneficial symbiosis. The human host provides a stable, nutrient-rich ecosystem, and commensal microorganisms contribute energy and cellular precursors in the form of short-chain fatty acids, prevent infections, and modulate and train the host immune system. This dynamic multipartner symbiosis can, however, become unbalanced through genetic and external factors, leading to severe diseases of the intestinal tract. Approximately 4 million people worldwide, mostly in the United States and Europe, are affected by chronic intestinal bowel disease (IBD), primarily Crohn's disease (CD) and ulcerative colitis (UC) (1). IBD has no single etiology but is rather the consequence of a detrimental interaction of intestinal microbiota, epithelium, and immune system in genetically susceptible individuals (2). Application of modern molecular

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methods that allow comprehensive analysis of the gut microbiota has shown that IBD patients have a distorted, low-diversity intestinal microbiota (reviewed in Packey and Sartor (3) and Reiff and Kelly (4)). A specific IBD microbiota has however not yet been revealed, which might in part be because of interindividual and interintestinal (ileum vs. colon, mucosa vs. lumen) variability, different disease characteristics or subtypes, and differences in data acquisition and analysis (5–7).

Standard IBD management employs anti-inflammatory and/or immunosuppressive medication or surgical intervention, but no cure is available (8,9). Alternative IBD treatment approaches aimed at modifying the composition of the intestinal microbiota in order to overcome gut dysbiosis have become of major interest in recent years. Recently, a meta-analysis of randomized controlled trials has revealed a significant benefit of antibiotic therapy in IBD patients (10). There is also some evidence that probiotics may facilitate and stabilize clinical remission, especially in patients with UC (11). However, it is not known whether a commercial probiotic preparation, which only consists of a low number and diversity of bacterial species, or a short-term antibiotic therapy is able to durably modify the intestinal microbial ecosystem. Additional treatment approaches aimed at modifying the composition of the intestinal microbiota in order to overcome dysbiosis have thus become of major interest in recent years, and transplantation of the fecal microbiota from healthy donors is being discussed as an alternative therapy option for IBD (12,13).

Fecal microbiota transplantation (FMT), also called fecal bacteriotherapy, has been occasionally used for >50 years for treatment of *Clostridium difficile*–associated diarrhea and pseudomembranous colitis with great success (14). Two recent systematic reviews of 317 patients across 27 case series (15) and 124 patients across 7 case series (16) highlighted a disease improvement or resolution rate of 92% and 83%, respectively, with very few adverse effects. In contrast, FMT has rarely been used for IBD management (17).

Given the high success rate of FMT and the underlying assumption that it correlates with efficient colonization of the recipient's intestine by the donor microbiota, it is surprising that hardly any information exists regarding the fate of the transplanted microbiota. Very few studies have compared the fecal microbiota composition of donor and recipient, and these have analyzed only a single or very few selected time points after FMT (12,18–20). Furthermore, with only one exception (19), 16S rRNA gene-targeted fingerprinting methods were used that lack the phylogenetic resolution for comprehensive comparative analysis of the diverse gut microbiota. Thus, it remains to be demonstrated whether and to what extent resolution of clinical symptoms correlates with colonization of the recipient's intestinal tract by the donor microbiota.

In the present pilot study, we addressed this and the following specific research questions regarding the fate of the intestinal microbiota after FMT. How does the composition of the new intestinal microbiota (the composite donor–patient microbiota) change in the short and long term after transplantation? Is colonization of the receiving intestinal environment an instant "all-or-none" event or a continuous process characterized by successive establishment of individual microorganisms? How stable is donor-derived microbiota over time, and what is the identity of microorganisms that become temporally stable residents in the new intestinal environment? Here, we report the first study of temporal microbiota dynamics in five UC patients followed up to 3 months after receiving FMT. Comparative fecal microbiota analysis was performed using highly parallel pyrosequencing of bacterial 16S rRNA gene amplicons retrieved at 5–9 time points after FMT. Microbiota data and clinical measures of disease severity were compared to identify indicator taxa for disease at various levels of phylogenetic resolution.

METHODS

Patients and identification of donors

FMT was offered as a therapeutic option in a compassionate use setting to patients of our outpatient clinic with moderately to severely active chronic UC (Mayo scores ≥6; see ref. (21)) and who were refractory and/or intolerant to standard IBD therapies including steroids, cyclosporine, thiopurines, and/or biologics (definitions of prior clinical treatment failure are specified in **Supplementary Methods** online), but who were not at imminent risk of colectomy. Diagnosis of UC was based on clinical, radiological, endoscopic, and histopathological criteria as recommended by the European Crohn's and Colitis Organisation (ECCO) (22). Treatment with infliximab, cyclosporine, thiopurine, methotrexate, and/or steroids was stopped at least 8, 4, 4, 4, and 2 weeks before FMT, respectively. Concomitant therapy with 5-aminosalicylates was allowed.

Patients were able to suggest a possible donor, but first-degree relatives of the patients and hospital or health-care workers were excluded. A complete medical and surgical history of each potential donor was obtained. Donors were considered to be suitable if they had no history of chronic gastrointestinal disease and if they had not been hospitalized for at least 3 months before FMT or received antibiotics or proton pump inhibitors for at least 6 months before FMT. Blood collection including complete blood count, chemistry, and iron profile was performed before FMT. Donor blood was negative for common viruses (hepatitis A, B, and C, HIV-1 and HIV-2, cytomegalovirus, *Epstein–Barr*, *Herpes simplex*, and *Varicella zoster*) and *Treponema pallidum*. Donor feces were negative for common enteric pathogens (*Yersinia* spp., *Salmonella* spp., *Shigella* spp., *Campylobacter jejuni*, *C. difficile* toxin, helminths, ova, parasites, and *Helicobacter pylori*), as determined with standard screening methods.

Patients and donors of fecal material were informed of the potential risks and benefits of the fecal transplantation and its experimental nature. All patients and donors gave their written informed consent to the protocol. The ethics committee of the Medical University of Vienna approved the retrospective review of patients' file.

Patient preparation

Before FMT, patient blood was collected for chemical and biological analyses and feces were analyzed to ensure that common enteric pathogens were absent (**Supplementary Table S1** online). Patients received antibiotic treatment (metronidazole, 500 mg twice a day) for 5–10 days until the evening before they were admitted to the hospital for FMT (except for the first FMT of patient 1). On the day of admittance, a nasojejunal tube was placed in the patient's jejunum using sedation with midazolam and propofol. The presence of cytomegalovirus colitis was excluded by concomitant diagnostic sigmoidoscopy and by PCR and immunohistochemistry of retrieved biopsy samples. In the afternoon of the same day, the position of the nasojejunal tube was verified by abdominal radiography. Polyethylene glycol (2 l) was subsequently administered via the nasojejunal tube for bowel lavage. Proton pump inhibition therapy (pantoprazole 20 mg), begun the evening before FMT, was applied twice a day during the transplantation procedure. Patients did not eat for 6 h before FMT.

Preparation of fecal suspension

Because protocols for donor stool preparation differ between published FMT studies, three preparation protocols were initially assessed (**Supplementary Methods**). A variation of one of these protocols (protocol B) was finally used as described below. Volunteers who passed the selection criteria donated stool samples on the day of FMT in the laboratories of the Medical University of Vienna (Department of Internal Medicine III, Division of Gastroenterology and Hepatology, Medical University Vienna, Wien, Austria). Fresh stool (60 g) was immediately mixed in a standard household blender with 250 ml of 0.9% sterile saline for several seconds until it developed a smooth consistency. The stool suspension was then filtered through a gauze pad to remove large particles. Of the filtered suspension, 100 ml was filled into an enema bag. The rest of the suspension (~150 ml) was diluted in 0.9% sterile saline to a volume of 400–425 ml. Afterwards, it was filtered several times through an increasing number of gauze pads (4 to >6 to >8) to remove small particles that could clog the nasojejunal tube. This stool suspension was poured into a sterile bottle and administered within 1 h.

Route of administration

Patients received FMT on three consecutive days (except for FMT1 of patient 1, **Figure 1**) via both nasojejunal tube and enema. Altogether, a median 24 g (17–25 g in median 250 ml) and 20 g (6–22 g in median 100 ml) stool suspension was administered via nasojejunal tube and enema, respectively. The nasojejunal tube was flushed with 20 ml saline solution after each procedure. Patients received two tablets of loperamide to diminish intestinal motility immediately after FMT and 6 h after procedure. After FMT, patients were encouraged to consume fluid food including a high-fiber diet (Nestlé Nutrition Resource 2.0 Faser, Florham Park, NJ). Blood samples were collected every day. One day after the last FMT, patients were discharged from hospital.

Clinical diagnostics

Blood and stool were collected and analyzed as outlined in **Supplementary Table S1** online. Clinical activity was assessed at each visit according to the (partial) Mayo score (21). Furthermore, at weeks 4 and 12, sigmoidoscopy and the H_2-glucose breath test to assess microbial overgrowth were scheduled. Fecal calprotectin was determined by enzyme-linked immunosorbent assay according to the manufacturer's instructions (Bühlmann Laboratories AG, Schönenbuch, Switzerland).

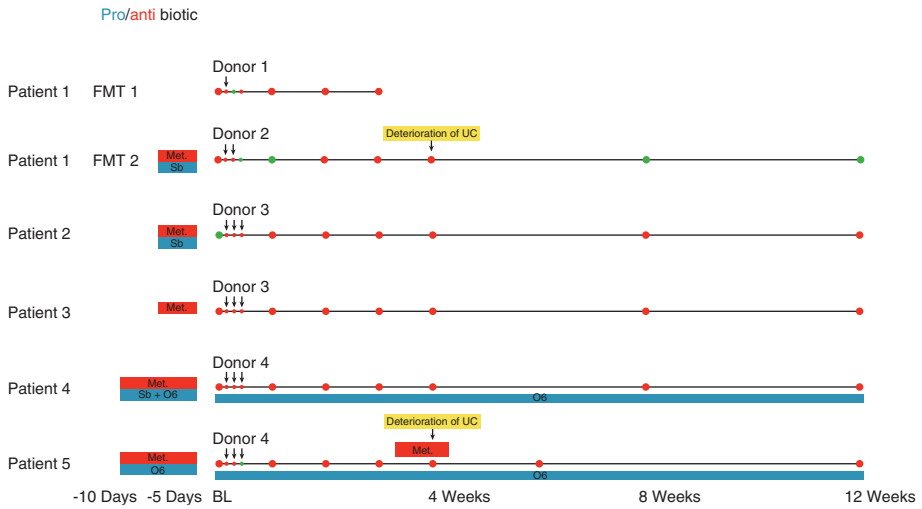

Figure 1. Schematic overview of fecal microbiota transplantation (FMT) for ulcerative colitis (UC) patients. Probiotics (in blue) and/or antibiotics (in red) were given for either 5 or 10 days before FMT. The pro/antibiotics given are as follows: metronidazole (Met.; 500 mg twice a day), *Saccharomyces boulardii* (Sb; Yomogi), and =Omnibiotic 6 (O6). FMT procedures are shown with an arrow and the donor is indicated above the arrow. Patient 1 (P1) received two FMTs with two different donors, patients 2 and 3 shared a donor, and patients 4 and 5 shared a donor. Red circles indicate sampling dates with microbiota data and green circles indicate dates with clinical data but no microbiota data. Patient 5 provided a stool sample at week 6 instead of the prespecified week 8. BL, baseline before FMT, which is a post-antibiotics baseline for all FMTs except for FMT 1 of P1.

Table 1. Demographic and clinical information of patients

Pt	Sex	Age	Disease duration (years)	Disease extent	Smoker	Prior therapy	Concomitant therapy	Post-Abx baseline total Mayo score
1	F	22	1.7	Left sided	Ex-smoker	AZA, CyA	5-ASA	11[a]
2	M	27	1.3	Extensive	No	AZA, CyA, IFX	None	10
3	M	44	4.3	Extensive	Yes	AZA, CyA, IFX, ADA, MTX	5-ASA	11
4	M	51	3.5	Extensive	Ex-smoker	AZA, IFX, GOL	5-ASA, PRO	11
5	F	27	9.7	Extensive	Yes	IFX, ADA, MTX	PRO	8

ADA, adalimumab; 5-ASA, 5-aminosalicylates; AZA, azathioprine; CyA, cyclosporine; F, female; GOL, golimumab; IFX, infliximab; M, male; MTX, methotrexate; PRO, probiotic therapy; Pt, patient.

[a]Before FMT1, no prior antibiotic treatment.

All prior immunosuppressive therapies are indicated excluding steroids, and include AZA, CyA, IFX, ADA, GOL, MTX, and 5-ASA. Concomitant therapies and total Mayo score at post-antibiotics baseline are also indicated. PRO is Omnibiotic 6, a mixture of *Lactobacillus acidophilus*, *Lactococcus lactis*, *Enterococcus faecium*, *Bifidobacterium bifidum*, *Lactobacillus casei*, and *Lactobacillus salivarius*.

Efficacy end points

Remission was defined as a total Mayo score of ≤2 with no individual subscore exceeding one point for UC patients, and response as a decrease from baseline in the total Mayo score of at least three points and at least 30%, with an accompanying decrease in the subscore for rectal bleeding of at least one point or an absolute subscore for rectal bleeding of 0–1 for UC patients (23).

Sampling of stool samples and bacterial community analyses

Stool samples of the patients were collected after antibiotic treatment and bowel lavage (post-antibiotics baseline), but before the first FMT, and on several time points after FMT (**Figure 1**). Donor stool samples, including both native stool and stool suspension for FMT, were collected. Stool samples were either immediately frozen at −80 °C or after transportation in cool bags (for weeks 1, 2, 4, 8, and 12).

DNA was extracted from fecal samples using a standard phenol–chloroform protocol with bead beating (24). Purified DNA served as the template for a two-step, low-cycle number PCR with primers targeting the 16S rRNA gene of most *Bacteria* (spanning the V6 to V9 regions, 909F, 5′-ACTCAAAKGAATWGACGG-3′ and 1492R, 5′-NTACCTTGTTACGACT-3′) (25). The two-step PCR produces amplicons tagged with an 8-nt sample-specific barcode sequence, but minimizes barcoded primer-associated bias (25). PCR products were purified with Agencourt AMPure beads (Beckman Coulter Genomics, Danvers, MA), quantified with PicoGreen (Quant-iT PicoGreen, Invitrogen, Carlsbad, CA), and pooled for sequencing. Pyrosequencing was performed on a GS FLX (Roche, Basel, Switzerland) instrument with Titanium chemistry by Eurofins MWG Operon (Ebersburg, Germany). The two-step PCR pyrosequencing approach was previously shown to reliably monitor relative differences in abundant 16S rRNA gene phylotypes (26).

Sequence data were quality-filtered using the Pyronoise algorithm in mothur, clustered into species-level phylotypes of 97% sequence identity using UCLUST, and checked for chimeras with Chimera Slayer (27–29). Samples with <200 reads ($n=3$) were excluded from downstream analysis. Reads were taxonomically classified using the Ribosomal Database Project naive Bayesian classifier. Relative comparisons of α-diversity (Chao 1 phylotype richness) and β-diversity (weighted UniFrac distances) were performed using QIIME with re-sampling at less than the size of the smallest library (1,000 subsamples of 200 reads) (30,31). Community similarity was calculated using the weighted UniFrac distance metric, which incorporates phylogenetic as well as relative abundance information (32). Statistical significance of shifts in the relative abundance of bacterial taxa as well as Pearson's correlation coefficients were corrected for multiple comparisons using the Holm–Bonferroni method, and adjusted P values are reported.

Pyrosequencing data are archived at National Center for Biotechnology Information (NCBI) Sequence Read Archive under Accession SRS350277.

RESULTS

Patients and donor characteristics

We report on five patients with moderately to severely chronic active UC who received FMT (**Table 1**). Characteristics of the four donors are shown in **Supplementary Table S2** online. In summary, two volunteers each donated stool to two patients and one patient received stool from two donors (**Figure 1**).

Safety of FMT

Patient 1 had no prior antibiotic or probiotic treatment regime before the first FMT. She experienced fever above 39 °C and greater than eightfold increase of C-reactive protein after the first FMT and further FMT was postponed for 5 weeks. Because of this experience, all other patients completed an antibiotic therapy with metronidazole before FMT. Patients 1 (FMT 2), 2, 4, and 5 underwent probiotic therapy before FMT with *Saccharomyces boulardii* (Yomogi, Ardeypharm GmbH, Herdecke, Germany, 2 tablets once a day) and/or a preparation of different viable

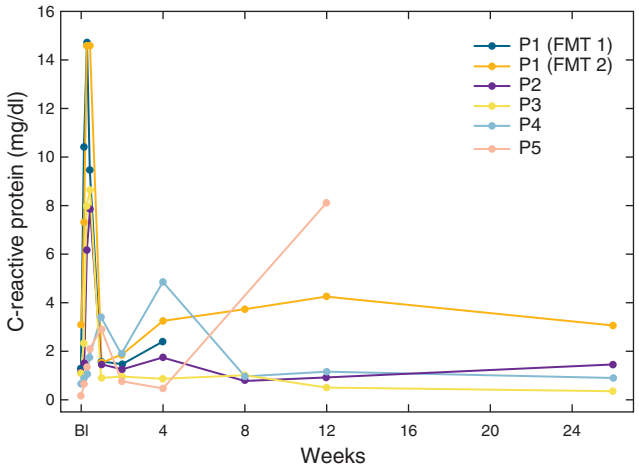

Figure 2. Levels of C-reactive protein (CRP) in the blood of patients. A transient increase of CRP was observed in all of the patients during fecal microbiota transplantation. CRP levels of <1 mg/dl are considered normal. CRP levels in patients 1–4 (P1 to P4) were measured after 26 weeks as a follow-up to the 12-week study. Patient 1 received two fecal microbiota transplantations (FMTs; FMT 1 and FMT 2). BL, baseline before FMT, which is a post-antibiotics baseline for all FMTs except for FMT 1 of P1.

bacteria (Omnibiotic 6, Institut Allergosan, Graz, Austria, twice a day) (**Figure 1**). Despite this, all patients developed fever and a temporary increase in C-reactive protein after FMT (**Figure 2**), although blood culture tests from patients who had temperatures above 38 °C revealed no detectable bacterial pathogens. Patients reported that diarrhea worsened during FMT, particularly the night after the first day of FMT. In addition, sore throat due to the irritation of the nasojejunal tube ($n=5$), flatulence ($n=2$), and vomiting ($n=1$) were reported. In the follow-up period, common cold ($n=3$), pancreatitis of unknown origin ($n=1$), itchiness ($n=1$), erythema ($n=1$), paresthesia of the hip ($n=1$), collapse due to orthostatic disorder ($n=1$), and blisters on the tongue ($n=1$) were reported, but no serious adverse event occurred. H_2-glucose breath tests were performed at week 4 (for all patients) and at week 12 (patients 2–4), but bacterial overgrowth was not evident in any patient (data not shown).

Efficacy of FMT

None of the patients achieved remission by week 12, but response was observed in one patient (patient 3), whose Mayo endoscopic subscore improved from 3 to 2 and total Mayo score from 11 to 6 (**Figure 3**). In the 7-month follow-up, he reported on 4–6 bowel movements per day without blood (partial Mayo score of 3). In patients 1 and 5, further deterioration of UC was observed 4 weeks after FMT (**Figure 3**). The change of fecal calprotectin is shown in **Table 2**.

Fecal microbiota in patients and donors at baseline

The post-Abx baseline microbiota of UC patients compared with donors was characterized by low phylotype richness ($P=0.03$) (**Supplementary Figure S1** and **Supplementary Table S3** online), overrepresentation of *Enterobacteriaceae* ($P<0.001$) and *Enterococccaceae* ($P=0.03$), and underrepresentation of *Lachnospiraceae* ($P<0.001$), *Ruminococcaceae* ($P<0.001$), and *Bacteroidaceae* ($P=0.01$) (**Supplementary Figure S2** online). For each donor, fecal samples from at least two different days were analyzed. In some cases (donors 2 and 3), both native stool and fecal suspension preparations for FMT were investigated. Stool composition was relatively stable between different days and preparations and similar between different donors (**Figure 4**). All donors had high levels of *Faecalibacterium prausnitzii* (14.3±9.0%), with no consistent differences in the abundance between the donors. All donors could be characterized as belonging to the recently described Enterotype 3 because of high levels of *Ruminococcus* species, moderate levels of *Bacteroides* species, and almost no detectable *Prevotella* species (33).

Fecal microbiota transplantation and alteration of patients' microbiota

As expected, FMT resulted in a transient increase in similarity to donor microbiota (**Supplementary Figure S3** online) and increase in phylotype richness (**Supplementary Figure S1**). Donor microbiota was detected in patients during and after FMT, but the magnitude and persistence of these changes in microbiota composition were highly variable between patients. A shift in the patient microbiota, characterized by higher similarities to the donor microbiota, was observed for patients 1 (FMT 1 and 2), 3, and 5 (**Figure 4**). Notably, the microbiota of patient 3, the sole clinical responder, was similar to donor microbiota even 12 weeks after FT. In contrast, in patients 1 and 5, this shift was transient and the microbiota showed increasing dissimilarity to the donor microbiota after 2–4 weeks of FMT. In patient 4, FMT resulted in no marked shifts in the composition of the microbiota, remaining dissimilar to the donor for the duration of the study. A post-Abx baseline sample was not available for patient 2 and thus it remains speculative that the relatively high similarity between the patient's microbiota after FMT and the donor was a consequence of the treatment (**Figure 4**).

Across the entire data set, Mayo scores displayed strong positive (Pearson's $r=0.76$, $P<0.001$) and negative correlation (Pearson's $r=-0.84$, $P<0.001$) with relative abundances of the bacterial families *Enterobacteriaceae* and *Lachnospiraceae*, respectively (**Supplementary Figures S4 and S5** online). At the phylotype level, the only phylotype with a strong correlation with Mayo scores was the dominant *Enterobacteriaceae* phylotype (OTU_2470; Pearson's $r=0.76$, $P<0.001$; **Supplementary Table S4** online).

Individual donor phylotypes reached high relative abundance in the recipient, but the number and temporal stability of putatively transplanted phylotypes differed between patients (**Table 3**). In accordance with the course of disease, patient 3 received the greatest fraction of abundant donor phylotypes, four of which remained consistently present in the patient's microbiota for 12 weeks (**Figure 5, Table 3,** and **Supplementary Table S4**

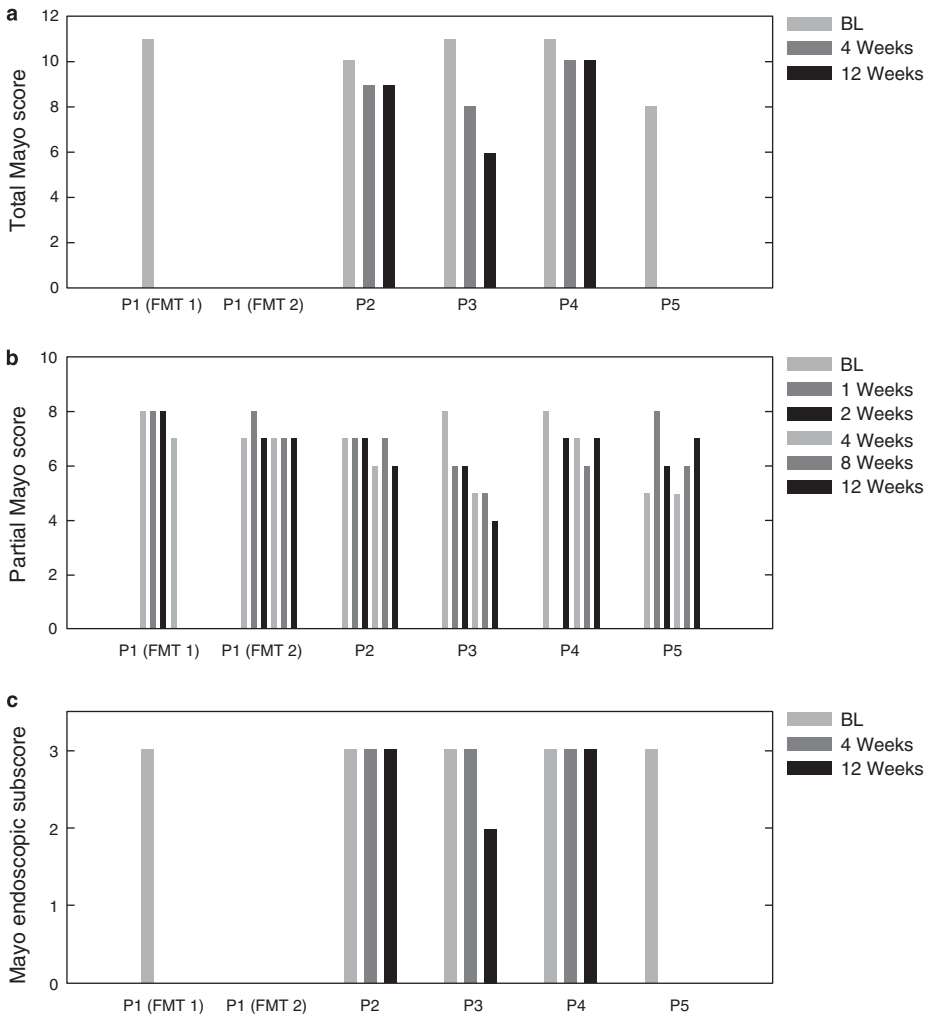

Figure 3. Mayo scoring of patients. Patients were scored at baseline before fecal microbiota transplantation (FMT; which is a post-antibiotics baseline for all FMTs except for FMT 1 of patient 1 (P1)) and at several time points from 1 to 12 weeks after FMT. Scoring was done according to the Mayo scoring criterion (21). (**a**) Total Mayo score, (**b**) partial Mayo score, and (**c**) Mayo endoscopic subscore. For patient 1, the first (FMT 1) and second FMT (FMT 2) are shown separately. Patient 3 (P3) showed a clinical response, but no patient achieved clinical remission 12 weeks after FMT. Follow-up sigmoidoscopy was not performed for patients 1 and 5.

Table 2. Fecal calprotectin in patients

Patient	Post-antibiotics baseline	Week 12	Early termination
1	11,808[a]	—	5,616
2	Not available	2,016	
3	636	238	
4	1,608	825	
5	2,484	—	2,808

Calprotectin concentration is given in μg/g.
[a]No antibiotic treatment preceded the first fecal microbiota transplantation (FMT 1).

online). Three phylotypes displayed high 16S rRNA sequence similarity (>99%) to known intestinal bacteria, whereas one was only distantly related to *Clostridium spiroforme* and could not be classified to the species level. The most abundant was the *F. prausnitzii* phylotype.

DISCUSSION

Studies of FMT in patients with active UC are rare and restricted to clinical case reports (17). Here, we give a detailed account of clinical outcomes of FMT in a pilot study of five patients with moderately to severely chronic active UC refractory to IBD standard therapies and additionally report, for the first time, post-FMT changes in the fecal bacterial community composition of these patients.

FMT is postulated to be safe (15,34). Although there were no serious adverse events, all of our patients developed fever and a temporary increase of C-reactive protein during FMT. A temporary systemic immune response related to FMT has not

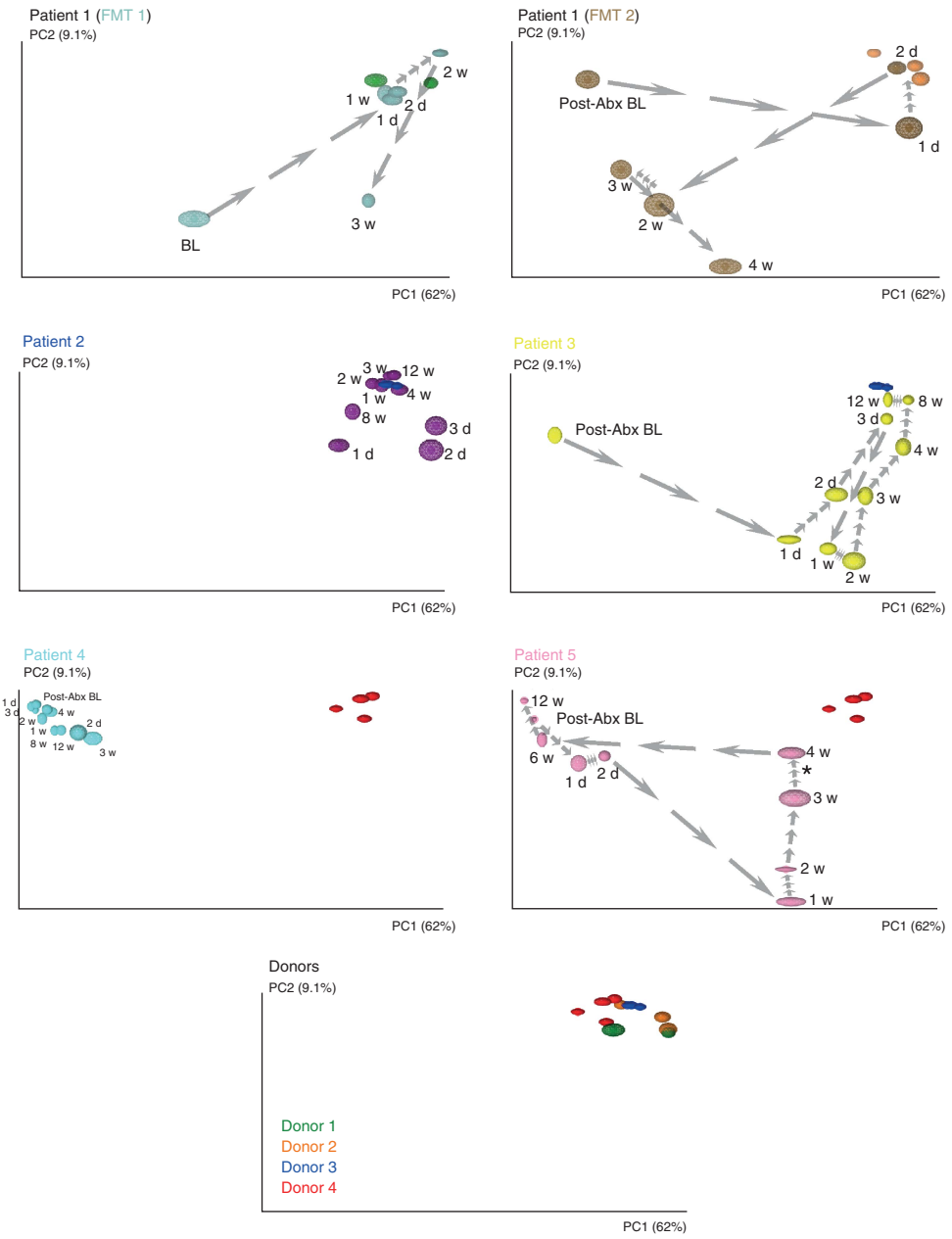

Figure 4. Principal coordinates analysis (PCoA) of the fecal microbiota of patients and donors. Community similarity was calculated using the weighted UniFrac distance metric, which incorporates phylogenetic as well as relative abundance information (32). PCoA was performed on the entire data set and subsets of points are shown in separate panels to emphasize individual fecal microbiota transplantation (FMT) procedures. The baseline microbiota of each patient before FMT is shown ("BL" is the post-Abx baseline for all FMTs except for FMT 1 of patient 1 (P1). The post-antibiotics baseline sample for patient 2 was unavailable) together with the microbiota of the donor for that patient. Trajectories are indicated with gray arrows when large differences over time were observed. Start of antibiotic treatment in patient 5 is indicated with an asterisk. Patient 5 provided a stool sample at week 6 instead of the prespecified week 8. The microbiota of all donors is plotted separately (lower panel) to show the similarity of these samples relative to patient microbiota. d, day; w, week.

previously been described in patients with *C. difficile* infection, independently of the route of fecal suspension administration. However, Vermeire *et al.* (35)reported high fever and abdominal tenderness on three of four patients with refractory CD treated with FMT via nasojejunal tube. The transient immune response in our study was apparently not caused by bacteremia as blood cultures remained sterile in all patients, although translocation of bacterial compounds such as lipopolysaccharides fostered by an inflammation-driven disruption of the intestinal mucosal barrier cannot be excluded. It has been speculated that instillation of donor stool via nasogastric tube might cause bacterial overgrowth (36), although this was not detectable in any patient

Table 3. Transfer of abundant donor phylotypes to patients

Phylotypes	Patients					
	1 (FMT 1)	1 (FMT 2)	2	3	4	5
Number	13	2	ND	12	0	7
Efficiency (%)	32	6	ND	52	0	24
Stability (%)	56 (100)	33 (33)	ND	40 (100)	NA	31 (50)

FMT, fecal microbiota transplantation; NA, not available; ND, not detected.

For the comparison of the transfer of abundant donor phylotypes to patients, a phylotype was considered to be transferred from the donor to the patient if it was detected with at least 1% relative abundance in both the donor and the patient at any time between weeks 1 and 12 after transplantation, but not detected in the patient before transplantation. Transfer efficiency was defined as the percentage of donor phylotypes that were transferred to the patient. Transfer stability was defined as the percentage of samples in which a phylotype was detected at >1% relative abundance between weeks 1 and 12 (or between week 1 and weeks 3–4 for patient 1 (FMT 1 and 2)). For transfer stability, the arithmetic average and maximum of all transferred phylotypes are shown.

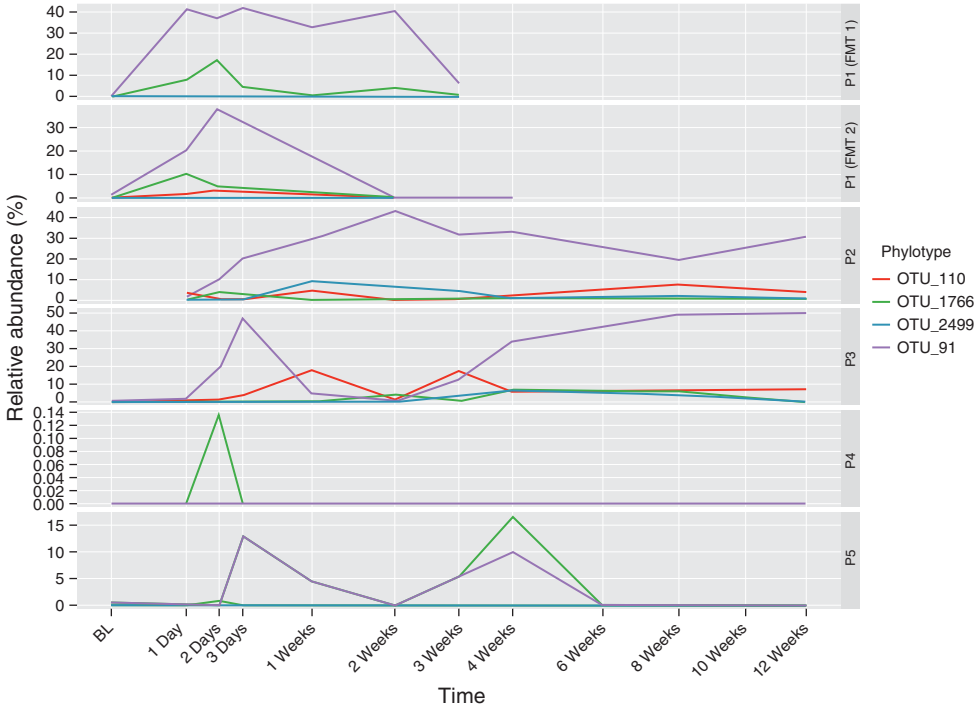

Figure 5. Dynamics of the abundant phylotypes transferred most stably to patient 3 (P3). Phylotypes that were transferred from the donor to patient 3 and were stable (>1% relative abundance in at least 50% of samples). The dynamics of these phylotypes in the other four patients are shown for comparison. Patients are indicated by different panels, and for patient 1 the first fecal microbiota transplantation (FMT 1) and the second FMT (FMT 2) are shown separately. Each panel is scaled separately to the relative abundance range shown in the panel. OTU_110 shares 94% sequence similarity (over 391 nt) with *Clostridium spiroforme* DSM 1552 (X73441). OTU_1766 shares 99% sequence similarity (over 391 nt) with *Bacteroides ovatus* strain 8 (GU968166). OTU_2499 shares 99% identity (over 413 nt) with *Roseburia faecis* strain M88/1 (AY804150). OTU_91 shares 99% sequence identity (over 419 nt) with *Faecalibacterium prausnitzii* strain ATCC 27768 (NR_028961). Because of their high relative abundance, all four phylotypes were also identified in the analysis of the abundant transferred phylotypes (**Table 3**). BL, baseline before FMT, which is a post-antibiotics baseline for all FMTs except for FMT 1 of patient 1 (P1).

at 4 and 12 weeks after transplantation, as measured via an H_2-glucose breath test.

FMT has primarily been used as a therapy for fulminant or refractory *C. difficile* infections. The reporting success rate approaches up to ~90%, although in most studies response was not clearly defined (15,34). Borody *et al.* (37)reported on six UC patients who were prepared for FMT with antibiotics (vancomycin, metronidazole, and rifampicin) and a single-bowel lavage. Our patients received metronidazole and a single-bowel lavage before the first FMT, except for patient 1 in FMT1. Probiotic therapy was also applied before the first FMT in four patients (**Figure 1**). Because several variants of the FMT procedure have

been described, we decided to combine nasojejunal and rectal approaches and therefore administered donor stool suspension via nasojejunal tube and enema daily for 3 days. In the case series of Borody *et al.*(37), patients were in clinical, endoscopic, and histological remission without any UC medication for 1–13 years after FMT. In our pilot study, however, only patient 3 responded clinically to FMT, whereas in two other patients (1 and 5) a clinical deterioration of UC was observed. One reason for the poor response rate might be patient selection. In contrast to the six patients reported by Borody *et al.* (37), our patients had a short disease duration of median 3.5 years and in this period all of the patients had already received at least three different immunosuppressive drugs, including steroids and biologics, which reflects a progressive course of disease. The relatively low calprotectin levels of patient 3 at baseline compared with other patients in the study support this interpretation (**Table 2**). Furthermore, patients with severe chronic diseases might require repeated FMT infusions to induce disease remission (13). Thus, patients with UC induced into remission via conventional medical therapy or those with mild disease might be more likely candidates for future FMT trials. The fact that patient 3 was the only subject who did not receive pretreatment with a probiotic might challenge the usefulness of probiotics in FMT protocols. More than 1 year after FMT, patient 3 continues to be in stable clinical remission without need of immunosuppressive therapy (data not shown).

The post-Abx baseline fecal microbiota of the five UC patients showed clear evidence of dysbiosis. UC communities lacked or were underrepresented in signatures of healthy microbiota such as key bacterial phyla (*Bacteroidetes*, *Firmicutes*, *Verrucomicrobia*) and had high relative abundance of *Enterobacteriaceae* (**Supplementary Figure S4** online), which is consistent with the microbiota observed under highly disrupted and inflamed conditions (5,38,39). Reduced microbial diversity in UC patients was likely because of the combined effects of intestinal inflammation and antibiotics treatment (40,41), although it was also observed in patient 1 (FMT 1), who did not receive antibiotics before FMT. The baseline microbial community may have also been altered because of concomitant 5-aminosalicylate treatment (42) and lavage (43).

FMT therapy generally (although not always) produced a major shift in the patient microbiota toward the donor microbiota in the first 3 days (**Figure 4** and **Supplementary Figure S3** online). In some cases, this shift was transient and probably partly because of the presence of recently introduced donor microbiota and was lost 1–4 weeks after FMT (patient 1 (FMT 1 and 2) and patient 4). In other cases, similarity with the donor microbiota was maintained over at least 12 weeks (patients 2 and 3). Abundant phylotypes transferred from donors were also detected in patients with an efficiency of transfer of 0–52%, although the temporal stability of these transferred phylotypes varied (**Table 3**). Patient 3, who responded positively to the treatment, maintained four stable abundant donor phylotypes over the 12-week period. These four abundant phylotypes had strikingly different dynamics in patient 3, with one peaking in abundance at weeks 1 and 3 (OTU_110, distantly related to *C. spiroforme*), two peaking at week 4 (*Roseburia faecis* OTU_2499, *Bacteroides ovatus* OTU_1766), and one only increasing in abundance later and peaking at 8–12 weeks (*F. prausnitzii* OTU_91) (**Figure 5**). This type of dynamics might indicate that the colonization of donor microbiota is a gradual process of sequential establishment of individual organisms analogous to observations of slow, and individual-specific, directional change of gut communities after a ciprofloxacin-induced perturbation (44). *B. ovatus* has been shown to protect mice from dextran sodium sulfate–induced colitis (45) and is a member of a mixture of 10 bacteria that have been shown to clear *C. difficile* infections (46). *R. faecis* is an acetate-consuming butyrate producer (47) and depletion of the genus *Roseburia* has been associated with ileal CD (7) and colorectal cancer (48). The late and most abundant succession colonizer (OTU_91) shares 99% sequence identity to *F. prausnitzii*, an anti-inflammatory, butyrate-producing commensal bacterium that has been suggested to be protective in colitis and CD (49), although exceptions have been reported (50,51).

Although the microbiota of both patients 2 and 3 shared similarity to their donor (donor 3), these two patients had different disease outcomes. This indicates that only presence of a fraction of the healthy donor-type microbiota is insufficient to clearly predict a positive clinical response. There were, however, strong correlations of two bacterial families, *Enterobacteriaceae* ($r = 0.76$, $P < 0.001$) and *Lachnospiraceae* ($r = -0.84$, $P < 0.001$), with Mayo scores across all patients, indicating that some bacterial groups can be diagnostic for health state. It was not possible to evaluate whether different donors had different efficiencies of transfer, although the lack of transfer to patient 4 is likely not because of a faulty microbiota of donor 4, as patient 5 showed a partial transfer (**Figure 4** and **Table 3**). Conclusive interpretations regarding efficacy and safety of FMT are not possible because of the small patient cohort in this pilot study. However, the combination of clinical outcomes with time-resolved microbiota analysis presented here is a novel approach that will inform future larger studies. In conclusion, the results from this small case series with five patients indicate that transferred donor fecal microbiota may be difficult to maintain stably in recipients with moderate-to-severe active UC, in contrast to the stable transfer of microbiota observed recently for FMT to treat recurrent *C. difficile* infections (52), and that FMT should not yet be offered to UC patients outside of study protocols because of lack of proven efficacy and safety. This study further demonstrates that time-resolved fecal microbiota analysis represents an effective option for monitoring colonization efficacy and assessing FMT therapy success. Changes in the relative abundance of the families *Enterobacteriaceae* and *Lachnospiraceae* in UC patients provide useful diagnostic indications of clinical response after FMT. Randomized, placebo-controlled trials with larger cohorts will be necessary to establish cause–effect relationships for the successfully transmitted donor phylotypes such as *F. prausnitzii*, *B. ovatus*, and *R. faecis*. In addition, further studies should be conducted to address: (i) whether adding these species alone has a therapeutic effect or whether a complex background bacterial

community is also necessary for efficient transplantation and (ii) whether phylogenetic and/or functional (in)compatibilities between donor and recipient microbiota are factors that govern the success and efficacy of FMT.

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CONFLICT OF INTEREST

Guarantor of the article: Walter Reinisch, MD.

Specific author contributions: S.A. and W.R. designed and performed the clinical study and interpreted clinical data; A.M and C.L. prepared fecal suspensions; C.D, P.P., G.N, and M.T. were clinical investigators; D.B. and A.L. designed and performed the microbial community analysis and interpreted microbiota data; and S.A., W.R., A.L., and D.B. wrote the manuscript. All authors contributed to and approved the final version of the manuscript.

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Potential competing interests: None.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Fecal microbiota transplantation (FMT) is effective in *Clostridium difficile* disease.
- ✓ Few case studies of FMT as a treatment for inflammatory bowel disease have been published.
- ✓ The temporal dynamics of gut microbial populations and the extent to which donor microorganisms establish in patients receiving FMT are not understood.

WHAT IS NEW HERE

- ✓ Abundant donor-derived bacteria were able to establish in ulcerative colitis patients. but the efficiency and temporal stability of donor microbiota colonization varied greatly between individual recipients.
- ✓ Bacterial taxa that are indicative for ulcerative colitis severity and FMT success were identified.
- ✓ This study identifies diagnostic microbial indicators of disease severity and FMT success in the treatment of ulcerative colitis and underscores the value of characterizing microbial dynamics to evaluate FMT.

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