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Potential role of fecal microbiota in patients with constipation

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Abstract

Background: We evaluated the safety and efficacy of fecal microbiota transplantation (FMT) for chronic functional constipation (CFC) ineffectively treated by conventional constipation medication.

Methods: Thirty-four patients with CFC underwent FMT treatment (three rounds, *via* gastroscopy). Clinical scales, including the Wexner constipation score as the main index of efficiency, were completed at baseline; after each treatment, and at 2 and 3 months of follow up. Secondary evaluation indices included the self-assessment of constipation symptoms, patient assessment constipation quality-of-life questionnaire, Bristol stool form scale, and Zung's self-rating depression and anxiety scales. Gastrointestinal motility, motilin, gastrin, nitric oxide (NO), and 5-hydroxytryptamine (5-HT) were assessed before and after treatment. Intestinal flora changes were assessed by 16S ribosomal ribonucleic acid (rRNA) sequencing.

Results: There were no serious adverse reactions. The clinical cure rate was 73.5% (25/34), clinical remission rate was 14.7% (5/34), and the inefficiency rate was 11.8% (4/34). Clinical scale data indicated that the FMT treatment was effective. Furthermore, FMT treatment promoted intestinal peristalsis, increased gastrointestinal motility, and increased serum NO and 5-HT levels. The 16S rRNA sequencing data indicated that high abundances of *Bacteroides, Klebsiella, Megamonas, Erysipelotrichaceae* and *Epulopiscium* may be the cause of constipation, and high abundances of *Prevotella, Acidaminococcus* and *Butyricimonas* may be the main factors in curing constipation.

Conclusion: Treatment with FMT regulates the intestinal microflora and changes the abundance of CFC-associated bacterial flora to improve constipation.

Keywords: chronic functional constipation, fecal microbiota transplantation, gastrointestinal motility, intestinal flora

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Introduction

Chronic functional constipation (CFC) is a common intestinal functional disease, characterized by a reduced number of bowel movements and dry stools. It can also be accompanied by anal swelling and defecation. Although there are numerous medications for constipation, the effect is inadequate for some patients; therefore, new types of treatment are necessary. The gut microbiota is closely related to intestinal function, and disturbances in the host microbiome have been proven to contribute to gut dysfunction. It remains unclear whether the microbiota participates in the pathogenesis of CFC. However, recent studies suggest that patients with constipation have a disorder of the intestinal flora.[1] The intestinal flora of patients with CFC is abnormal in terms of numbers and composition.[2] Furthermore, although it is unknown which strains affect intestinal peristalsis, the diversity of the intestinal flora is altered in patients with CFC, mainly due to reductions in *Bifidobacterium* and

Lactobacillus, and significant increases in *Bacteroidetes, Fusobacterium*, and *Enterobacter*.[3,4] Thus, it is worth exploring whether constipation can be improved by regulating the intestinal flora.

The pathogenesis of the intestinal flora involved in constipation is unclear. However, intestinal bacterial metabolites, such as endotoxin and short-chain fatty acids, are closely related to intestinal smooth muscle contraction and intestinal peristalsis function.[5] Short-chain fatty acids promote the secretion of 5-hydroxytryptamine (5-HT) and accelerate the rate of colonic transmission. Furthermore, endotoxin is a component of Gram-negative bacteria cell lysis, which promotes the production of nitric oxide (NO); NO diffuses into smooth muscle cells and activates soluble uridine cyclase to cause cyclic guanosine monophosphate (CGMP) elevation, reducing intracellular $Ca^{2}+$ concentration and causing smooth muscle relaxation.[6] Consistent with this, patients with CFC have decreased serum 5-HT levels and elevated NO levels.[7] Furthermore, motilin and gastrin participate in intestinal smooth muscle contraction and relaxation, thus playing an important role in regulating intestinal motility. Motilin is produced by duodenal and jejunal mucosal M cells, and acts on the smooth muscle cells of the gastrointestinal tract, stimulating gastrointestinal motility. Gastrin is a gastrointestinal motility hormone secreted by the gastric antrum and duodenum and promotes intestinal motility mainly by enhancing intestinal smooth muscle contraction. Patients with constipation have decreased serum motilin and gastrin levels.[8,9]

In the present study, we evaluated the clinical efficacy and mechanism of fecal microbiota transplantation (FMT) by extracting and analyzing the microbiota in feces from donors and patients with CFC, before and after FMT therapy. We hypothesized that if FMT can improve constipation, it may be because the metabolites of some beneficial bacteria in the transplant promote the contraction of the intestinal smooth muscle *via* the normalization of gastrointestinal hormones, such as those mentioned above.

Methods

Study design

The purpose of this study was to explore the therapeutic effect of FMT on patients with CFC ineffectively treated by conventional constipation medication. For the benefit of the included patients, a medication treatment control was not set; all included patients were treated with FMT. Therefore, this was a prospective self-control study, intended to evaluate the treatment efficacy by comparing patients before and after FMT treatment. Consecutive patients from the Gastroenterology Department of The First Affiliated Hospital of Chengdu Medical College, between April 2016 and October 2017, were enrolled. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Chengdu Medical College, and all patients and donors provided informed consent before entering the study. The study was registered at ClinicalTrials.gov [ClinicalTrials.gov identifier: NCT03018613].

Patient inclusion

We recruited patients aged 30–75 years who were diagnosed with CFC according to Rome III criteria (with confirmation by typical clinical, endoscopic, and histopathological findings) and were not responsive to medication treatment (cellulose preparations, laxatives, microecological preparations, and other conventional drugs). All included patients were able to communicate well with the researcher. The exclusion criteria were as follows: serious primary heart, liver, brain, kidney, or other serious diseases; severe anemia or severe systemic infection; history of thyroid disease; pregnancy or lactating; unable to provide full informed consent due to a mental disorder; history of abdominal or perianal surgery; history of digestive diseases (tumor, inflammatory bowel disease); history of an intestinal pathogen infection; and use of prebiotics, probiotics, or proton-pump inhibitors since January 2016.

Donor screening

Fecal donations for FMT were obtained from volunteers (four students aged 23–27 years). The donors were healthy individuals without diseases or pathologic conditions potentially associated with changes in the gut microbiota. Individuals who had used antibiotics or probiotics within the past month were not considered. All donors were required to complete a Donor Questionnaire form. In order to prevent the transmission of infectious diseases from the donor to the recipient, all donors underwent stool tests, including

Table 1. Patient groupings.

Group 1 (donor 1)	Group 2 (donor 2)	Group 3 (donor 3)	Group 4 (donor 4)
Patient 17	Patient 1	Patient 6	Patient 10
Patient 18	Patient 2	Patient 7	Patient 11
Patient 19	Patient 3	Patient 8	Patient 12
Patient 24	Patient 4	Patient 9	Patient 13
Patient 25	Patient 5	Patient 14	Patient 16
Patient 27	Patient 28	Patient 15	Patient 21
Patient 33	Patient 29	Patient 20	Patient 22
Patient 34	Patient 30	Patient 26	Patient 23
	Patient 32	Patient 31	

Table 2. Donors' clinical index scores.

Donor	WCS	SACS	PAC-QOL	BSFS	SDS score	SAS score
Donor 1	0	0	1	4	23	22
Donor 2	0	0	0	4	21	25
Donor 3	0	0	1	5	21	20
Donor 4	0	0	0	4	23	21

BSFS, Bristol stool form scale; PAC-QOL, patient assessment constipation quality-of-life questionnaire; SACS, self-assessment of constipation symptoms; SAS, self-rating anxiety scale; SDS, self-rating depression scale; WCS, Wexner constipation score.

bacterial culture and identification, fecal flora ratio examination, human rotavirus antigen determination, parasite egg detection to screen for most parasites, and *Cryptosporidium*, *Cyclospora*, and *Giardia* antigen detection. Microscopic and serologic tests, including tests for hepatitis A, B, and C viruses, human immunodeficiency virus antibodies, syphilis, herpes simplex virus, and Epstein–Barr virus. We also had strict dietary requirements; the fecal donors had a healthy diet and regular daily exercise, with no smoking, alcohol, or ingestion of unhealthy foods, such as preserved and canned food.

The four donors provided stool samples for 34 patients. Patients were divided into four groups, and the patients in each group received stool samples from the same donor (Table 1). The donors' clinical index scores (which were within normal ranges) are provided in Table 2.

FMT procedure

Patients received a bowel lavage (polyethylene glycol 4000) for colonoscopy preparation on the day before FMT. Donors were instructed to collect their feces (median amount, 50 g) on the day of the scheduled transplant. A total of 250 ml of extracted fecal suspension was prepared with a 0.9% NaCl solution using a conventional blender. The filtered fecal microbiota suspension was administered, into the descending duodenum *via* an inserted gastroscopy infusion catheter. After the procedure, the patient maintained a low head position for 60 min. Bowel movements were avoided for 0.5 h, food intake was forbidden for 1 h, and activity was prohibited for 2 h. All patients completed three rounds of FMT treatment, with the rounds spaced 3 weeks apart.

During both the treatment and follow-up period, the patients were instructed to not take medications for constipation.

Assessments

Clinical index scores. Before treatment, after each FMT treatment, and at 2 and 3 months of follow up, patients completed the following clinical scales to comprehensively assess the effect of FMT: the Wexner constipation score (WCS), self-assessment of constipation symptoms (SACS), patient assessment constipation quality-of-life questionnaire (PAC-QOL), Bristol stool form scale (BSFS), Zung's self-rating depression scale (SDS), and Zung's self-rating anxiety scale (SAS). The WCS scale was considered the main evaluation index to determine the treatment efficiency, while the SACS, PAC-QOL, BSFS, SDS, and SAS were considered secondary evaluation indices.

Electrointestinography (EGEG-5D Gastrointestinal Electrogram Tester, Hefei Aoyuan Technology Development Co., Ltd, China) was used to detect intestinal peristalsis by recording the average preprandial frequency, average postprandial frequency, average preprandial amplitude, and average postprandial amplitude before and after the entire course of FMT treatment.

Gastrointestinal hormone analysis. As motilin, gastrin, 5-HT, and NO are released into the blood after being produced in the intestinal tract, measuring the level of serum gastrointestinal hormones reflects their secretion from the intestine. Fasting venous blood was taken from patients before treatment and at 3 weeks after the first treatment, and centrifuged at 3000 r/min for 15 min at 4°C. The serum was separated and detected by the nitrate reductase method. Serum 5-HT, motilin, and gastrin were detected by enzyme-linked immunosorbent assay.

Intestinal flora analysis. A 16S ribosomal ribonucleic acid (rRNA) sequencing analysis was performed on donor stool samples (representing healthy intestinal flora) and patient stool samples before and after each treatment round. We extracted DNA from the stools of healthy donors and patients, and DNA pre-amplification and sequencing was carried out by Tianjin Novo Zhiyuan.

Statistical analysis

Measurement indicators are represented as mean and standard deviation, and counting indicators are presented as number and percentage of each category. The comparison between the two groups before and after treatment of measurement indicators was performed using paired t test. p values < 0.05 were considered statistically significant. Statistical analysis was performed to use SPSS 21 software (IBM Corp., Armonk, NY, US).

Results

Clinical outcomes

During the study, one patient withdrew (lost to follow up); thus 34 patients (12 men and 22 women) were finally analyzed. The WCS scale is a validated and internationally adopted questionnaire, used to quantify the severity of constipation, and was the main treatment efficiency evaluation index in the present study. This scale consists of questions examining various clinical expressions of constipation, with scores ranging from 0 (best) to 30 (worst). The score in healthy people is <8 points. In patients, scores <8 indicate a cure, 8–10 indicate remission, and scores >10 indicate ineffective treatment. From the end of the first treatment to the end of the follow-up period, the average values on the WCS scale among all patients at each treatment stage were <8, indicating that the FMT treatment was effective [Table 3, Figure 1(a)] At the end of the third treatment, the clinical cure rate was 73.5% (25/34, i.e. the WCS value was <8 in 25 patients), the clinical remission rate was 14.7% (5/34, i.e. the WCS value ranged from 8 to 10 in 5 patients), the overall efficacy was rate 88.2% (30/34, i.e. the WCS value was <10 in 30 patients), and the inefficiency rate was 11.8% (4/34, i.e. the WCS value was >10 in 4 patients).

The SACS, PAC-QOL, BSFS, SDS, and SAS were used as secondary evaluation indices. The SACS evaluates the severity of constipation by three aspects: fecal traits, rectal symptoms, and abdominal symptoms. The higher the score, the more severe the constipation. The SACS score before FMT treatment was 19.29 ± 6.53, the score after the first treatment was 8.36 ± 6.69, the score after the second treatment was 6.65 ± 5.04, and the score after the third treatment was 6.85 ± 4.77. The 2-month follow-up score was 4.33 ± 2.99 and the 3-month follow-up score was 3.83 ± 3.27 (Table 3). Compared with that before treatment, the SACS score was significantly decreased after FMT treatment [Figure 1(b)], indicating improved symptoms of constipation.

Table 3. Scores on clinical scales used to evaluate the clinical efficacy of fecal microbiota transplantation.

Project	Before treatment	After first treatment	After second treatment	After third treatment	2-month follow up	3-month follow up
WCS	13.53 ± 4.55	7.00 ± 3.25 ($p < 0.001$)	6.65 ± 3.34 ($p < 0.001$)	6.56 ± 3.69 ($p < 0.001$)	5.10 ± 2.39 ($p < 0.001$)	5.25 ± 3.22 ($p < 0.001$)
SACS	19.29 ± 6.53	8.36 ± 6.69 ($p < 0.001$)	6.65 ± 5.04 ($p < 0.001$)	6.85 ± 4.77 ($p < 0.001$)	4.33 ± 2.99 ($p < 0.001$)	3.83 ± 3.27 ($p < 0.001$)
PAC-QOL	55.41 ± 18.91	30.82 ± 19.63 ($p < 0.001$)	27.00 ± 13.93 ($p < 0.001$)	27.29 ± 15.45 ($p < 0.001$)	23.62 ± 14.21 ($p < 0.001$)	17.00 ± 14.18 ($p < 0.001$)
BSFS	2.41 ± 1.88	3.23 ± 1.63 ($p = 0.031$)	3.80 ± 1.51 ($p < 0.001$)	3.62 ± 1.10 ($p = 0.003$)	3.43 ± 1.43 ($p = 0.005$)	3.83 ± 0.94 ($p < 0.001$)
SDS score	55.44 ± 10.89	50.09 ± 12.33 ($p = 0.062$)	45.75 ± 10.74 ($p < 0.001$)	46.97 ± 9.20 ($p = 0.001$)	43.38 ± 7.97 ($p < 0.001$)	41.08 ± 7.48 ($p < 0.001$)
SAS score	44.71 ± 9.86	38.91 ± 9.92 ($p = 0.018$)	34.90 ± 6.66 ($p < 0.001$)	36.50 ± 7.08 ($p < 0.001$)	33.48 ± 4.40 ($p < 0.001$)	31.75 ± 4.09 ($p < 0.001$)

Data are expressed as the mean ± standard deviation. $p < 0.05$, the difference was statistically significant compared with before-treatment values. BSFS, Bristol stool form scale; PAC-QOL, patient assessment constipation quality-of-life questionnaire; SACS, self-assessment of constipation symptoms; SAS, self-rating anxiety scale; SDS, self-rating depression scale; WCS, Wexner constipation score.

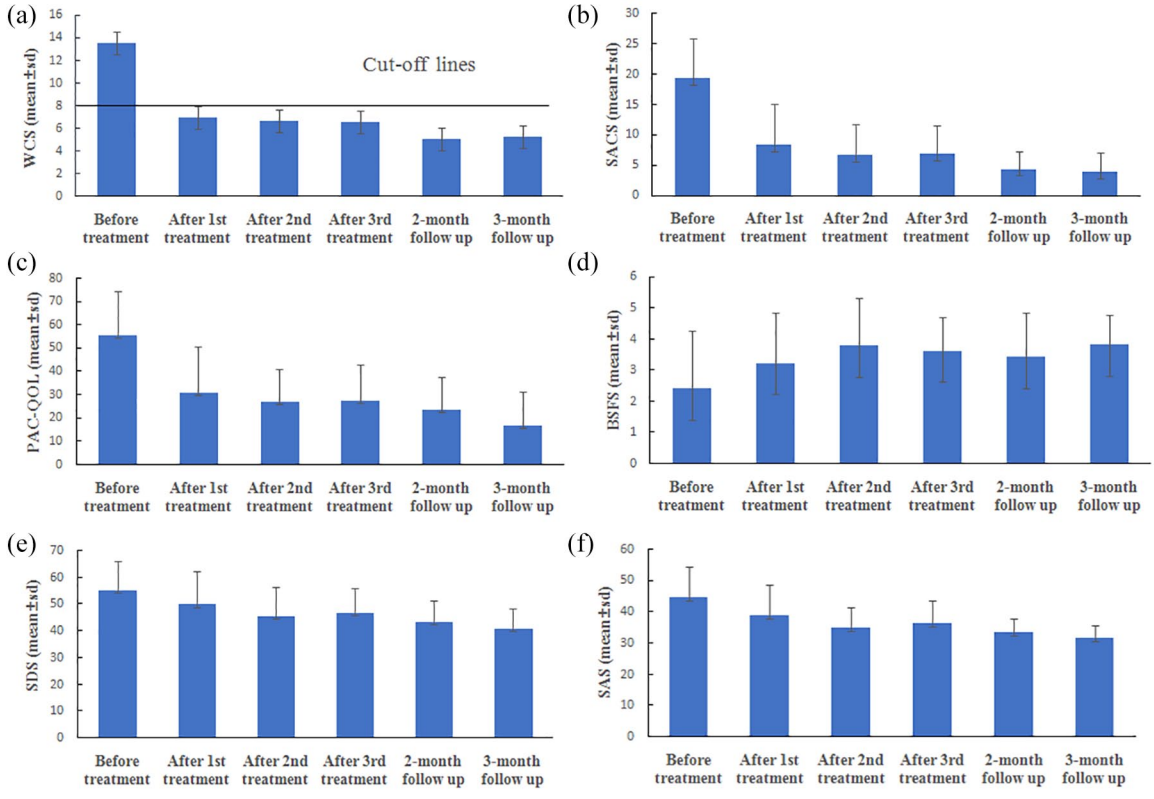

Figure 1. The clinical evaluation indices used to comprehensively evaluate the clinical efficacy of FMT.
(a) Wexner constipation score (WCS); (b) self-assessment of constipation symptoms (SACS); (c) patient assessment constipation quality-of-life questionnaire (PAC-QOL); (d) Bristol stool form scale (BSFS); (e) Zung's self-rating depression scale (SDS); and (f) Zung's self-rating anxiety scale (SAS).
FMT, fecal microbiota transplantation.

On the PAC-QOL questionnaire, higher scores indicate lower QOL. Compared with that before treatment, the PAC-QOL score was significantly decreased after FMT treatment [Table 3, Figure 1(c)]. At the 3-month follow up, the lowest PAC-QOL score was 17.00 ± 14.18, indicating that with the progression of FMT treatment, the QOL of patients improved significantly.

The BSFS mainly evaluates stool traits; stools rated 1 or 2 were defined as hard, 6 or 7 were defined as loose, and 3–5 were defined as normal. After FMT treatment, the average BSFS score among patients was >3, indicating a normal stool habit, without constipation [Table 3, Figure 1(d)].

Studies have shown that constipation can cause anxiety and depression, and that improvement in depression and anxiety can partially improve symptoms of constipation. Therefore, the SAS and SDS were used to analyze the effect of FMT treatment on depression and anxiety of patients with CFC. On the SDS, scores <50 indicate no depression, 50–59 indicate mild to moderate depression, 60–69 indicate moderate to severe depression, and >70 indicates severe depression. Compared with that before treatment, the SDS score was significantly decreased after FMT treatment. At the 3-month follow up, the lowest SDS score was 41.08 ± 7.48 [Table 3, Figure 1(e)]. On the SAS, scores <50 points indicate no anxiety, 50–60 indicate mild anxiety, 61–70 indicate moderate anxiety, and >70 indicate severe anxiety. The SAS score showed a continuous downward trend after FMT treatment. The SAS score was 44.71 ± 9.86 before treatment and 31.75 ± 4.09 at the 3-month follow up. These results indicate that the FMT treatment could relieve the depressive symptoms of patients with CFC [Table 3, Figure 1(f)].

In summary, through a comprehensive analysis of clinical indices, we found that FMT was an effective treatment in patients with CFC ineffectively treated by conventional medications.

Current research has found that gastrointestinal motility disorders are closely related to chronic constipation. Gastrointestinal motility disorders include delayed gastric emptying, slowed small bowel emptying, gastrointestinal coordinated movement disorders, and rectal contradictive contractions.[10] We tested the gastrointestinal electrogram before and after FMT treatment to evaluate the effect of FMT on gastrointestinal motility. The amplitude and frequency were used to evaluate gastrointestinal motility. The normal frequency is 7–12 times/min, and the normal amplitude is 90–190 μV. Within normal ranges, higher frequency and amplitude indicate better gastrointestinal motility. Although the average frequency and amplitude of gastrointestinal electrogram in patients with constipation are, in theory, lower than normal, our results showed that the average frequency and amplitude of the gastrointestinal electrogram were within normal preprandial and postprandial ranges for all groups (Table 4). However, compared with that before treatment, the average amplitude was increased after FMT treatment (Table 4), indicating that the FMT treatment could promote intestinal peristalsis and increase gastrointestinal motility.

Differences in the expression of NO, 5-HT, motilin, and gastrin with FMT treatment

The pathogenesis of the intestinal flora involved in constipation is unclear. The expression of NO, 5-HT, motilin, and gastrin were determined to evaluate whether FMT can treat constipation by regulating the expression of these molecules. The serum NO and 5-HT levels were significantly increased after FMT treatment [Figure 2(a)]. Serum motilin and gastrin levels were also increased after FMT treatment; however, the difference failed to reach statistical significance [Figure 2(b)].

Results of a Venn diagram analysis

A 16S rRNA sequencing analysis was performed to compare the intestinal flora before and after FMT treatment, as well as to compare the intestinal flora between donors and patients. After the removal of chimeras, the filtered high-quality sequences were grouped into 13,260 operational taxonomic units (OTUs; Supplemental Table 1). The Venn diagram in Figure 3 reflects the total number of OTUs in each group, the overlap OTUs between all groups, and unique OTUs in each group. The results showed the pretreatment group had the highest number of OTUs (9567), while the donor group and the three treatment groups had approximately the same number of OTUs (over 6000; Figure 3). Additionally, the results indicated that the pretreatment group had the highest number of unique OTUs (5012) compared with that in the donor group. However, with FMT treatment, the number of unique

Table 4. Gastrointestinal electrogram data before and after fecal microbiota transplantation.

Time	Average preprandial amplitude (μv)	Average postprandial amplitude (μv)	Average preprandial frequency (cpm)	Average postprandial frequency (cpm)
Before treatment	133.63 ± 41.39	186.06 ± 74.68	8.38 ± 4.58	8.55 ± 4.20
After first treatment	163.91 ± 84.78 ($p = 0.066$)	223.29 ± 77.49 ($p = 0.048$)	8.64 ± 1.01 ($p = 0.748$)	9.13 ± 0.67 ($p = 0.429$)
After second treatment	195.34 ± 53.81 ($p < 0.001$)	219.75 ± 48.39 ($p = 0.031$)	9.31 ± 0.84 ($p = 0.248$)	9.10 ± 1.09 ($p = 0.463$)
After third treatment	182.11 ± 53.84 ($p < 0.001$)	205.66 ± 83.54 ($p = 0.312$)	8.64 ± 0.77 ($p = 0.745$)	8.91 ± 0.43 ($p = 0.621$)

Data are expressed as the mean ± standard deviation.
cpm, counts per min; μV, microvolt.

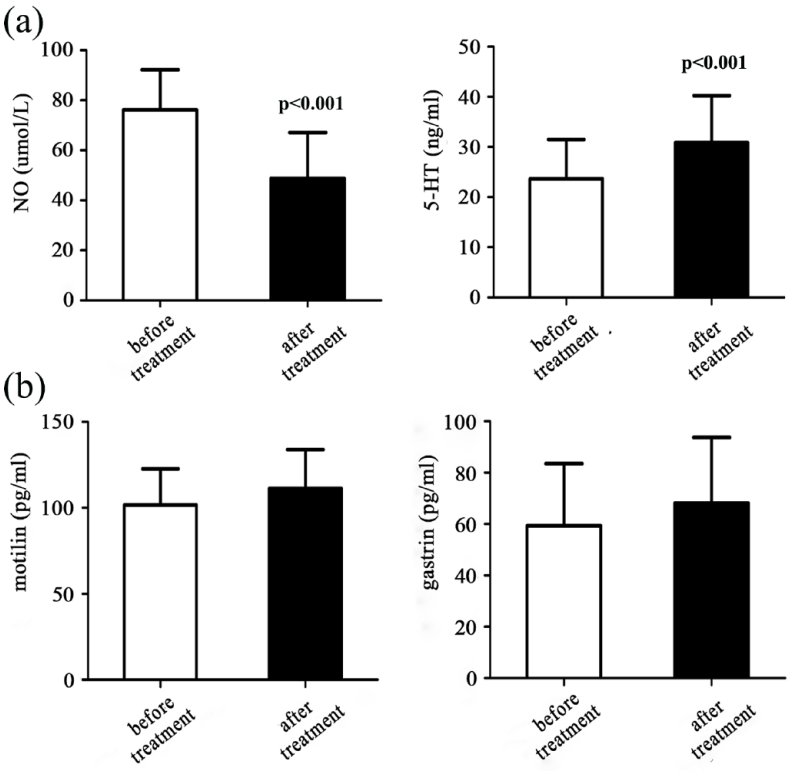

Figure 2. Changes in the expression of NO, 5-HT, motilin, and gastrin with FMT treatment.
(a) Serum NO and 5-HT levels before and after FMT treatment (mean ± SD) and (b) serum motilin and gastrin levels before and after FMT treatment (mean ± SD).
FMT, fecal microbiota transplantation; NO, nitric oxide; 5-HT, 5-hydroxytryptamine; SD, standard deviation.

OTUs in the treatment groups relative to that in the donor group gradually decreased (3142, 2707, 2619, respectively; Figure 3).

Relative abundance of intestinal flora in patients with CFC and donors

The analysis of variance and least-squares difference statistical analyses revealed no significant differences in intestinal microbial composition between patients with CFC and healthy donors. At the phylum level, all samples mainly comprised *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* (accounting for 98.2% of the total sequence). *Actinobacteria*, *Fusobacteria*, and *Verrucomicrobia* accounted for 0.7%, 0.5%, and 0.4% of the total sequence, respectively [Figure 4(a)]. At the genus level, all samples were dominated by *Bacteroides*,

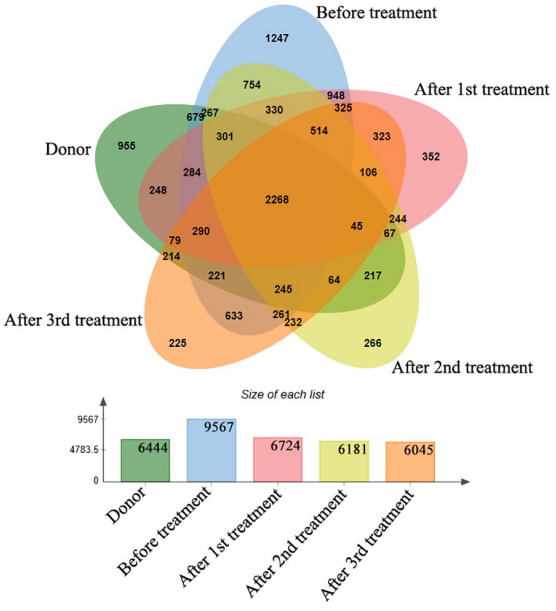

Figure 3. Venn diagrams of the shared and unique operational taxonomic units (OTUs) among all groups. Each ellipse in the figure represents a sample set. The number in the overlap region represents the number of OTUs shared between the sample sets. The number without overlap indicates the number of OTUs unique to the sample set. The lower histogram shows the total number within each sample set.

Prevotella, and *Ruminococcaceae* (accounting for 24.1%, 14.7%, and 13.0% of the total sequence, respectively). Additionally, *Lachnospira, Enterococcus, Megasphaera, Veillonella*, and *Streptococcus* accounted for 3.8%, 1.2%, 1%, 1%, and 0.8% of the total sequence, respectively [Figure 4(b)].

Although there were no significant differences in gut microbiota composition at the phylum level among all samples, there were significant differences in the composition of the flora at various treatment stages compared with that in the pretreatment group. *Bacteroidetes* and *Firmicutes* were the main phyla in the intestinal flora of the donors, with proportions of 41.8% and 53.5%, respectively. At pretreatment, the proportion of *Bacteroidetes* was 47.6% in the patients, which was higher than that in the donors, but at the 3-month follow up, the proportion was similar to that in the donor group. In contrast, the proportion of *Firmicutes* (44.4% at pretreatment) did not change significantly with treatment [Figure 4(c)]. At the genus level, the relative abundance of *Bacteroides* was generally higher in the patients at pretreatment than in the donors. The proportion of *Bacteroides* at the genus level was generally decreased after each treatment and at each follow up, and gradually approached that of the donor group. *Prevotella* accounted for 13.7% of the total sequence at pretreatment and showed an overall increasing trend with treatment. *Enterobacteriaceae* accounted for 2.8% at pretreatment, which was higher than that in the donors, and showed an overall downward trend with treatment, gradually becoming similar to that in the donors [Figure 4(d)].

Result of the linear discriminant analysis effect size analysis

The linear discriminant analysis (LDA) effect size (LEfSe) is used to analyze species that have significant differences in abundance between groups (biomarkers). As shown in Figure 5, a total of 27 significant different genera were identified by the LEfSe analysis of the donors and patients at different treatment stages. Among these, the donors were dominated by *Ruminococcaceae, Lachnospiraceae, Blautia, Anaerostipes, Dorea, Faecalibacterium*, and *Asticcacaulis*. At pretreatment, the patients were dominated by *Klebsiella, Megamonas, Erysipelotrichaceae, Epulopiscium*, and *Dorea*, among others. In contrast, the patients were dominated by *Bacteroides* and *Phascolarctobacterium* after the first treatment, by *Megamonas* after the third treatment, and by *Acidaminococcus* and *Butyricimonas* at the 2-month follow up (Figure 5).

Mild discomfort after FMT treatment

None of the 34 patients with CFC experienced a serious adverse reaction during the treatment and follow-up periods. Six patients experienced mild discomfort, including five patients with mild abdominal pain and discomfort after treatment, without requiring medical treatment; all abdominal pain symptoms were relieved within 24 h. One patient developed abdominal distension and increased anal exhaust; this patient rested and was hospitalized for observation. The abdominal discomfort spontaneously improved within 48 h.

Discussion

Related studies have shown that CFC is associated with an intestinal flora imbalance. The intestinal flora can reach 1000~1500 species,[11] a disruption in this balance can lead to disease. The present study showed that FMT is an effective

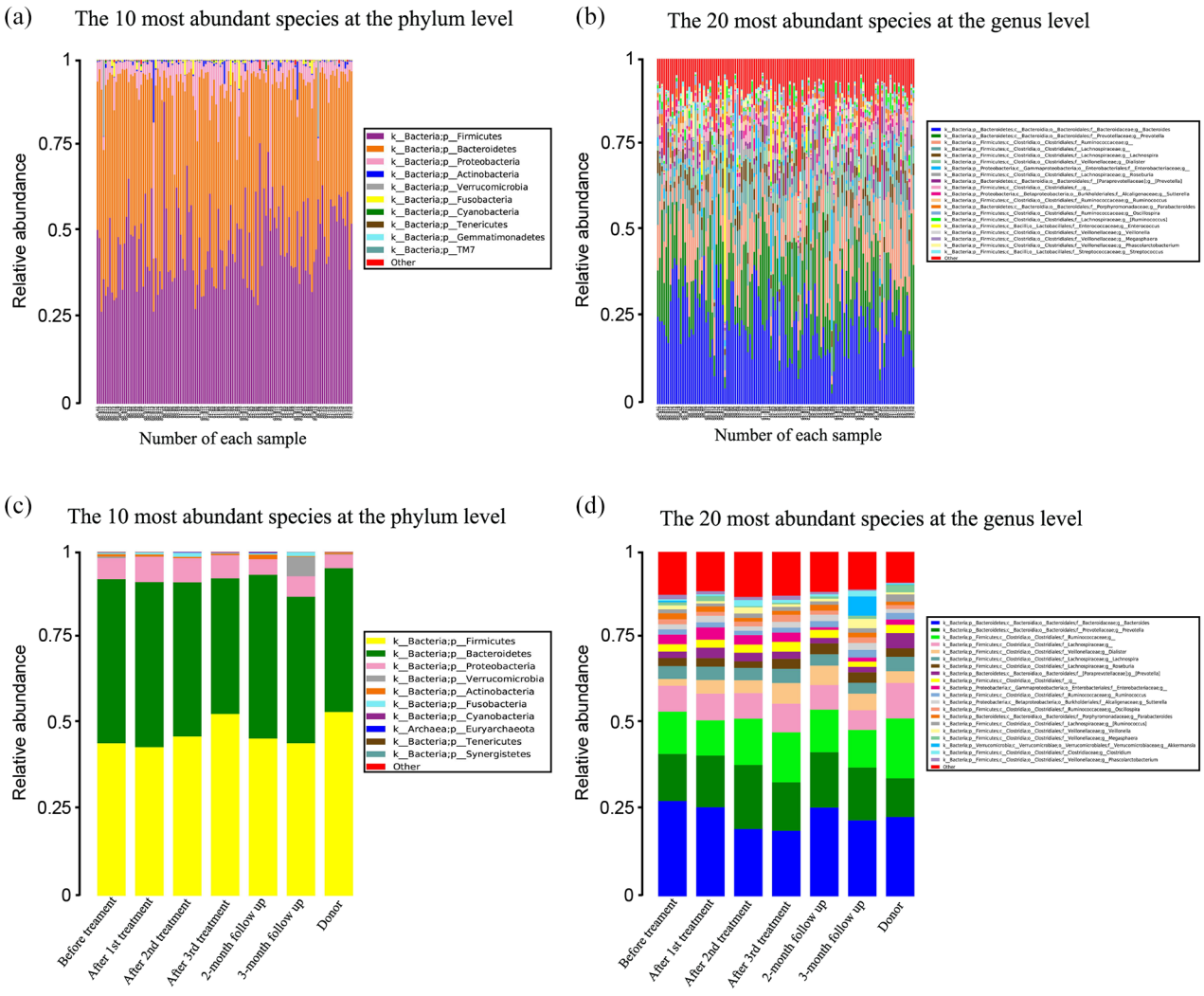

Figure 4. Relative abundances of fecal microbiota at phylum and genus level in patients with chronic functional constipation before and after treatment.

Different colors represent different bacterial species. (a) The 10 most abundant species at the phylum level. The abscissa indicates the sample name, and the vertical bar represents a sample; (b) the 20 most abundant species at the genus level. The abscissa indicates the sample name, and the vertical bar represents a sample; (c) the 10 most abundant species at the phylum level. The abscissa represents the group, and the vertical bar represents a group; and (d) the 20 most abundant species at the genus level. The abscissa represents the group, and the vertical bar represents a group.

treatment in patients with CFC ineffectively treated by conventional medications. The clinical cure rate was 73.5% (25/34), clinical remission rate was 14.7% (5/34), and inefficiency rate was 11.8% (4/34). SACS data suggested that the symptoms of constipation improved significantly. PAC-QOL data indicated that with the progression of FMT treatment, the QOL of patients was improved significantly. BSFS data showed that the patients had normal stool habits, without constipation. SAS and SDS data indicated that FMT could relieve the depressive symptoms of patients with constipation.[12]

Serum motilin, gastrin, 5-HT, and NO are gastrointestinal hormones closely related to gastrointestinal motility.[13] Serum motilin and gastrin levels are decreased in patients with CFC.[14,15] 5-HT activates 5-HT receptors in intestinal submucosal neurons, promoting intestinal peristalsis.[16,17] Studies have shown that an elevated NO level can aggravate the symptoms of constipation.[18,19] The present study found that serum 5-HT levels were significantly increased and NO levels were significantly decreased after FMT treatment compared to pretreatment levels. In contrast, there were no significant changes in

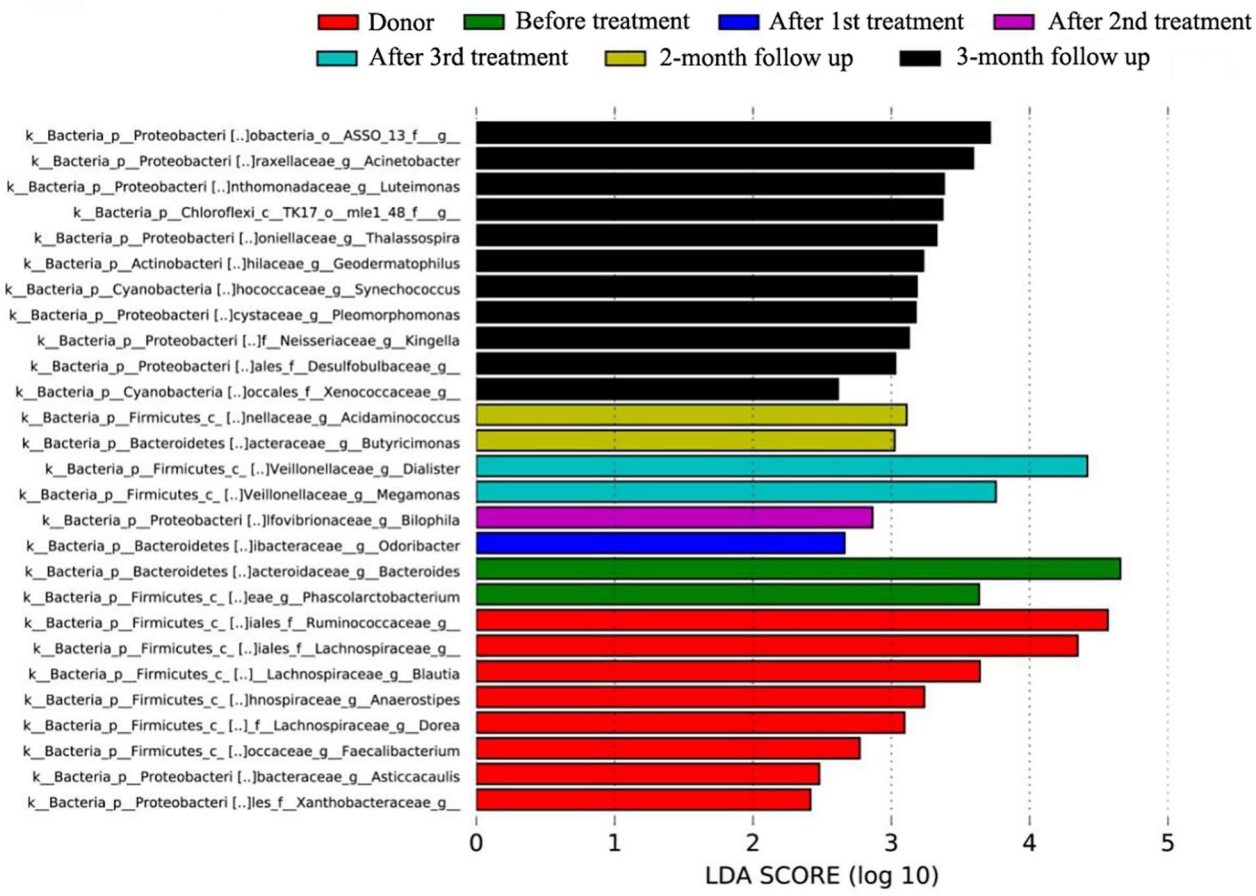

Figure 5. Diagram of the linear discriminant analysis (LDA) score.
The LDA score chart is shown for species with an LDA score greater than 2, which can be used as a biomarker for each group.

serum motilin and gastrin levels. These results may be due to a reconstitution of the structure of the intestinal flora after FMT, with a reduced number of bacteria producing NO. An increase in short-chain fatty acids among intestinal bacterial metabolites can promote the release of 5-HT, accelerating the movement of the colon.[20] However, FMT has little effect on motilin and gastrin in transmission. At present, there are no reports of an association between intestinal flora and serum motilin and gastrin levels. Thus, we speculate that the intestinal flora cannot directly stimulate the secretion of motilin and gastrin from the gastrointestinal mucosa.

The present study found that *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* mainly comprised the intestinal flora of both patients with CFC and donors; however, there were still some differences. Patients with CFC had an increased abundance of *Enterobacteriaceae*, and a reduced abundance of *Prevotella*, consistent with previous studies.[2] However, the abundance of *Enterobacteriaceae* decreased after FMT treatment, and the abundance of *Prevotella* increased after FMT treatment, gradually becoming similar to that in the donors. Therefore, we speculate that *Enterobacteriaceae* and *Prevotella* may be intestinal bacteria associated with secret seizures.

The LEfSe analysis showed that at pretreatment, the patients were dominated by *Bacteroidetes* and *Phascolarctobacterium*. After the third treatment, the patients were dominated by *Megamonas*, and at the first follow up, the patients were dominated by *Acidaminococcus* and *Butyricimonas*. The main metabolite of *Megamonas* and *Acidaminococcus* is acetic acid. Acetic acid promotes intestinal peristalsis and relieves constipation. Additionally, *Butyricimonas* is a butyrate-producing bacterium. Butyrate plays an important role in maintaining the function of the large intestine.[21] As the

abundances of *Megamonas, Acidaminococcus*, and *Butyricimonas* were increased after treatment, and the FMT treatment showed clinical efficacy, these three bacteria may play an important role in protecting the intestine and improving constipation.

Conclusion

In the present study, FMT treatment was shown to improve constipation symptoms and the constipation-related QOL, promote bowel movements, and improve anxiety and depression in patients with CFC. The study results suggest that the mechanism of FMT improvement in constipation involves the normalization of gastrointestinal hormones. FMT may rebuild the intestinal flora structure with normal function, producing short-chain fatty acids, which promote 5-HT secretion and intestinal peristalsis. Furthermore, FMT was shown to increase the production of acetic acid and butyrate bacteria, and reduce the production of NO, which could promote intestinal peristalsis. Thus, FMT is a promising new therapy for CFC. However, FMT itself is not a widely used method; thus, it is difficult to enroll patients. In addition, the number of patients with chronic intractable constipation that is ineffective or poorly treated by conventional constipation drugs in our hospital was not very large, further limiting the sample size of the study. In the follow-up research, we plan to include a control arm and expand the sample size.

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Author contributions

YT, YZ, and XL2 were involved in the conception and design of the study. YT, YZ, and JL provided laboratory expertise. YT, KZ, LZ, and XL1 acquired the data. YT and YZ conducted data analysis. YT, YZ, LZ, ZH, JZ, and XL2 interpreted the data. YT drafted the manuscript. YZ, YZ, and XL2 critically revised the manuscript for intellectual content, approved the final version, and agreed to be accountable for all aspects of the work (XL1: Xiaohui Li, XL2: Xiao-an Li).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Ethics approval and consent to participate

The trial was registered with the America Clinical Trials Registry [ClinicalTrials.gov identifier: NCT03018613]. All patients and donors gave written informed consent after counseling about the study and its potential risks and benefits.

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Supplemental material

Supplemental material for this article is available online.

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