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CLINICAL TRIALS AND THERAPEUTICS

Fecal microbiota transplantation through mid-gut for refractory Crohn's disease: Safety, feasibility, and efficacy trial results

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Abstact

Background and Aim: The gut microbiota plays a pivotal role in the intestinal diseases. Fecal microbiota transplantation (FMT) might be a rescue therapy for refractory inflammatory bowel disease. This study aimed to evaluate the safety, feasibility, and efficacy of FMT through mid-gut for refractory Crohn's disease (CD).

Methods: We established standardized laboratory protocol and clinical work flow for FMT. Only refractory CD patients with Harvey–Bradshaw Index (HBI) score ≥ 7 were enrolled for this study. All included patients were treated with single FMT through mid-gut and assessed during follow-up.

Results: Metagenomics analysis showed a high concordance between feces sample and purified fecal microbiota from same donors. Standardized fecal microbiota preparation and clinical flow significantly simplified the practical aspects of FMT. Totally, 30 patients were qualified for the present analysis. The rate of clinical improvement and remission based on clinical activity at the first month was 86.7% (26/30) and 76.7% (23/30), respectively, which was higher than other assessment points within 15-month follow-up. Patients' body weight increased after FMT, and the lipid profile improved as well. FMT also showed a fast and continuous significant effect in relieving the sustaining abdominal pain associated with sustaining CD.

Conclusion: This is a pilot study with the largest sample of patients with refractory CD who underwent single FMT. The results demonstrated that FMT through mid-gut might be a safe, feasible, and efficient rescue therapy for refractory CD.

Introduction

The gut microbiota is considered to constitute a "microbial organ" which plays a pivotal role in the intestinal diseases.[1] The gut metagenomic sequencing showed that over 99% of the genes are bacterial.[2] Previous studies from our group[3] and others[4,5] strongly support the link between intestinal bacteria and inflammatory bowel disease (IBD). IBD is a chronic inflammatory disorder of the gastrointestinal tract that includes both ulcerative colitis (UC) and Crohn's disease (CD). In IBD patients, the adaptive immune system is hyper-reactive to the commensal intestinal microflora in genetically susceptible individuals, and the intestinal flora species decreases by about 30 –50%.[6]

Patients with severe CD usually present with systemic symptoms such as abdominal pain, diarrhea, fatigue, anorexia, and malnutrition, sometimes with stricturing, fistula, or perforating complications.[7] The conventional approach for CD treatment is administration of multiple courses of corticosteroids and immunomodulators prior to escalation to monoclonal antibodies, while there are many disadvantages of these drugs.[8] Alternative CD treatment approaches aimed at modifying the composition of the intestinal microbiota in order to overcome gut dysbiosis have become a major interest in recent years.[9] Transplantation of the fecal microbiota from healthy donors is one of the potential alternative therapy options for IBD.[1,9–11]

The concept of fecal microbiota transplantation (FMT) for treatment of human intestinal diseases was firstly recorded in traditional Chinese medicine (TCM) in the fourth century.[12] In modern medicine, FMT has been used for successfully treating thousands of cases with *Clostridium difficile* infection[13,14] and limited cases with IBD.[9] All documented 12 reports of FMT for IBD until May 2013 had demonstrated a success rate close to 90% in improving UC[15] but had been rarely used for CD management.[15] Recently, three separate cases showed that clinical remissions were achieved by FMT in three severe CD patients.[16–18] One was reported by us about a patient with severe entercolonic fistulizing Crohn's disease.[16] This case has sustained clinical remission with normal work productivity over one year, and the follow-up is ongoing.

However, despite the long and successful track record, as well as great clinical need, the availability of FMT for many patients remains very limited due to unstandardized procedure for IBD and lack of systemic clinical study as well as the basic research. In 2012, we established the standardized FMT program, which has evolved since to overcome or minimize some of the associated challenges in isolation of gut flora and clinical work flow. This study explored the safety, feasibility, and efficacy of single standardized FMT through mid-gut for treatment of refractory CD.

Methods

Patient recruitment. All patients and donors agreed to participate in a clinical trial of FMT for moderate to severe CD (NCT01793831) at The Second Affiliated Hospital of Nanjing Medical University. Standardized FMT was offered to patients, whom had to be refractory to standard CD treatment options. At present time, there is no agreement on definition of refractory CD.[19] To define the refractory CD, we had made detailed standards, and patients were included or excluded according Table S1. The study started November 2012, and the end point of follow-up was February 2014.

Donor screening. Patients were asked to self-identify potential donor, such as relative, family member, friend, or the physician recommended fecal microbiota from our bacteria bank. A complete medical and surgical history of each potential donor was obtained. Donors were considered to be suitable if they had been screened by our exclusion criteria (Table S2). The age of donor ranged from 8 to 15-year olds, except one who was 28 years old. Eight patients shared donors with others because they couldn't supply a valid donor themselves. The volunteers who had passed the selection criteria were given two bags of Forlax (macrogol 4000) orally before defecation.

Patient preparation and FMT procedure. As shown in Table S3, at least one week prior to FMT, all conventional treatment for CD was stopped. Before FMT, patient condition was assessed thoroughly, including disease duration, disease localization, disease behavior, treatment and surgical history, body weight, as well as Harvey–Bradshaw Index (HBI). Peripheral blood was collected for chemical and biological analyses. Patients were prepared according to the preparation of custom gastroscopy. The detailed procedure of FMT had been shown in Table S3. Laboratory purified fresh fecal microbiota suspension was input into patients' mid-gut by a tube within gastroscope under anesthesia, and the entire procedure should be done within 1 h. If the fecal microbiota was obtained from bacteria bank under −80 °C, it should be thawed at room temperature first and then proceed to FMT immediately. After FMT, patients were encouraged to eat according to food instruction made by us, which was based on the literature by Macdertmott.[20] Mesalazine 3.0 g daily was given as a sustain treatment for three months and then the dose reduced to 1.5–2.5 g daily, no other medication was used. Although mesalazine had limited benefit in moderate to severe CD, the reason for using here was to provide adjunctive anti-inflammation effect for the patients who might achieve improvement to wild condition or get remission after FMT. To avoid the observation bias, Mesalazine was started from one week before FMT. Blood and stool samples were collected before and after FMT.

Here, we input the microbiota by mid-gut instead of duodenum, it is because of the possible route via duodenum below Vater papilla, or proximal jejunum post-gastrodunodenal surgery. The endoscopic parameters were collected, including the time of injecting bacteria suspension, whether there was reflux from small intestine to stomach and other adverse events.

Stool sample collection and microbial DNA extraction. Fecal samples were obtained from four clinical scanned donors (Table S2) after signing an informed consent form, and were isolated for microbiota at the laboratory. The fecal samples and isolated microbiota samples were frozen immediately and underwent DNA extraction using standard methods at BGI-Shenzhen.[2] For sequencing and data processing, Illumina GAIIx & HiSeq 2000 (San Diego, CA) were used.[2]

Surveillance post FMT. The whole process of this study was shown in Figure S1. The surveillance points started from the first day to the third day, one week, one month, three months, and later every three months after FMT. The activity of the disease was assessed by HBI based upon abdominal symptoms, examination findings, and presence of extraintestinal manifestations.[21] Clinical improvement was defined as decrease of HBI > 3. Clinical remission was defined as HBI score ≤ 4. All the patients who achieved clinical remission were also included in the analysis of clinical improvement. Blood and stool samples were collected at the same time and were analyzed by flow cytometry and laboratory examination, clinical activity was also assessed at each visit. For those patients living in remote provinces, some follow-up information was obtained through telephone and Internet. The surveillance was stopped in case of disease recurrence or getting worse.

Safety. Adverse events were recorded during FMT and after FMT. For assessment of longer term safety, all involved patients had more than six months of follow-up. Intensity and relationship of adverse events with FMT was described using Common Terminology Criteria for Adverse Events (version 3.0). Intensity of adverse events was classified as mild, moderate, severe, or disabling. Relationship of adverse events with FMT was categorized as unrelated, possible, probable, or definitely related to FMT.

Statistical analysis. Data were analyzed by using SPSS (Chicago, IL) or GraphPad (La Jolla, CA). Analyses included paired student's *t*-test for paired data and Fisher's exact test for categorical data. Two tailed *P* value was calculated with each test. *P* values < 0.05 were considered significant.

Ethical considerations. This clinical study (NCT01793831) had been approved by the Second Affiliated Hospital of Nanjing Medical University ethical committee in November 2012. All patients and donors were informed of the benefits and potential risks of standardized FMT and laboratory screening. Their written informed consent was obtained.

Results

Patient and donor characteristics. This study includes 49 patients who received standardized FMT through mid-gut for CD. All patients were Chinese living in China. Nineteen patients were excluded from analysis, including 11 patients with less than six-month follow-up, three patients with stoma, three for undefined IBD, one for accompanying with glycogen storage disease, and one for *C. difficile* infection. Finally, 30 patients were analyzed in this study (Table 1). Scanned for stool donation were 23 donors.

Sequencing of purified fecal microbiota and feces. The shotgun sequencing justified the similarity of bacterial composition between the purified fecal microbiota and original feces from four donors (Fig. 1). The purified fecal microbiota seemed higher than that of original feces, while there was no significant difference. This indicates that purification of fecal microbiota should be a good way to enrich bacteria for having sensitive sequencing results. The mapping rate to mOTU database was higher than that of original feces. This indicates that the purified fecal microbiota should be quite similar but less complex than original feces.

Safety of FMT and endoscopic procedure. There were no severe or obvious adverse events during endoscopic infusion after FMT and long-term follow-up. The injection of metoclopramide saved the time of endoscopic infusion (3.5 ± 2.16 min *vs* 6.0 ± 1.23 min, $P = 0.0192$). The rate of flora suspension reflux from duodenum to stomach in group with metoclopramide was lower than that in group without metoclopramide ($P < 0.05$, data not shown). The effect of metoclopramide might potentially avoid the adverse events during procedure, such as vomiting during endoscopy, adverse events after longer time anesthesia. Two (2/30) patients were observed with fever within 1 h to 6 h after FMT. This was considered as doubtful adverse event, because these patients also had fever after their colonoscopy under anesthesia before FMT. Within 1 h to 6 h after FMT, 23.3% (7/30) of patients had increased diarrhea. Although no pain and fecal ileus occurred, the criteria had to be mentioned that those patients with severe stricture in rectum were

Table 1 The characteristics of included patients and donors

	Items	Results
Patient	Total number	30
	Age, m ± SD (range)	38.0 ± 13.83 (15–71)
	Sex, male % (*n*)	64.5 (19)
	Disease duration (years, m ± SD)	7.4 ± 5.3
	Harvey Bradshaw Index (m ± SD)	11.7 ± 4.5
	Location, % (*n*)	
	L1 (with or without p)	26.7% (8)
	L2 (with or without p)	10% (3)
	L3 (with or without p)	63.3% (19)
	Weight (kg, m ± SD)	48.8 ± 10.1
	Using immunomodulator, yes % (*n*)	66.7% (20)
	Using steroid (> 10 mg/d prednisone equivalent), yes % (*n*)	56.7%(17)
	Using anti-TNF, yes % (*n*)	20% (6)
	With history of surgery, yes % (*n*)	60% (18)
	Frequency of surgery (*n*)	
	Intestinal surgery	20
	Anal surgery	11
Donor	Total number	23
	Age, m ± SD (range)	14.3 ± 5.2 (5–28)
	Sex, male % (*n*)	56.5 (13)
	One donor to ≥ 2 recipients, % (*n*)	26.1% (6)
	Genetic background, yes % (*n*)	26.1% (6)

Immunomodulator here did not include steroid.
Genetic background with recipient: yes, with brother, sister or parent-child relationship between donor and recipient.
L1, ileal; L2, colonic; L3, ileocolonic; P, perianal disease (according to Montreal classification of CD).

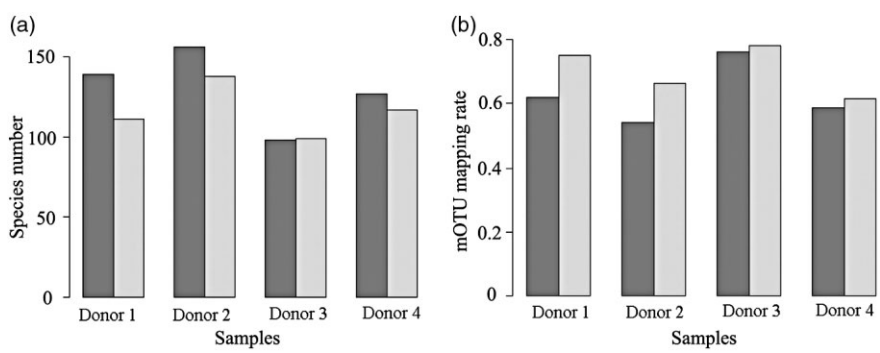

Figure 1 Metagenomics analysis. (a) The species number of bacteria was evaluated by paired Wilcox test, and the results showed that there were no significant differences between the purified fecal microbiota and original feces from four donors. (b) The mapping rate to mOTU database between two groups further supported the similarity. The feces sample ($n = 4$) and purified microbiota samples ($n = 4$) were from four healthy donors. ■, purified; □, fecal.

excluded for FMT. Except of the above, there were no more adverse events during the whole FMT procedure and 6–15-month follow-up.

Response to FMT. CD-related abdominal pain, stool frequency, bloody purulent stool, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), fistula, and other parameters were assessed for all patients after FMT. Endoscopy, magnetic resonance imaging or computed tomography scan was generally performed six months after FMT. HBI at each assessment point from one week post-FMT dramatically decreased compared with the baseline HBI before FMT (Fig. 2). Both rates of clinical improvement (86.70%) and clinical remission (76.7%) reached the peak of 15-month follow-up at the first month post-FMT. The longest follow-up until the endpoint of this study was 15 months, and the shortest follow-up included in this analysis was 6 months (Table 2). Those patients weren't involved in later assessment for having not reached the required assessment time point. Twenty-six of 30 patients had abdominal pain or related back pain (defined as CD-related pain). Figure 2 showed an immediate significant relief at several hours post-FMT. And body weight increased significantly at three-month assessment point after FMT.

As shown in Figure 3a–c, the level of ESR decreased one month post-FMT compared with the value before FMT ($n = 27$, $P < 0.01$), CRP in peripheral blood decreased at 1 week after FMT ($n = 20$, $P < 0.01$), and the serum immunoglobulin M (IgM) increased significantly 1 month after FMT ($n = 18$, $P < 0.01$). Blood lymphocytes of 17 patients were analyzed by FCM at later phase. Three patients were excluded from the analysis for having been treated with immunomodulators or steroid when presented to our hospital, which may disturb the normal lymphocyte system. As shown in Figure 3d–i, T lymphocyte, $CD3^+CD4^+$ cell, and ratio of $CD4^+/CD8^+$ was increased at three days post-FMT ($P < 0.05$, $n = 14$), while there were no significant change in B lymphocyte, NK cells, and $CD3^+$ $CD8^+$ cells before and after FMT treatment.

Changes of blood biochemistry. Plasma hemoglobin, albumin, and blood lipid were analyzed (Table 3). Three months after FMT, the level of plasma hemoglobin, albumin, total cholesterol, HDL-C, and LDL-C all showed significant increase compared with that before FMT ($P < 0.05$).

FMT-related factors. We tested the correlation between the potential impact factors (including donors' age, relative genetic background, or close contact with recipients, fecal bacteria's form) and patients' clinical response at six months post-FMT. As seen in Table 4, relative genetic background or close contact with recipients did not significantly affect the outcome of patients' clinical response at six months after FMT. There were no differences in patients' clinical response between the two age groups of donors, which were divided by the mean age of donors (14-year olds). The efficacy of FMT using fresh fecal microbiota seemed higher than that using frozen fecal microbiota, however, the difference on the clinical improvement or clinical remission was not significantly.

Discussion

The intestinal microbiota plays important roles in the regulation of human physiology,[2] include metabolism, nutrition absorption, and

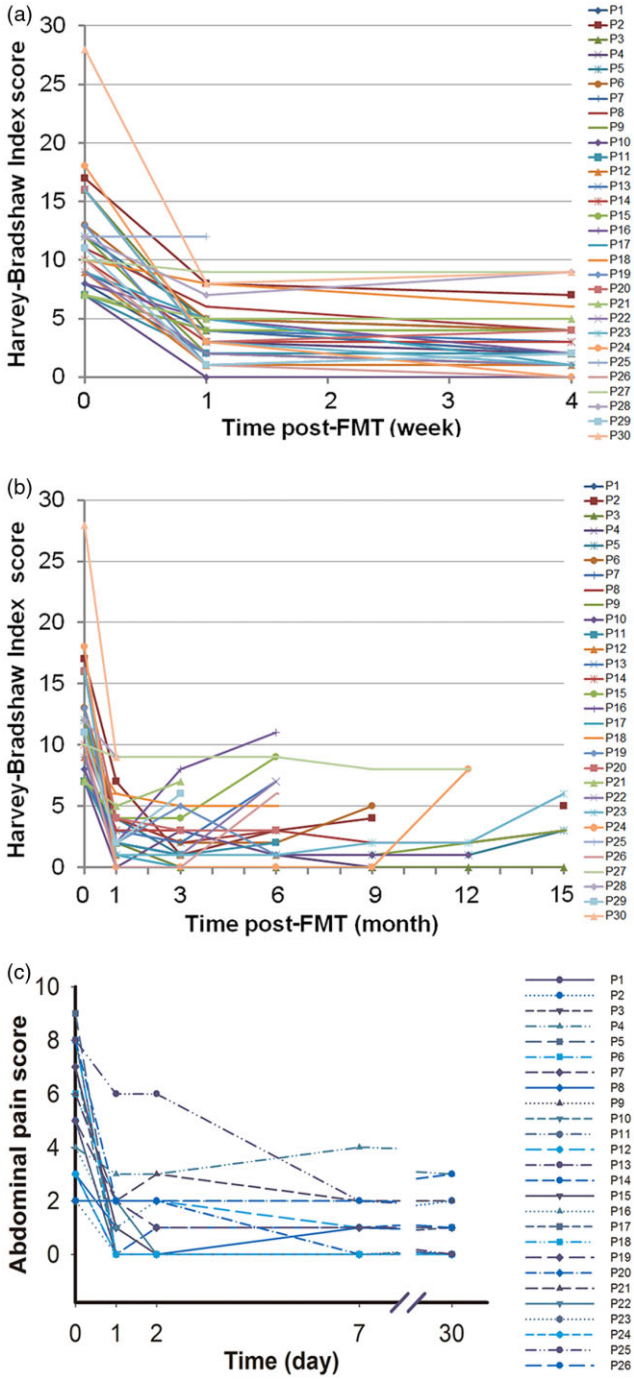

Figure 2 Harvey–Bradshaw Index (HBI) score analysis and abdominal pain remission. (a) The HBI score at the baseline before fecal microbiota transplantation (FMT), and surveillance point at first week and first month post-FMT. (b) The change of patients' HBI scores from basic value before FMT to 15months post-FMT during the follow-up. (c) FMT relieved abdominal pain ($n = 26$) and sustained the effects (trend of longer follow-up than one month not shown), four cases without pain before and after FMT were not included.

Table 2 Clinical response to FMT

Clinical response	Post-FMT	Clinical improvement (*n*)	Clinical remission (*n*)
Activity based HBI	1 week	83.3% (25/30)	60% (18/30)
	1 month	86.7% (26/30)	76.7% (23/30)
	3 month	80% (24/30)	70% (21/30)
	6 month	66.7% (20/30)	60% (18/30)
	9 month	57.1% (12/21)	52.3% (11/21)
	12 month	60% (9/15)	53.3% (8/15)
	15 month	85.7(6/7)	57.1(4/7)
Body weight (before *vs* post)	1 month	48.9 ± 10.1 *vs* 49.9 ± 10.2* (30)	/
	3 month	48.3 ± 9.9 *vs* 50.1 ± 11.3* (23)	/
	6 month	48.8 ± 9.9 *vs* 51.8 ± 10.9** (22)	/

Paired *t*-test, $*P < 0.05$, $**P < 0.001$.

The comparison of HBI between post-FMT in each objective endpoint from one week to 15 months and baseline is significantly (paired *t*-test, $P < 0.005$, data not shown in this table). The rates of clinical improvement include the patients who achieved clinical remission. Body weight (mean ± SD).

FMT, fecal microbiota transplantation; HBI, Harvey–Bradshaw Index.

Table 3 Chemical changes after FMT during follow-up

Parameter (normal range)	*n*	Before FMT	3 months post-FMT	*P*
Hemoglobin (110–160, g/L)	24	101.0 ± 23.09	114.0 ± 21.80	0.0016**
Albumin (39.7–49.4, g/L)	24	32.95 ± 8.96	38.83 ± 7.95	0.0067**
Total cholesterol (3.0–5.7 mmol/L)	18	3.29 ± 1.01	3.67 ± 0.77	0.0327*
Triglycerides (0.4–1.7 mmol/L)	18	1.26 ± 0.82	1.54 ± 0.85	0.2934
HDL-C (1–2 3.1 mmol/L)	18	0.98 ± 0.28	1.15 ± 0.30	0.048*
LDL-C (< 3.1 mmol/L)	18	1.78 ± 0.63	2.02 ± 0.51	0.0066**

$*P < 0.05$; $**P < 0.01$.

Paired *t*-test, the value (mean ± SD) of each assessment point after FMT compared with that before FMT.

FMT, fecal microbiota transplantation; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol.

immunological reaction.[22–24] Here, we established a new standardized FMT protocol to rebuild refractory CD patients intestinal microbiota circumstance, including the crude purification of microbiota from feces, preparation of recipient before transplantation, endoscopic protocol through mid-gut, volume of purified microbiota for a single therapy, and the food plan before and after the FMT.

Shotgun sequencing showed that this method successfully maintained the bacteria composition in the purified microbiota compared with the original feces microbiota. The selected 30 cases in this study had failed or had been proven not suitable to biotherapeutics, immunosuppressant, steroids, aminosalicylic acid, or surgery. After FMT treatment, the overall clinical improvement and clinical remission of these cases was 83.3% and 60% at the first week, respectively. This strongly showed the fast clinical response to FMT through mid-gut. The highest rates of clinical improvement (86.7%) and clinical remission (76.7%) after FMT were at the first month. The follow-up within the 15 months further showed the sustained clinical efficacy. The reason why the highest efficacy appeared at first month post-FMT remained unknown. Further metagenomic analysis with patients' feces microbiota will provide more evidences. A possible explanation was the host intestine may exert colonization resistance to the transplanted microbiota at beginning, after one month's remodeling, a new balance was rebuilt between the foreign microbiota and host microbiota, which would exert maximal efficacy. But the balance might be vulnerable to the effect of host's genetic, intestinal environment, dietary, and other pathogenesis, which might ultimately cause the disease relapse.

One of aims of therapy for CD is to regain work and productivity improvement.[25] FMT therapy significantly improved the productivity outside of work in the present population of patients. Unfortunately, the work ability was not included for assessment.

The more surprising finding in this study was the role of FMT through mid-gut in relieving CD-related pain, which used to be a clinically challenge for a physician. Although the result should be confirmed by a large placebo group, this implied definitely novel clues on translational research from gut flora remodeling to pain management.

The body weights, as well as the hemoglobin, albumin, and lipid profile of patients with CD were improved after FMT. Of the involved patients, 35.5% (11/30) had dry, itchy, or burst skin, which was defined as "skin lesions," 72.7% (8/11) of them had lesions relieved within two weeks after FMT (data not shown). Seven in 10 patients who had sexual life were reported to have significant improvement in the quality of sexual life in three months after FMT (data not shown). These interesting phenomenona might relate to the improvement of nutrition status after FMT.

The decreased CRP and ESR one week post-FMT showed the fast anti-inflammation effect. FCM test demonstrated the

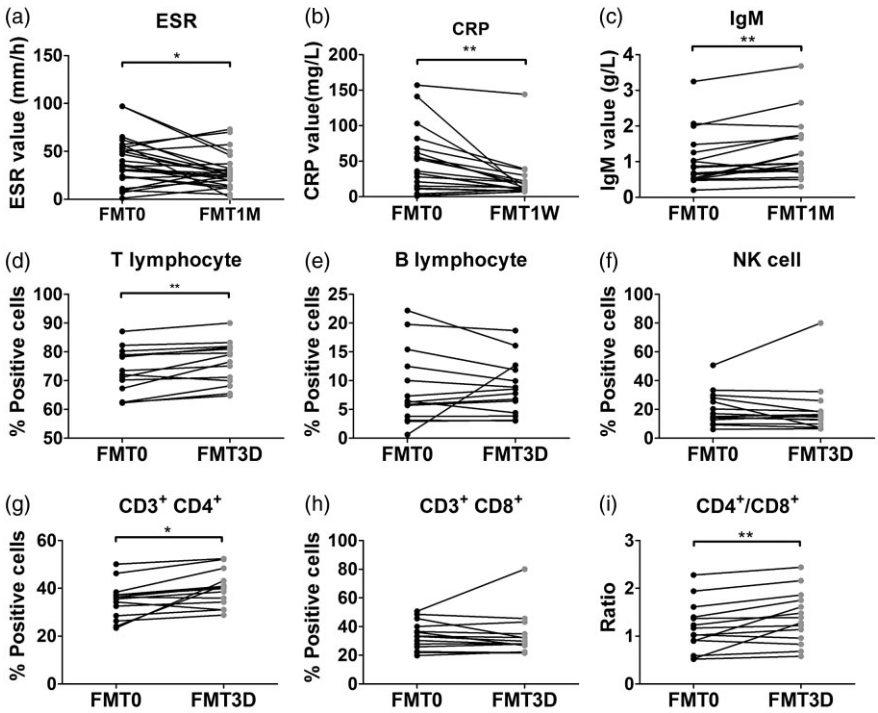

Figure 3 Assessment of inflammation and immunology function after fecal microbiota transplantation (FMT). (a) The level of ESR decreased one month post-FMT respectively, $n = 26$, $P < 0.01$; (b) CRP decreased one week post-FMT, $n = 20$, $P < 0.01$; (c) Serum immunoglobulin M (IgM) increased significantly one month post-FMT, $n = 18$, $P < 0.01$; (d–i) T lymphocyte, $CD3^+$ $CD4^+$ cell, and ratio of $CD4^+/CD8^+$ was increased at three days post-FMT ($P < 0.05$, respectively, $n = 14$), while the difference of B lymphocyte, NK cells, and $CD3^+$ $CD8^+$ cells between before and after FMT treatment were not significant.

Table 4 Impact factors on FMT efficacy

Donor and fecal bacteria information		Six months post-FMT	
		Clinical improvement (*n*)	Clinical remission (*n*)
Relative genetic background	yes	66.7% (4/6)	66.7% (4/6)
	no	62.5% (15/24)	50% (12/24)
	P	1.0000	0.6567
Close contact with recipient	yes	66.7% (4/6)	66.7% (4/6)
	no	62.5% (15/24)	50% (12/24)
	P	1.0000	0.6567
Age of donor (years)	≤ 14	75% (9/12)	66.7% (8/12)
	> 14	55.6% (10/18)	44.4 (8/18)
	P	0.4425	0.2839
Bacteria form	fresh	69.6% (16/23)	56.5% (13/23)
	frozen	42.9% (3/7)	42.9% (3/7)
	P	0.3717	0.6746

Relative genetic background: yes, brother, sister or parent–child relationships between donor and recipient; no, no genetic relationships. Fisher's exact test for all analysis.
FMT, fecal microbiota transplantation.

$CD3^+CD4^+$ cells, T lymphocyte, and ratio of $CD4^+/CD8^+$ three days post-FMT significantly increased than those before FMT. The serum IgM levels also increased at one month post-FMT. These immunological changes demonstrated the remodeling of gut flora increased the homeostatic immunological ability status.

There have been very few possible adverse events reported for FMT therapy in the literature.[26,27] Consistently, there is only very minor possible adverse event in the present study, suggesting FMT therapy is a relative safe procedure. Spontaneous resolved short-term fever in a few patients may be a "doubtful" adverse event related to FMT, which has been reported by previous studies.[14,28] And serious strictures of colon might be a potential high risk of fecal ileus after FMT. However, an effective health education and strict instruction on daily food might be important to sustain the efficacy of FMT.

The present clinical evidences indicated that the genetic relationship or close contact between the donor and patients, the age of donor within the selected range and the dose of bacteria were not associated with the efficacy of FMT. However, the fresh fecal

microbiota appeared to have higher rate of clinical improvement and clinical remission than frozen microbiota. But this was a case series; a small sample analysis may not be powerful to detect a difference in the dose of bacteria for FMT.

There are several limitations in this pilot study. It was not a rigorous clinical trial designed to test efficacy of particular FMT methodology *versus* another. The subgroup of disease location responded to FMT were not analyzed. Instead, it was an attempt to standardize FMT, as the procedure protocol evolved in the course of our clinical experience. A multicenter randomized clinical trial with larger sample size would be important to provide more evidences. Longer follow-up is necessary for assessment of safety and efficacy. Endoscopy evaluation was not performed for each case within six-month follow-up because of potential risk. We are currently trying to identify the bacteria species which are therapeutically most important to patients by characterizing the microbial composition of donor material and recipients' fecal samples collected during follow-up.

In conclusion, this is a pilot study with the largest sample of patients with refractory CD who underwent standardized single FMT through mid-gut. This study reported a new standardized laboratory protocol and specific clinical work flow. The strength lies in that this study evaluated serial objective endpoints and outcomes apart from clinical remission which included biochemical improvement, weight, and markers of immune system. The results demonstrated that standardized FMT through mid-gut might be a safe, feasible, and efficient rescue therapy for refractory CD.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1 Study design and procedures involved in the research.

Table S1 Inclusion and exclusion criteria for refractory CD in the present study.

Table S2 Exclusion criteria of donor for stool.

Table S3 Schedule and protocol of standardized fecal microbiota transplantataion.

Supplementary figure 1. Study design and procedures involved in the research.

Supplementary table 1. Inclusion and exclusion criteria for refractory CD in the present study

Inclusion and exclusion criteria for refractory CD in the present study
Inclusion criteria
All patients must be moderate to severe activity (basic HBI ≥ 7), and meet at least one or more standards listed below： 1. Age ≤ 16 year old, recurrence > 2/ year; 2. Accumulative intestinal lesions exceeded 100 cm, recurrence > 2/ year; 3. Perianal disease or intestinal fistula, no emergency, recurrence > 2/ year; 4. Recurrence after intestinal operation; 5. Recurrence after steroid therapy , recurrence > 2/ year; 6. Recurrence after immunomodulator therapy, or failure to immunomodulator therapy; 7. Recurrence after biologic therapy, or failure to biologic therapy; 8. Recurrence > 2/ year, with diabetes, failure to 5-ASA.
exclusion criteria
The patients would be excluded from the current analysis for: 1. Age < 14 years; 2. HBI < 7; 3. Accompanied with other severe disease (involve C.diff infection); 4. Follow-up less than 6 months.
The standards were set up according to Monteral classification of CD. Activity of CD based on HBI: moderate = 5-8; severe ≥ 8.

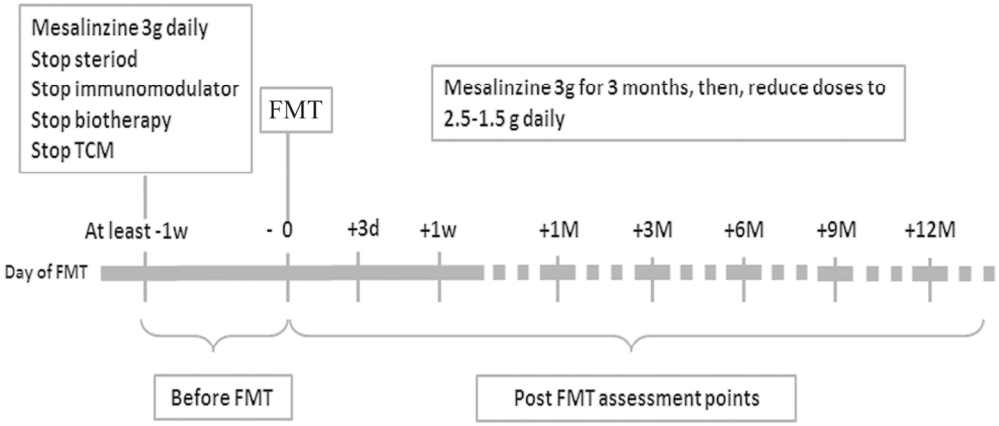
Supplementary table 2. Exclusion criteria of donor for stool

Exclusion criteria of donor
History of drug use
Received antibiotics, laxative or diet pills within the past 3 months; Received immunomodulators or chemotherapy.
History of diseases
History of all known infectious diseases, morbid obesity, diabetes, IBD, IBS, chronic diarrhea, constipation, colorectal polyps or cancer, immunocompromise, metabolic syndrome, allergy, and chronic fatigue syndrome. Major GI surgery (eg, gastric bypass) or systemic autoimmunity and other diseases or conditions potentially associated with specific changes in intestinal microbiota.
Positive laboratory examination
Regular blood cell test, CRP, ESR, biochemical tests, blood virus: Hepatitis A IgM, Hepatitis B surface antigen, Hepatitis B core IgG and IgM, Antibodies to hepatitis B surface antigen, Hepatitis C antibody, HIV types 1 and 2 antibody, Syphilis.
Stool testing
Stool regular test, stool ova and parasites test.
Others
High-risk sexual behaviors, incarcerated, gotten any tattoos or body piercings exposure to epidemic area within the past 3 months.

IBD: inflammatory bowel disease; IBS: irritable bowel syndrome; GI: gastrointestinal; CRP: C- reactive protein; ESR: erythrocyte sedimentation rate; HIV: human immunodeficiency virus.

Supplementary table 3. Schedule and protocol of standardized fecal microbiota transplantataion

Schedule and protocol of standardized fecal microbiota transplantataion	
Patient recruitment	Patients with refractory CD (Table 1)
Patient preparation	Start mesalazine 3g daily and stop steroid, immunomodulator, biotherapy, TCM > 1 week prior to FMT (Figure 1). Fasting 4-6 hrs before FMT; Esomeprazole Magnesium 40 mg by iv. and metoclopramide 10 mg by im. 1hr before FMT
Donor screening	Healthy relatives or family members are recommended; Screened by our exclusion criteria (Table 2)
Donor preparation	Take Forlax (Macrogol 4000) 2 bags by oral before defecation
Fecal microbiota purification	1. *Feces collection:* Collect all fresh feces with a sterilized container and transferred into blender. 2. *Fecal suspension Preparation:* Add 500-1000 mL 0.9% saline to blender, mix with feces thoroughly to make suspension, then filter using the scan within the blender and collect suspension. 3. *Filter:* Filter using a special micro-strainer for 4 times and collect suspension. 4. *Centrifuge:* Transfer suspension to 50 mL centrifuge tubes and centrifuge with 1200g for 3min. 5. *Wash:* Discard the supernatant; Add sterilized saline to 50ml, mixed gently by up and down, centrifuge again. 6. *Repeat centrifuge and wash steps for 3-5 times* 7. *Discard the most of supernatant, the ultimate deposit is the crudely purified fecal microbiota.* 8. *Fecal microbiota suspension:* Dilute the flora with 1.5-fold 0.9% normal saline; mix gently by up and down. The fresh concentrated fecal microbiota suspension must be administered to the patient intestine immediately. 9. *Bacteria storage:* The suspension also can be stored with 10% sterile pharmaceutical grade glycerol at -80℃. 10. *Endoscopic procedure:* 150-200 mL liquid suspension (include ~ 60cm^{3}fecal flora and ~ 100 mL normal saline) is transplanted into patient's mid-gut through tuble in the gastroscope under anesthesia.
Follow-up	*Each ssessment point*



Supplementary figure 1. Study design and procedures involved in the research.
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