Journal of Translational Medicine

RESEARCH

Open Access

Fecal microbiota transplantation restores gut microbiota diversity in children with active Crohn's disease: a prospective trial

Biao Zou[1†], Shengxuan Liu[1†], Chen Dong[1], Hexiao Shen[2], Yongling Lv[2], Jiayi He[1], Xuesong Li[1], Mengling Ruan[1], Zhihua Huang[1] and Sainan Shu[1*]

Abstract

Background Clinical data on oral fecal microbiota transplantation (FMT), a promising therapy for Crohn's disease (CD), are limited. Herein, we determined the short-term safety and feasibility of FMT for pediatric patients with active CD.

Methods In this open-label, parallel-group, single-center prospective trial, patients with active CD were treated with oral FMT capsules combined with partial enteral nutrition (PEN) (80%). The control group comprised pediatric patients with active CD treated with PEN (80%) and immunosuppressants. Thirty-three patients (11.6 ± 1.82 years)—17 in the capsule and 16 in the control groups—were analyzed. Data regarding the adverse events, clinical reactions, intestinal microbiome composition, and biomarker parameters were collected and compared post-treatment.

Results At week 10, the clinical and endoscopic remission rates did not differ between the two groups. By week 10, the mean fecal calprotectin level, C-reactive protein level, erythrocyte sedimentation rate, simple endoscopic score for CD, and pediatric CD activity index decreased significantly in the capsule group (all $P < 0.05$). The main adverse event was mild-to-moderate constipation. Core functional genera, *Agathobacter*, *Akkermansia*, *Roseburia*, *Blautia*, *Subdoligranulum*, and *Faecalibacterium*, were lacking pre-treatment. Post-treatment, the implantation rates of these core functional genera increased significantly, which positively correlated with the anti-inflammatory factor, interleukin (IL)-10, and negatively correlated with the pro-inflammatory factor, IL-6. The combination of these six functional genera distinguished healthy children from those with CD (area under the curve = 0.96).

Conclusions Oral FMT capsules combined with PEN (80%) could be an effective therapy for children with active CD. The six core functional genera identified here may be candidate biomarkers for identifying children with CD.

Trial registration: ClinicalTrials.gov, retrospectively registered, ID# NCT05321758, NCT05321745, date of registration: 2022-04-04.

Keywords Oral fecal microbiota capsules, Crohn's disease, Efficacy and safety, Core functional genera, Children

†Biao Zou and Shengxuan Liu are joint first authors.

*Correspondence:
Sainan Shu
snshu@tjh.tjmu.edu.cn
[1] Pediatric Department, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, No. 1095, Jiefang Road, Wuhan 430030, Hubei, China
[2] School of Life Science, Hubei University, Wuhan 430030, Hubei, China

Background

Crohn's disease (CD) is a chronic, non-specific inflammatory disease of unknown etiology. Over the last decade, the incidence of CD has gradually increased worldwide, with a rising prevalence among younger patients [1, 2]. CD poses more significant challenges for pediatric patients than adults [3]. However, treatment options for

CD in children are limited. Traditional CD treatment regimens focus on controlling inflammation using corticosteroids and immunosuppressants. Although these treatment options have been continuously developed and improved, there are still some limitations, such as adverse events (AEs), immune tolerance, drug resistance, and easy recurrence [4–6].

In recent years, studies have confirmed that the qualitative and quantitative characteristics of the gut microbiota in patients with CD differ significantly from those in healthy individuals [7]. This disparity is primarily evidenced by a reduction in biodiversity among CD patients, which is characterized by a decrease in protective flora and a notable increase in pathogenic flora [7, 8]. The dysbiosis of gut microbiota may be the key trigger point for the development of CD [8, 9]. Therefore, restoring intestinal microbiota homeostasis has attracted much attention and become a novel therapeutic approach for the management of CD.

Fecal microbiota transplantation (FMT) is an advanced microbial treatment that restores the patient's gut microbiota by introducing healthy microbiota from a donor individual to the patient [10]. With advancements in FMT, it has become a transformative treatment for CD [11, 12]. FMT promotes competition between donor and recipient microbes in the gut. Moreover, an increase in the number of donor microbes may potentially enhance overall colonization in the recipient's gut. This may be achieved by increasing the microbial load for a single FMT or the number of fecal infusions [13]. Our previous studies have confirmed that the periodic administration of FMT plus partial enteral nutrition (PEN, 80%) is clinically efficacious in children with CD [14]. However, selecting convenient and efficient administration routes to implement multiple rounds of FMT remains unexplored.

Driven by its clinical practicality and flexibility, FMT using oral capsules garnered increasing attention [15, 16]. Oral FMT capsules are preferred and beneficial for many patients because they are easier to administer, less invasive, and have aesthetic appeal, high adherence, and tolerance [16]. Theoretically, oral FMT capsules are particularly suitable for the treatment of children with CD who are unable to adapt to long-term outpatient and inpatient treatments due to academic commitments and potential psychological stress. However, clinical data on the use of oral FMT in children with CD are lacking.

Therefore, we designed an open-label, parallel-group, controlled study to explore the clinical efficacy and AEs of oral FMT capsules combined with PEN (80%) in patients with active CD. Additionally, the microbiomes of the recipients before and after transplantation were analyzed to investigate the implantation rate of donor bacterial genera after oral administration of FMT capsules.

Methods

Study design and population

This study was approved by the Medical Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (TJ-IRB20220127) and conducted between January 2022 and December 2023. Written informed consent for FMT treatment was obtained from the legal guardians of all patients. Pediatric patients with active CD who were treated with oral FMT capsules combined with PEN (80%) were enrolled, and those with active CD who were treated with PEN (80%) and immunosuppressants simultaneously were enrolled as controls. The primary outcome was the efficacy and safety of oral FMT capsules in treating active CD in children. The secondary outcome was the degree of the implantation rate of donor bacterial genera.

Patients who met the following criteria were included in the study: (1) those with active CD aged 6–15 years; (2) those who agreed to undergo colonoscopy during enrollment (including consent from their guardians); (3) those taking biologics (e.g., infliximab, adalimumab), provided they showed no response or a less effective response after four induction treatments; and (4) those with no change in medication and dose for at least 1 week prior to transplantation. The exclusion criteria were as follows: (1) clinical remission; (2) presence of lesions limited to the small intestine or with stenosis, obstruction, and perforation; (3) a change in medication within the last week; and (4) refusal to undergo regular follow-up visits and endoscopic evaluation.

The definitions of PEN (80%) (enteral nutrition formula in Supplementary Table 1), simple endoscopic score for CD (SES-CD), and endoscopic remission are detailed in our previous study [14]. Patients treated with oral FMT capsules combined with PEN (80%) were included in the capsule group, and those treated with PEN (80%) combined with immunosuppressants (corticosteroids, thalidomide, and azathioprine) were included in the control group (Fig. 1). Biological remission was defined as normal C-reactive protein (CRP) levels (< 3 mg/L), and a biological response was defined as abnormal baseline levels of CRP that decreased to normal or decreased by ≥ 50% [17].

Active CD in children was defined as a pediatric CD activity index (PCDAI) > 10, SES-CD > 3, and a fecal calprotectin (FCP, cutoff < 200 μg/g) level at least twice the upper limit of normal. The PCDAI score was used to assess the severity of CD and the efficacy of CD treatment in the patients [18]. A clinical response was defined as a

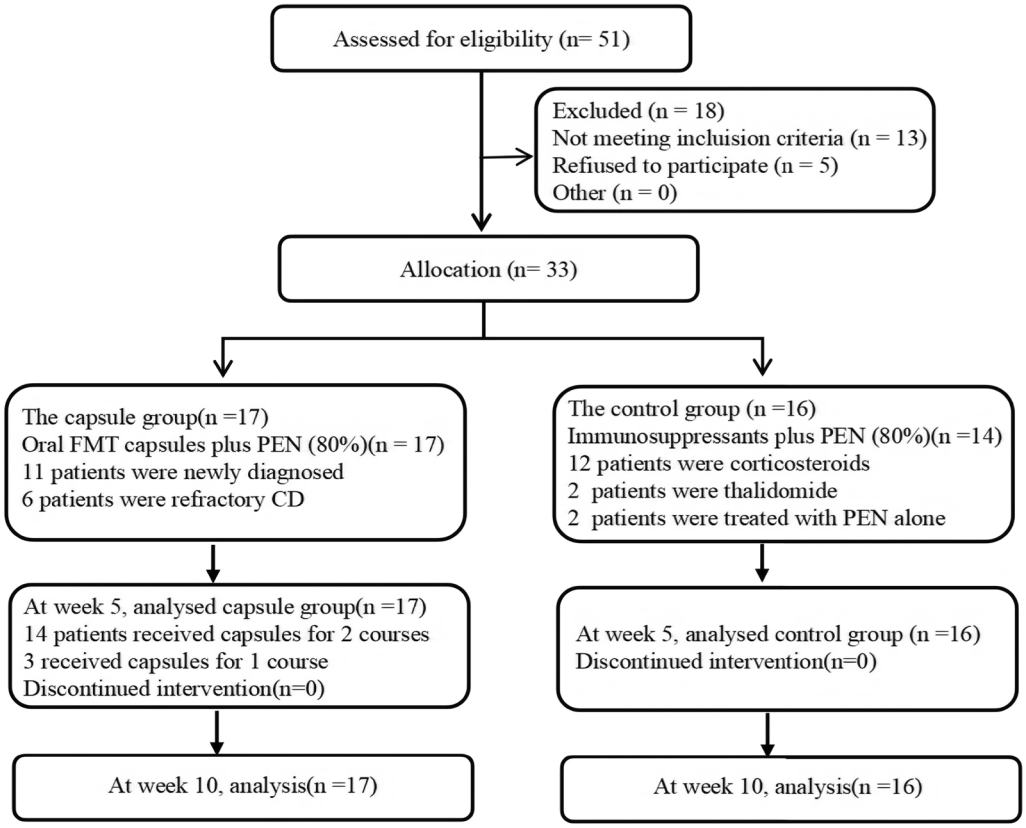

Fig. 1 Trial profile of patient inclusion. PEN: Partial enteral nutrition

12.5-point reduction in the PCDAI. A PCDAI score < 10 was defined as clinical remission, 10.0–27.5 as mild activity, 30.0–37.5 as moderate activity, and 40.0–100.0 as severe activity.

The baseline was defined as the time the treatment regimen was initiated. Colonoscopies were performed by trained pediatric endoscopists at baseline and 10 weeks after treatment. Cases of patients requiring additional medication within 10 weeks were considered clinical failures.

Data collection

Outpatient and hospitalization data were collected. In addition to anthropometric measurements, including height, weight, body mass index (BMI), and weight percentiles assessed before and after treatment, clinical data were collected at baseline (week 0) and at 2, 5, and 10 weeks post-treatment to evaluate both efficacy and safety. This clinical data encompassed erythrocyte sedimentation rate (ESR), CRP, serum cytokine (flow cytometry), vitamin D, and FCP levels; PCDAI; SES-CD; and FMT-related AEs.

Preparation of FMT capsules

The donors were healthy children of a similar age to the patients, and the screening criteria and fresh fecal bacteria were prepared as previously described [14]. The prepared fresh bacterial liquid was centrifuged at 4 °C, and the supernatant was removed. The lyophilized protective agent was added, and the bacteria were quickly transferred to a freeze-drying machine for 24 h. The freeze-dried bacterial powder was encapsulated in capsules made from the currently available common acid-resistant hydroxypropyl methylcellulose packaging and released into the cecum based on the pH conditions (Supplementary Table 2). The prepared capsules were sealed and stored in the refrigerator at − 80 °C initially. Information on capsule samples is provided in the Supplementary Methods (Supplementary Table 3).

FMT protocol

Each capsule contained 99 mg of lyophilized bacterial powder. All children in the capsule group were administered oral capsules (No. 3 capsule; 150 mg/capsule; 60 capsules per course; total dosage, $3*10^{13}$ bacteria per course; stored at -80 °C for 6 months and − 20 °C for

more than 1 month). Some younger children initially needed short-term swallowing training to swallow the capsules. Five capsules were administered twice a day for 6 consecutive days. The capsules were taken on an empty stomach after awakening in the morning and before going to bed at night. The capsules were removed from refrigeration 10–30 min before administration, thawed to room temperature (25 °C), and then swallowed with warm water (37 °C) 2 h before breakfast and 2 h after dinner. Five capsules were removed at a time for thawing, and the remaining capsules remained in the refrigerator.

The patients were advised to eat only liquid food and ensure an intake of 1–1.5 L of water per day. A laxative (compound polyethylene glycol 4000, 64 g) was administered one night before FMT capsule treatment. The patients were administered periodic capsule therapy at weeks 0 (baseline) and 5. All combined PEN (80%) therapies and other concomitant treatments remained unchanged during treatment.

Microbiota composition analysis

Fecal samples were collected from healthy donors and recipients before treatment and at weeks 5 and 10 after treatment. The samples were kept frozen at − 80 °C until DNA extraction. The procedures regarding DNA extraction, 16S rRNA gene amplification, and processing and sequencing platforms were described previously [14]. In short, Microbial DNA was extracted using a PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA) according to the manufacturer's protocol and the hyper-variable V3-V4 region was amplified using 338F and 806R primers. Library construction and sequencing were conducted on an Illumina MiSeq platform (Illumina, San Diego, United States). Species composition analyses, alpha diversity analyses, beta diversity measurements, and linear discriminant analysis effect size (LEfSe) multilevel discriminant analysis of genus differences were performed on the QIIME2 v.2020.2 platform of Majorbio Cloud Platform (Supplementary Methods). Sequencing data were recovered as FASTQ files and deposited in the National Genomics Data (GSA: CRA015829) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa.

Statistical analysis

Continuous variables are presented as mean (± standard deviation). Non-continuous parameters are presented as frequencies and percentages. An independent sample-T test or Mann–Whitney U test was used to compare differences between groups. Paired Wilcoxon rank sum test or paired t-test were used to compare the samples before and after treatment. Categorical variables were expressed as frequency and percentage, and chi-square test was used for comparison between groups. Statistical analysis between more than two groups was performed using the Kruskal–Wallis H test. Associations between clinical parameters and bacterial genera were tested using Spearman's correlation analysis. SPSS 25 was used for the statistical processing of all data, and GraphPad Prism 9 was used for statistical mapping. The level of statistical significance was set at $P < 0.05$.

Results

Patient's demographic and clinical characteristics

Overall, 33 patients were enrolled in this study, 17 in the capsule and 16 in the control groups. The median age of patients in the capsule group was 11.8 ± 1.8 years (range 9–15 years), with 10 males and seven females. The median age of patients in the control group was 11.1 ± 2.0 years (range 8–14 years), with nine males and seven females. According to the PCDAI score, in the capsule group, two patients were classified as having severe activity, six had moderate activity, and nine had mild activity. The control group had two, five, and nine patients with severe, moderate, and mild activities, respectively.

In the capsule group, 11 patients were newly diagnosed and received oral capsules combined with PEN (80%) treatment. The other six patients had refractory CD, with immunosuppressive treatment exhibiting poor efficacy in one patient and biological agents exhibiting poor efficacy in five. In the capsule group, except for the addition of oral capsules combined with PEN (80%) treatment, biological treatment was maintained (the dose and duration remained unchanged). Among the patients in this group, 14 received capsules for two courses, and three received capsules for one course (Table 1).

The control group included 16 patients, all newly diagnosed patients, 12 of whom were treated with combined corticosteroids (prednisone, 2 mg/kg, maximum dose 40 mg/d). After 4 weeks of corticosteroid therapy, the dose was gradually decreased to one tablet per week. Two patients were treated with thalidomide, and two were treated with PEN alone (80%). The demographics of the two groups at baseline were not significantly different (Table 2).

Response to FMT

In the second week of treatment, 10 and 7 patients in the capsule group achieved biological remission and biological response, respectively. In the control group, 12 and 4 achieved biological remission and biological response, respectively, with no statistically significant differences between the two groups. At week 10, clinical remission was observed in 13 (76.5%) and 12 (75%) patients in the capsule and control groups, respectively, while six patients in both groups achieved endoscopic remission

Table 1 Clinical data of patients in capsule group

Patient	Age (year)	Sex	Group type	Concomitant medications	Donor age (year)	Donor sex	Patient-donor relationship	AEs	Course of capsule
P1	11	Male	Newly diagnosed	PEN	9	Female	Nonrelative	Abdominal pain, constipation	Two courses
P2	10	Male	Newly diagnosed	PEN	7	Male	Nonrelative	/	Two courses
P3	12	Male	Newly diagnosed	PEN	8	Female	Nonrelative	/	Two courses
P4	11	Female	Refractory CD patients	PEN + Infliximab	8	Male	Nonrelative	Abdominal pain、constipation	Two courses
P5	13	Female	Refractory CD patients	PEN + Infliximab	9	Female	Nonrelative	/	Two courses
P6	11	Male	newly diagnosed	PEN	8	Male	Nonrelative	/	One course
P7	10	Male	newly diagnosed	PEN	7	Male	Nonrelative	Constipation, Fatigue	Two courses
P8	10	Female	newly diagnosed	PEN	7	Male	Nonrelative	Constipation	Two courses
P9	13	Female	newly diagnosed	PEN	13	Male	Nonrelative	Alopecia, Constipation	Two courses
P10	12	Female	newly diagnosed	PEN	9	Female	Nonrelative	/	One course
P11	15	Male	Refractory CD patients	PEN	9	Female	Nonrelative	Constipation, Fatigue	Two courses
P12	11	Female	newly diagnosed	PEN	7	Male	Nonrelative	Constipation	One course
P13	15	Male	Refractory CD patients	PEN + Infliximab	8	Male	Nonrelative	Constipation	Two courses
P14	12	Male	Refractory CD patients	PEN + Adalimumab	8	Male	Nonrelative	/	Two courses
P15	9	Female	newly diagnosed	PEN	8	Male	Nonrelative	Alopecia	Two courses
P16	12	Male	newly diagnosed	PEN	14	Male	Nonrelative	Constipation	Two courses
P17	15	Male	Refractory CD patients	PEN + Infliximab	14	Male	Nonrelative	/	Two courses

CD: Crohn's disease; PEN: Partial enteral nutrition; AEs: adverse events

with no statistical significance (Supplementary Table 4 and Supplementary Fig. 1).

At week 10, the relevant parameters of the two groups showed significant improvements compared with those before treatment. At week 10, the FCP level, PCDAI, ESR, CRP level, and SES-CD were compared between the two groups. No statistical differences were observed between them (Table 3).

Compared with baseline, at week 10, the mean height, weight, BMI and weight percentile of children in both groups were significantly improved ($P < 0.005$), as illustrated in Fig. 2, while the levels of CRP, FCP and ESR in the capsule group were significantly declined ($P < 0.05$). The PCDAI and SES-CD also decreased significantly ($P < 0.05$), and the vitamin D levels increased significantly ($P < 0.05$), as illustrated in Fig. 3.

After oral administration of the capsule, the levels of the pro-inflammatory factors, interleukin (IL)-1 and IL-6, significantly decreased (both $P < 0.05$). The levels of other pro-inflammatory factors also reduced; however, the level of the anti-inflammatory factor, IL-10, showed a significant upward trend ($P < 0.05$). Although comparable changes were noted in the control group, the increase in IL-10 and the decrease in IL-6 were more pronounced in the capsule group (Fig. 4).

Safety

No serious AEs were observed after oral administration of the FMT capsules. However, mild AEs were observed in 10 patients (58.8%), including constipation (nine patients), mild abdominal pain, fatigue, and alopecia, most of which occurred approximately 10 days after the first course of oral FMT capsules. Most of these symptoms were self-limiting and did not require pharmacological intervention. Among the patients, four with constipation required treatment with lactulose to improve their symptoms. Seven days later, the constipation symptoms improved. After the second course of oral capsules, three patients developed constipation again, while the other patients did not experience any discomfort (Table 1).

Table 2 The baseline characteristics of the study population

Parameters	Capsule group	Control group	P value
Total number	17	16	–
Age(year), M ± SD	11.8 ± 1.8	11.1 ± 2.0	0.53
Sex, male, n (%)	10 (58.8%)	9 (56.2%)	1
Vitamin D, M ± SD	16.4 ± 7.5	16.4 ± 5.9	0.226
FCP (μg/g), M ± SD	2399 ± 510	2253 ± 449	0.148
ESR (mm/h), M ± SD	18.5 ± 8.7	20.8 ± 6.2	0.312
CRP (mg/L), M ± SD	17.7 ± 6.7	21.1 ± 4.2	0.175
Disease severity			0.424
Severe % (n)	2 (11.7%)	2 (12.5%)	
Moderate % (n)	6 (35.2%)	9 (56.2.5%)	
Mild % (n)	9 (52.9%)	5 (31.2%)	
Disease location			0.769
L1 Terminal ileum	1 (5.9%)	2 (12.5%)	
L2 Colon	6 (35.3%)	6 (37.5%)	
L3 Ileocolonic	10 (58.8%)	8 (50.0%)	
Disease behaviour			0.485
B1 nonstricturing-nonpenetrating	17 (100%)	15 (93.7%)	
B2 stricturing	/	1 (6.2%)	
B3 penetrating	/	/	
Perianal involvement	2 (11.7%)	2 (6.2%)	1
PCDAI score, M ± SD	21.7 ± 10.4	23.4 ± 6.3	0.283
SES-CD score, M ± SD	10.5 ± 3.7	9.6 ± 2.9	0.476

Mann–Whitney U test: Vitamin D, FCP, ESR, CRP, PCDAI score, SES-CD score; T test: Age; Chi-Square Test: Sex, Disease severity, Disease location, Disease behaviour, Perianal involvement

FCP: fecal calprotectin; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; PCDAI: Pediatric CD Activity Index; SES-CD: Simple Endoscopic Score for CD; M: mean; SD: standard deviation

Table 3 Comparison of clinical parameter between the two groups at week 10

	Capsule group	Control group	P value
FCP (μg/g), M ± SD	475 ± 602	468 ± 478	0.383
ESR (mm/h), M ± SD	11.5 ± 8.7	9.1 ± 10.2	0.523
CRP (mg/L), M ± SD	5.9 ± 5.5	6.1 ± 10.2	0.098
PCDAI score, M ± SD	8.24 ± 9.3	8.28 ± 10.5	0.67
SES-CD score, M ± SD	3.41 ± 3.3	4 ± 3.8	0.202

FCP: fecal calprotectin; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; PCDAI: Pediatric CD Activity Index; SES-CD: Simple Endoscopic Score for CD; M: mean; SD: standard deviation; Mann–Whitney U test

Improvement in microbial dysbiosis following oral FMT administration

To dynamically evaluate the differences in intestinal flora composition in the capsule group, 58 fecal samples were collected for 16S rRNA sequencing, including 16 from healthy donors (HDs), 17 before treatment (FMT 0W), eight at week 5 (FMT 5W), and 17 at week 10 (FMT 10W). The differences in the phylum, genus, and species levels in the patient and donor stool samples are presented in Supplementary Tables 5, 6, and 7, respectively.

Patients with CD experienced a remarkable increase relative to the abundance of Proteobacteria (24.14 ± 31.53% vs. 6.49 ± 5.654%, P = 0.8009) and a decrease in the relative abundances of Firmicutes (52.8 ± 28.48% vs. 65.04 ± 13.4%, P = 0.1089) and Bacteroidetes (12.45 ± 19.04% vs. 19.38 ± 12.54%, P < 0.05) compared with the HDs (Fig. 5A and Supplementary Table 8). After oral capsule treatment, the relative abundance of Proteobacteria decreased, while the relative abundance of Firmicutes and Bacteroidetes increased (Supplementary Fig. 2 and Supplementary Table 9).

Before capsule treatment, the alpha diversity index of the HDs significantly exceeded that of patients with CD (all P < 0.01), indicating evident flora disturbances and disorders in the intestinal flora of children with CD, with notably reduced intestinal flora diversity and abundance. Following oral capsule treatment, the species abundance and diversity of the gut microbiota of patients with CD

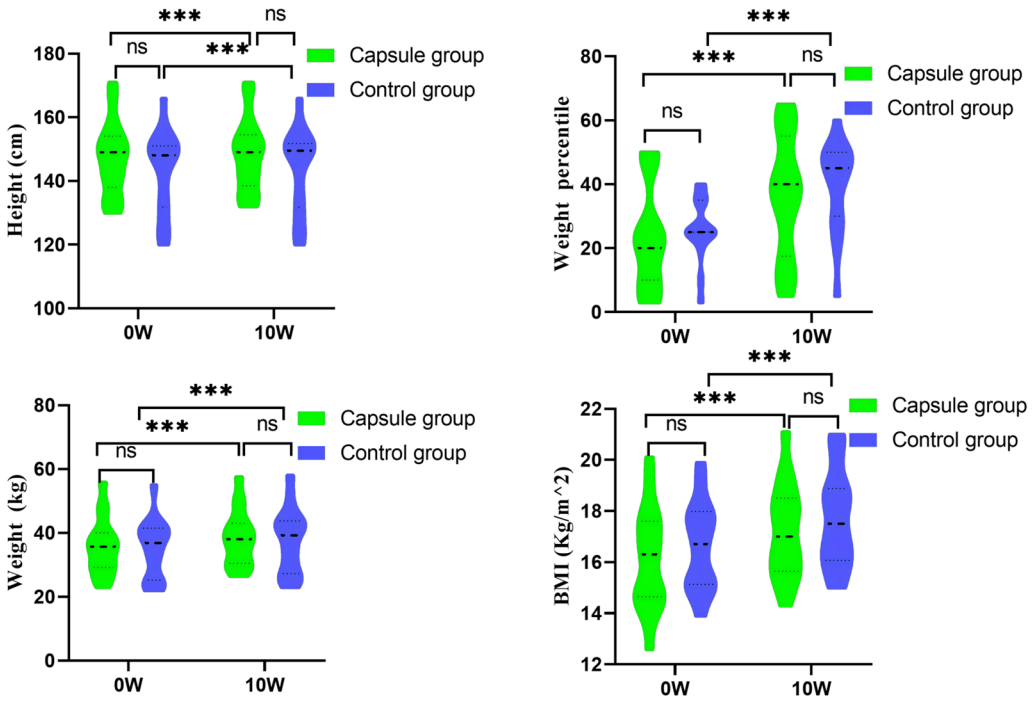

Fig. 2 The trends of weight, weight percentile, height and BMI of the two groups before and after treatment (The comparison between the two groups was the Mann–Whitney test, Paired Wilcoxon rank sum test was used before and after treatment, ***P < 0.001)

significantly improved (all P < 0.05), approaching levels observed in the HDs (Fig. 5B).

The intestinal flora displayed a substantial difference in beta diversity between the HDs and patients with CD. Oral capsules significantly improved the differences between patients with CD and HDs after treatment, with samples clustered closely in the principal coordinate analysis space (Fig. 5C).

Healthy and unhealthy gut flora exhibit different ecological characteristics. The Gut Microbiome Health Index (GMHI) in the healthy group was significantly higher than that of the group with CD. Compared with the gut microbiota of the HDs, that of patients with CD had a significantly lower GMHI (Fig. 5D). However, the GMHI of patients with CD increased significantly after capsule treatment (P < 0.001). As illustrated in Fig. 5E, the microbial dysbiosis index (MDI) was significantly increased in pre-FMT recipients compared with that of the HDs and post-FMT group (P < 0.001).

Implantation of the core functional genera

LEfSe was used to identify different genera at different classification levels between HDs and patients with CD (Supplementary Fig. 3). Compared with the HDs, the pre-FMT group had lower proportions of the following beneficial bacterial genera: *Agathobacter*, *Akkermansia*, *Roseburia*, *Blautia*, *Subdoligranulum*, and *Faecalibacterium* (pre-FMT vs. HD group, P < 0.05). In contrast, *Enterobacter*, *Enterococcus*, *Clostridioides*, and *Escherichia-Shigella* were more abundant in the pre-FMT group than in the HD group (P < 0.05) (Supplementary Fig. 4 and Supplementary Table 6).

After treatment, the colonization rate of the six above-mentioned beneficial bacterial genera increased significantly (Fig. 6A). After the periodic administration of oral capsules at week 5, the colonization rate re-increased significantly at week 10, whereas competitive inhibition decreased the abundance of harmful bacterial genera, such as *Enterococcus* and *Clostridioides* (pre-FMT vs. post-FMT; P < 0.05) (Supplementary Fig. 4).

The correlation heatmap analysis revealed that IL-10 was positively correlated with the following genera: *Subdoligranulum*, *Akkermansia*, *Faecalibacterium*, and *Blautia*. In contrast, a negative association was observed between *Agathobacter*, *Akkermansia*, *Roseburia*, *Blautia*, *Subdoligranulum*, and *Faecalibacterium* and the IL-2, IL-1, and IL-6 levels (Fig. 6B).

These six beneficial bacterial genera were used as predictors to generate the area under the curve (AUC). Comparison between the HDs and patients with CD revealed that the AUC values of the six bacterial genera exceeded 0.88: *Roseburia* (AUC = 0.92),

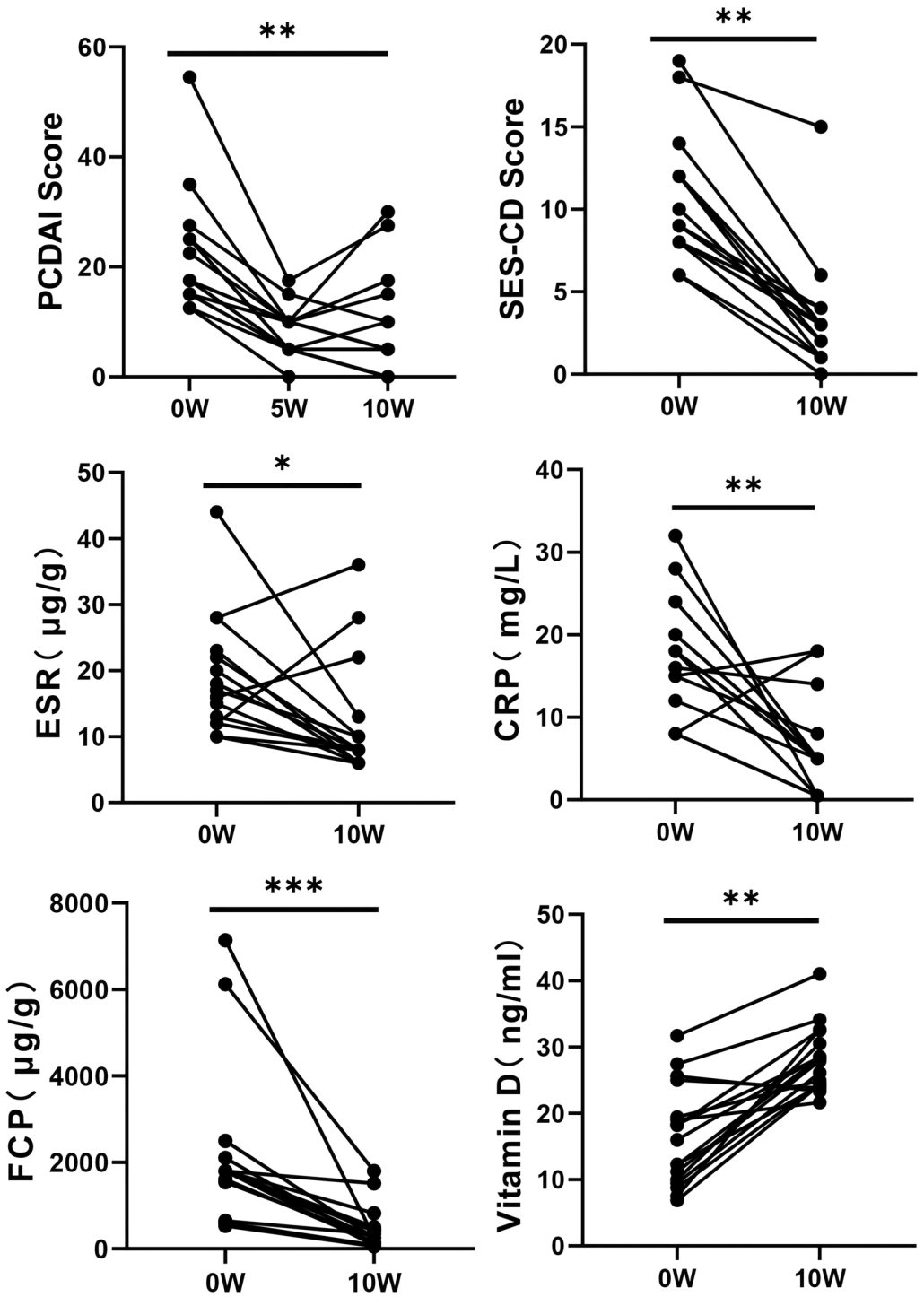

Fig. 3 Changes in the parameters before and after treatment in the capsule group. Changes in the C-reactive protein (CRP) level, erythrocyte sedimentation rate (ESR), fecal calprotectin (FCP) level, vitamin D level, pediatric Crohn's disease activity index (PCDAI), and simple endoscopic score for Crohn's disease (SES-CD) between the baseline and week 10

Faecalibacterium (AUC = 0.9), *Akkermansia* (AUC = 0.92), *Blautia* (AUC = 0.88), *Agathobacter* (AUC = 0.94), and *Subdoligranulum* (AUC = 0.96). When assessing the combined effects of the six bacterial genera, the AUC was 0.98 (Fig. 6C), showing that the predictive effect was advanced. Using these genera

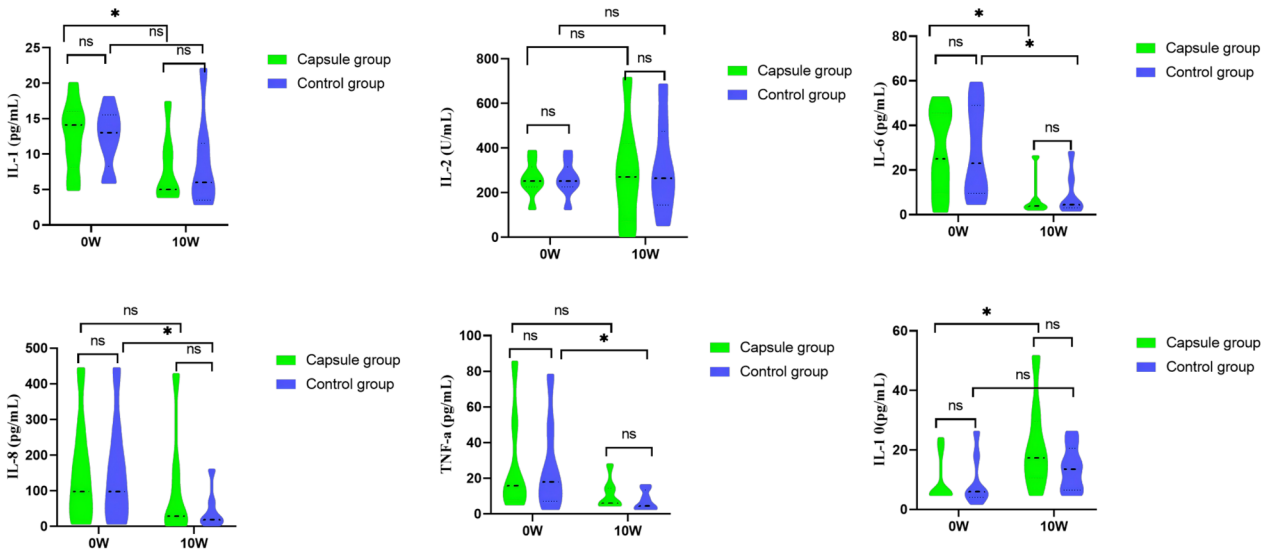

Fig. 4 Changes in the serum cytokine before and after treatment of the two groups. (The comparison between the two groups was the Mann-Whitney test, Paired Wilcoxon rank sum test was used before and after treatment, ***P < 0.001

as predictors of efficacy before and after CD treatment, we found a significant improvement in prediction accuracy when the six bacterial genera were combined (AUC = 0.93) (Fig. 6D). This suggests that these six bacterial genera may play a crucial role in the clinical and mucosal improvement of CD. Moreover, their combined use has a synergistic effect and could be used as a potential gut marker to diagnose CD.

Discussion

To the best of our knowledge, this was the first study to evaluate the effect of oral FMT capsules combined with PEN (80%) in pediatric patients with active CD and explore the implantation rate of core functional bacteria. In this prospective trial, the repeated and periodic administration of oral FMT capsules in pediatric patients with CD was effective, safe, and well tolerated. By week 10, 76.5% of the patients achieved clinical remission comparable to the use of immunosuppressants with PEN (80%). Before FMT administration, patients with CD demonstrated reduced species diversity and abundance compared to the HDs. After FMT administration, the species diversity and abundance significantly improved along with a significant increase in vital functional genera, similar to the HDs. This indicates a high success rate of core functional genera implantation. Despite the small sample size, the present study adds to the knowledge base of FMT and provides early evidence of its effectiveness.

Oral FMT capsules have demonstrated clinical efficacy for recurrent *Clostridium difficile* infection (CDI) [19, 20]. Kao et al. studied different transplantation routes for patients with CDI and found that the oral capsule approach was not inferior to the colonoscopy approach [17].

Data on the use of oral FMT capsules for treating inflammatory bowel disease are limited. Studies have shown that daily oral FMT capsules can prolong changes in the intestinal bacterial community structure in patients with ulcerative colitis [21], which is a promising FMT delivery route. Our study revealed that oral FMT capsules combined with PEN (80%) could help induce clinical remission and achieve endoscopic remission in children with active CD, comparable to PEN (80%) with immunosuppressants. It is noteworthy that the capsule group and the control group exhibited not only a comparable level of clinical efficacy but also a parallel trend towards improvement in their nutritional status. Although the number is limited, it is significant. Given the safety concerns with immunosuppressants, we propose oral FMT capsules combined with PEN (80%) as a viable alternative for active CD in children.

Regarding safety, common AEs associated with FMT are primarily minor transient gastrointestinal complications such as bloating, diarrhea, and abdominal pain [22–24]. Our previous studies confirmed that the short- and long-term safety of FMT is relatively good [25]. However, serious AEs associated with other routes of administration have been reported, including aspiration and intestinal perforation [22]. While AEs following oral capsule administration are infrequent, some studies have reported nausea, abdominal discomfort, and vomiting [26]. Haifer et al. reported that oral FMT capsules

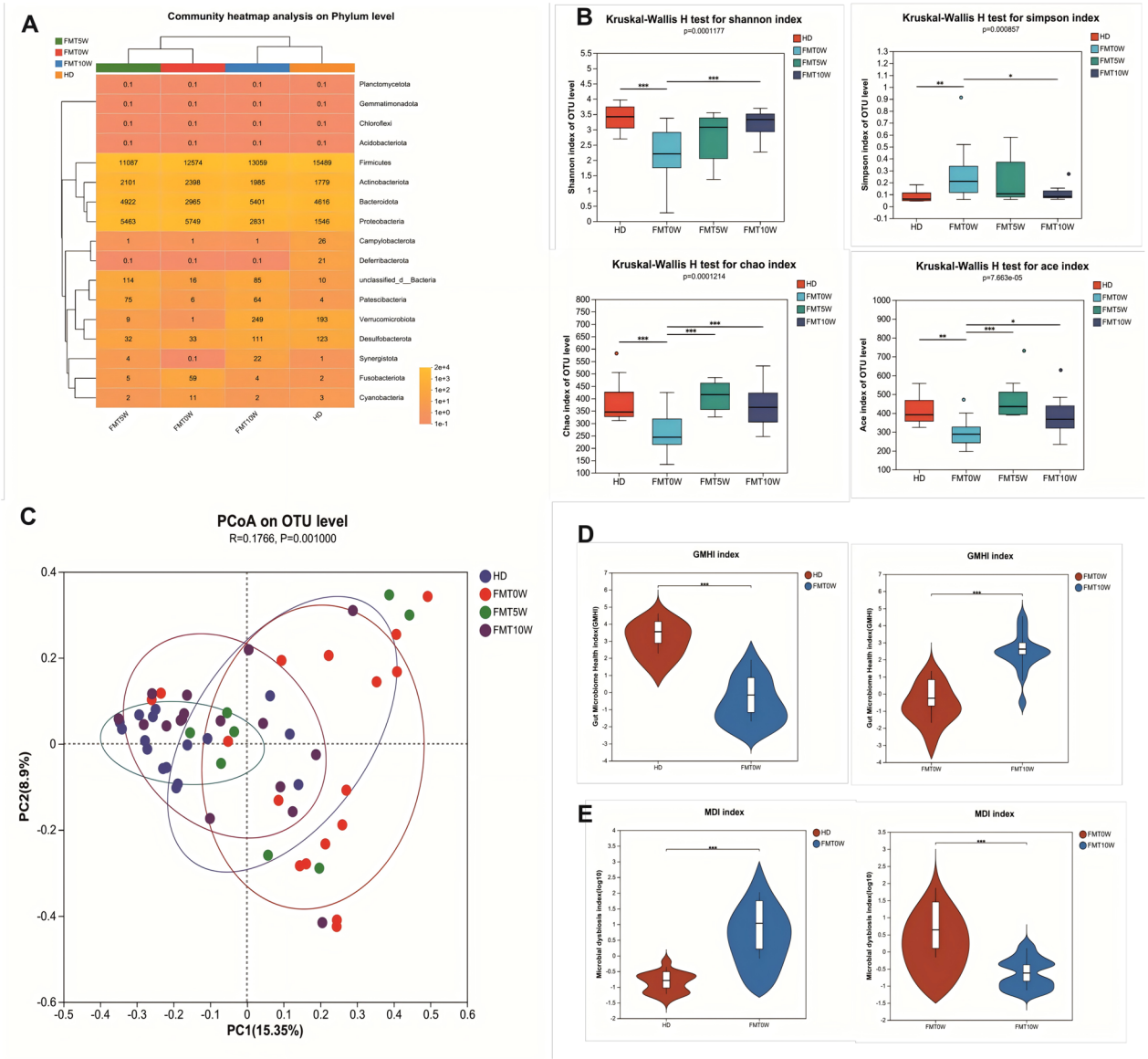

Fig. 5 The dynamic changes in the gut microbiota before and after treatment in the capsule group. **A** Phylum level changes between the healthy donors and patients with CD at various time points. **B** Alpha diversity indices (Chao1, ACE, Shannon and Simpson) changes between the healthy donors and patients with CD at various time-points. There was a significant reduction in the alpha diversity in patients in the FMT 0W group compared with donors and patients in the FMT 10W group (Kruskal–Wallis H test, $P < 0.001$, with *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). **C** Principal coordinate analysis (PCoA) comparisons of the microbial communities between the healthy donors and patients with CD at various time-points. The FMT 10W and donor samples were significantly clustered together than the FMT 0W samples. **D** Comparison of the Gut Microbiome Health Indices (GMHIs) in the different groups. The GMHIs of patients in the FMT 0W group were significantly lower than those of the healthy donors and patients in the FMT 10W group (Wilcoxon signed-rank test, $P < 0.001$). **E** Comparison of the microbial dysbiosis index (MDI) in the different groups. The MDI of patients in the FMT 0W group was significantly lower than that of the healthy donors and patients in the FMT 10W group (Wilcoxon signed-rank test, $P < 0.001$). HD: healthy donor; FMT 0W: before treatment; FMT 5W: at week 5 after oral capsule treatment; FMT 10W: at week 10 after oral capsule treatment

are safe and do not cause significant AEs in patients with ulcerative colitis [21]. Our research shows that most children tolerate FMT capsules, and their use is safe, with constipation being the primary AE. This may stem from the apparent short-term fluctuation in the microflora following the ingestion of numerous capsules and the low water intake at school. Constipation typically resolves in children after diet adjustment, and symptoms may improve after 1 week of lactulose administration.

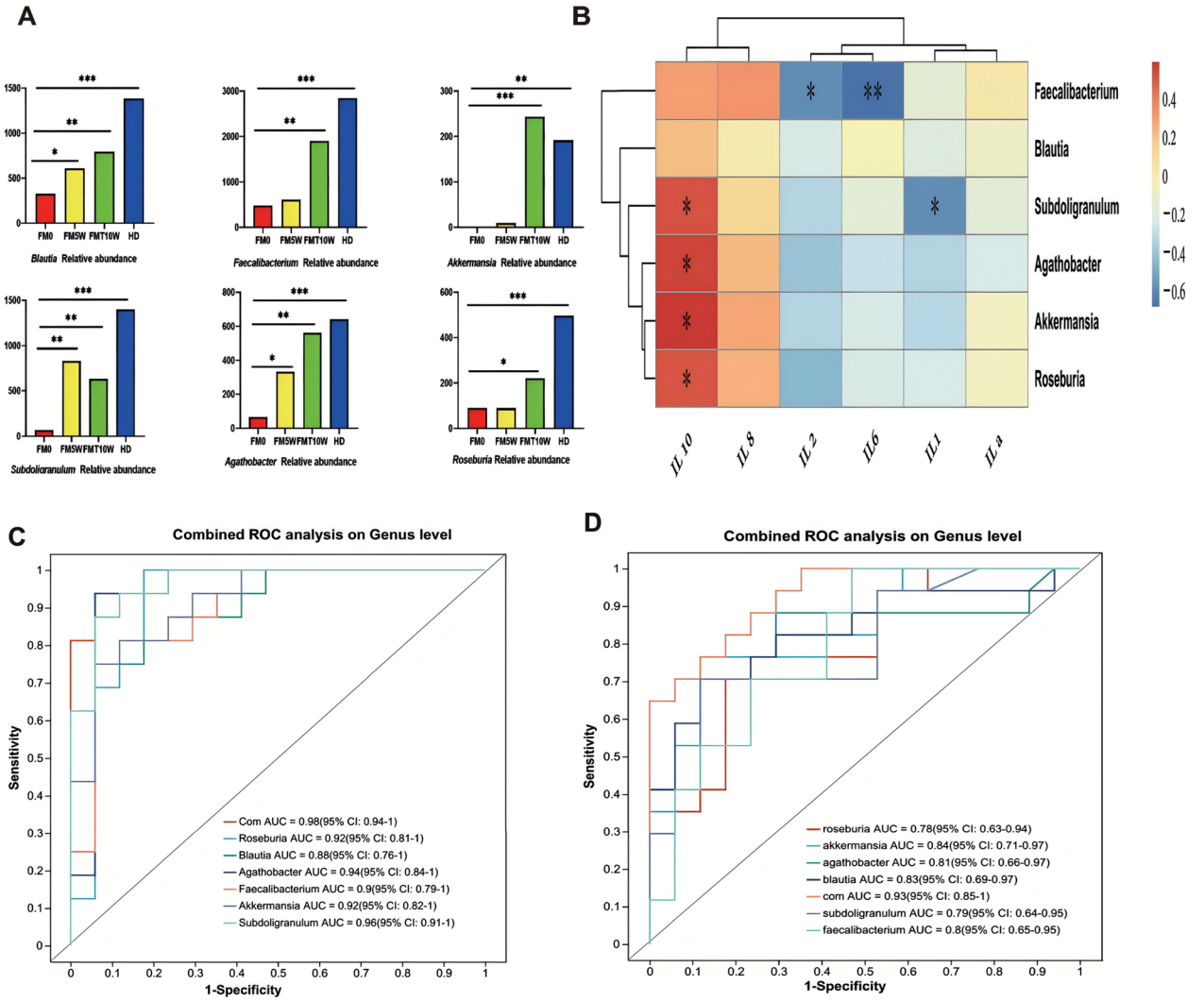

Fig. 6 Implantation of the core functional genera (*Agathobacter*, *Akkermansia*, *Roseburia*, *Blautia*, *Subdoligranulum*, and *Faecalibacterium*) after oral capsule administration. **A** The relative abundance of the core functional genera between the healthy donors and patients with CD at various time-points. The level of significance was determined using the Wilcoxon rank-sum test, with *P < 0.05, **P < 0.01, ***P < 0.001. **B** Spearman correlation analysis revealed a correlation between core functional bacteria and cytokines. The red and blue areas' represent positive and negative correlations, respectively, with *P < 0.05, **P < 0.01, ***P < 0.001. **C** The six core functional genera were assessed as potential markers to distinguish patients with CD from healthy children. The combined effects of the six bacterial genera showed an AUC of 0.98, indicating good performance. **D** The six core functional genera were assessed as potential gut markers to predict the efficacy of the CD therapy. The combined effects of the six bacterial genera showed an AUC of 0.93, indicating good performance. HD: healthy donor; FMT 0W: before treatment; FMT 5W: at week 5 after oral capsule treatment; FMT 10W: at week 10 after oral capsule treatment

Our study found significant differences in the intestinal flora of patients with CD before treatment compared to healthy controls, with significantly reduced diversity and abundance of the flora, consistent with extensive research data [26]. At week 10, the β-diversity and α-diversity had improved, the composition and abundance of the phyla, genera, and species resembled that of the HDs, and the GMHI and MDI had significantly improved. Moreover, our study revealed that oral capsules are as effective as traditional FMT delivery in restoring bacterial diversity.

Evidence reveals that the clinical success of FMT may be related to the extent to which the donor microbe is implanted in the recipient's gut. Quantifying this is the first step in determining the conditions under which implantation can be maximized [13]. In the present study, Harmful bacteria such as *Enterococcus* was highly abundant in the gut of children with

CD, while various beneficial bacterial genera that produce short-chain fatty acids (SCFAs) with anti-inflammatory properties were significantly lacking, including *Faecalibacterium*, *Blautia*, *Subdoligranulum*, *Agathobacter*, *Akkermansia*, and *Roseburia*. *Enterococcus* is an opportunistic pathogen that can cause systemic infection [27]. *Faecalibacterium*, *Akkermansia*, and *Roseburia* are representatives of the next generation of probiotics, which can not only produce SCFAs, but also prevent cancer [28]. *Agathobacter* and *Subdoligranulum* all produce butyrate, which helps suppress inflammation in the gut [29]. *Blautia* stimulates mucus growth by producing SCFAs and prevents disease infection [30]. After treatment, the colonization rate of these functional genera increased significantly, and simultaneously, the colonization rate of harmful bacterial genera, such as *Enterococcus* and *Clostridioides*, decreased significantly due to competitive inhibition. The cooperative implantation rate of the bacterial genera was higher after periodic oral capsule administration and was comparable to that of the gut microbiota of healthy controls. Our study found that multiple periodic administration of oral capsules can increase the number of donor microbes, compete better with the recipient's microbes, and help donor flora colonize the recipient's gut. This underscores the potential of the periodic administration of oral FMT capsules as a viable strategy to achieve long-term microbial regulation.

The AUC values indicated that the six beneficial bacterial genera mentioned above could not only help distinguish children with CD from healthy children but could help predict the clinical efficacy of CD treatment. Therefore, we concluded that the six beneficial bacterial genera were the "core functional genera" of CD. The clinical symptoms of the children improved with the colonization of the core functional genera.

Previous studies have reported that therapeutic FMT can directly regulate congenital and adaptive immune responses [31]. Moreover, positive immune regulation is associated with an upregulation of IL-10 levels and a decrease in the ESR and IL-6 and CRP levels [32]. We hypothesized that the mechanism underlying FMT's improvement of intestinal inflammation may be related to the six core functional genera. Studies have shown that *Faecalibacterium* and *Akkermansia* stimulate IL-10 secretion [33, 34]. Moreover, *Blautia*, *Agathobacter*, *Subdoligranulum*, and *Roseburia* are major producers of butyric acid, which contributes to the secretion of anti-inflammatory cytokines, such as IL-10 [28, 35]. Our study found that IL-10 was positively correlated with these genera, whereas IL-2, IL-1, and IL-6 were negatively correlated. With clinical improvement in children with CD, the ESR, CRP and FCP levels, and other parameters improved, and the intestinal mucosa was significantly repaired. Therefore, we speculate that these core functional genera may be key bacterial genera leading to clinical and mucosal improvement in CD.

The findings of this study suggest that the identified core functional genera may serve as potential biomarkers for the diagnosis and treatment of CD. Currently, the etiology of CD remains unclear, and there is a significant lack of characteristic diagnostic markers. Finding and verifying appropriate functional genera as biomarkers for CD diagnosis and treatment is helpful to accurately predict the treatment outcome [36, 37]. This study not only provides new insights into the pathogenesis of CD, but also offers a new therapeutic option. In the future, with the further study of the mechanism of these core functional genera, we are expected to develop precision therapy based on intestinal flora regulation of CD.

The capsules in our study were produced and administered uniformly. Moreover, we monitored patients over a long period and conducted a detailed microbiome analysis. However, this study had a few limitations. First, the sample size was limited and the trial lacked randomization, which may limit the strength of our findings. Second, 6 refractory patients were enrolled in the capsule group, while none were in the control group, and many patients in the control group were receiving medication, this discrepancy may introduce bias into the study. Finally, future studies are needed to verify the role of core functional bacteria through animal experiments.

Conclusions

We observed bacterial implantation after oral administration of FMT capsules matched the clinical symptoms. Moreover, we identified six core functional genera that may be candidate biomarkers for distinguishing children with CD from healthy children.

Abbreviations

AEs	Adverse events
AUC	Area under the curve
CD	Crohn's disease
CRP	C-reactive protein
ESR	Erythrocyte sedimentation rate
FCP	Fecal calprotectin
FMT	Fecal microbiota transplantation
GMHI	Gut Microbiome Health Index
HD	Healthy donor
IL	Interleukin
LEfSe	Linear discriminant analysis (LDA) Effect Size
MDI	Microbial dysbiosis index
PCDAI	Pediatric Crohn's disease activity index
PEN	Partial enteralnutrition
PCoA	Principal coordinate analysis
SES-CD	Simple endoscopic score for CD

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-024-05832-1.

Supplementary Material 1.

Acknowledgements
The authors thank all the patients who kindly participated in this study. We thank Maintainbiotech. Ltd. (Wuhan) for providing FMT oral capsules.

Author contributions
SS designed the research project; BZ writing—review and editing the manuscript; SL, XL and CD contributed to data collection; HS, YL conducted the FMT treatment; JH and MR contributed to interpretation of data; SS and ZH contributed to data analysis.

Funding
No funding.

Availability of data and materials
The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author. The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive in National Genomics Data (GSA: CRA015829) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa.

Declarations

Ethics approval and consent to participate
This study was approved by the Medical Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (TJ-IRB20220127). Written informed consent has been obtained from the patients to publish this paper.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 18 September 2024 Accepted: 31 October 2024
Published online: 06 March 2025

References

1. Ng SC, Shi HY, Hamidi N, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. Lancet. 2017. https://doi.org/10.1016/S0140-6736(17)32448-0.
2. Roberts SE, Thorne K, Thapar N, et al. A systematic review and meta-analysis of paediatric inflammatory bowel disease incidence and prevalence across Europe. J Crohns Colitis. 2020. https://doi.org/10.1093/ecco-jcc/jjaa037.
3. Kuenzig ME, Fung SG, Marderfeld L, et al. Twenty-first century trends in the global epidemiology of pediatric-onset inflammatory bowel disease: systematic review. Gastroenterology. 2022. https://doi.org/10.1053/j.gastro.2021.12.282.
4. Jeong DY, Kim S, Son MJ, et al. Induction and maintenance treatment of inflammatory bowel disease: a comprehensive review. Autoimmun Rev. 2019. https://doi.org/10.1016/j.autrev.2019.03.002.
5. Parigi TL, D'Amico F, Abreu MT, et al. Difficult-to-treat inflammatory bowel disease: results from an international consensus meeting. Lancet Gastroenterol Hepatol. 2023. https://doi.org/10.1016/S2468-1253(23)00154-1.
6. Magro F, Cordeiro G, Dias AM, Estevinho MM. Inflammatory bowel disease—non-biological treatment. Pharmacol Res. 2020. https://doi.org/10.1016/j.phrs.2020.105075.
7. Pittayanon R, Lau JT, Leontiadis GI, et al. Differences in gut microbiota in patients with vs without inflammatory bowel diseases: a systematic review. Gastroenterology. 2020. https://doi.org/10.1053/j.gastro.2019.11.294.
8. Brusaferro A, Cavalli E, Farinelli E, Cozzali R, Principi N, Esposito S. Gut dysbiosis and paediatric Crohn's disease. J Infect. 2019. https://doi.org/10.1016/j.jinf.2018.10.005.
9. Sokol H, Landman C, Seksik P, et al. Fecal microbiota transplantation to maintain remission in Crohn's disease: a pilot randomized controlled study. Microbiome. 2020. https://doi.org/10.1186/s40168-020-0792-5.
10. Ooijevaar RE, Terveer EM, Verspaget HW, Kuijper EJ, Keller JJ. Clinical application and potential of fecal microbiota transplantation. Annu Rev Med. 2019. https://doi.org/10.1146/annurev-med-111717-122956.
11. Lopetuso LR, Deleu S, Godny L, et al. The first international Rome consensus conference on gut microbiota and faecal microbiota transplantation in inflammatory bowel disease. Gut. 2023. https://doi.org/10.1136/gutjnl-2023-329948.
12. Porcari S, Baunwall SMD, Occhionero AS, et al. Fecal microbiota transplantation for recurrent *C. difficile* infection in patients with inflammatory bowel disease: a systematic review and meta-analysis. J Autoimmun. 2023. https://doi.org/10.1016/j.jaut.2023.103036.
13. Porcari S, Benech N, Valles-Colomer M, et al. Key determinants of success in fecal microbiota transplantation: from microbiome to clinic. Cell Host Microbe. 2023. https://doi.org/10.1016/j.chom.2023.03.020.
14. Zou B, Liu S, Li X, et al. Repeated and multiple fecal microbiota transplantations plus partial enteral nutrition as the first-line treatment in active pediatric Crohn's disease. Front Cell Infect Microbiol. 2023. https://doi.org/10.3389/fcimb.2023.1083236.
15. Staley C, Hamilton MJ, Vaughn BP, et al. Successful resolution of recurrent clostridium difficile infection using freeze-dried, encapsulated fecal microbiota; pragmatic cohort study. Am J Gastroenterol. 2017. https://doi.org/10.1038/ajg.2017.6.
16. Kao D, Roach B, Silva M, et al. Effect of oral capsule- vs colonoscopy-delivered fecal microbiota transplantation on recurrent clostridium difficile infection: a randomized clinical trial. JAMA. 2017. https://doi.org/10.1001/jama.2017.17077.
17. Verstockt B, Mertens E, Dreesen E, et al. Influence of drug exposure on vedolizumab-induced endoscopic remission in anti-tumour necrosis factor [TNF] naive and anti-TNF exposed IBD patients. J Crohns Colitis. 2020. https://doi.org/10.1093/ecco-jcc/jjz151.
18. Cozijnsen MA, Ben Shoham A, Kang B, et al. Development and validation of the mucosal inflammation noninvasive index for pediatric Crohn's disease. Clin Gastroenterol Hepatol. 2020. https://doi.org/10.1016/j.cgh.2019.04.012.
19. Du C, Luo Y, Walsh S, Grinspan A. Oral fecal microbiota transplant capsules are safe and effective for recurrent *Clostridioides difficile* infection: a systematic review and meta-analysis. J Clin Gastroenterol. 2021. https://doi.org/10.1097/MCG.0000000000001495.
20. Youngster I, Sauk J, Pindar C, et al. Fecal microbiota transplant for relapsing *Clostridium difficile* infection using a frozen inoculum from unrelated donors: a randomized, open-label, controlled pilot study. Clin Infect Dis. 2014. https://doi.org/10.1093/cid/ciu135.
21. Haifer C, Paramsothy S, Kaakoush NO, et al. Lyophilised oral faecal microbiota transplantation for ulcerative colitis (LOTUS): a randomised, double-blind, placebo-controlled trial. Lancet Gastroenterol Hepatol. 2022. https://doi.org/10.1016/S2468-1253(21)00400-3.
22. Marcella C, Cui B, Kelly CR, Ianiro G, Cammarota G, Zhang F. Systematic review: the global incidence of faecal microbiota transplantation-related adverse events from 2000 to 2020. Aliment Pharmacol Ther. 2021. https://doi.org/10.1111/apt.16148.
23. Ooijevaar RE, van Nood E, Goorhuis A, et al. Ten-year follow-up of patients treated with fecal microbiota transplantation for recurrent *Clostridioides difficile* infection from a randomized controlled trial and review of the literature. Microorganisms. 2021. https://doi.org/10.3390/microorganisms9030548.
24. Yau YK, Lau LHS, Lui RNS, et al. Long-term safety outcomes of fecal microbiota transplantation: real-world data over 8 years from the Hong Kong FMT registry. Clin Gastroenterol Hepatol. 2024. https://doi.org/10.1016/j.cgh.2023.09.001.

25. Zou B, Liu SX, Li XS, et al. Long-term safety and efficacy of fecal microbiota transplantation in 74 children: a single-center retrospective study. Front Pediatr. 2022. https://doi.org/10.3389/fped.2022.964154.
26. Wu R, Xiong R, Li Y, Chen J, Yan R. Gut microbiome, metabolome, host immunity associated with inflammatory bowel disease and intervention of fecal microbiota transplantation. J Autoimmun. 2023. https://doi.org/10.1016/j.jaut.2023.103062.
27. Cheng Y, Ling Z, Li L. The intestinal microbiota and colorectal cancer. Front Immunol. 2020;11:615056. https://doi.org/10.3389/fimmu.2020.615056.
28. Kircher B, Woltemate S, Gutzki F, Schlüter D, Geffers R, Bähre H, Vital M. Predicting butyrate- and propionate-forming bacteria of gut microbiota from sequencing data. Gut Microbes. 2022;14(1):2149019. https://doi.org/10.1080/19490976.2022.2149019.
29. Kaźmierczak-Siedlecka K, Skonieczna-Żydecka K, Hupp T, Duchnowska R, Marek-Trzonkowska N, Połom K. Next-generation probiotics—do they open new therapeutic strategies for cancer patients? Gut Microbes. 2022;14(1):2035659. https://doi.org/10.1080/19490976.2022.2035659.
30. Holmberg SM, Feeney RH, Prasoodanan PKV, Puértolas-Balint F, Singh DK, Wongkuna S, Zandbergen L, Hauner H, Brandl B, Nieminen AI, Skurk T, Schroeder BO. The gut commensal *Blautia* maintains colonic mucus function under low-fiber consumption through secretion of short-chain fatty acids. Nat Commun. 2024;15(1):3502. https://doi.org/10.1038/s41467-024-47594-w.
31. Amoroso C, Perillo F, Strati F, et al. The role of gut microbiota biomodulators on mucosal immunity and intestinal inflammation. Cells. 2020. https://doi.org/10.3390/cells9051234.
32. Burrello C, Garavaglia F, Cribiù FM, et al. Therapeutic faecal microbiota transplantation controls intestinal inflammation through IL10 secretion by immune cells. Nat Commun. 2018. https://doi.org/10.1038/s41467-018-07359-8.
33. Falony G, Joossens M, Vieira-Silva S, et al. Population-level analysis of gut microbiome variation. Science. 2016. https://doi.org/10.1126/science.aad3503.
34. Mulhall H, DiChiara JM, Huck O, Amar S. Pasteurized *Akkermansia muciniphila* reduces periodontal and systemic inflammation induced by *Porphyromonas gingivalis* in lean and obese mice. J Clin Periodontol. 2022. https://doi.org/10.1111/jcpe.13629.
35. Liu S, Zhao W, Lan P, Mou X. The microbiome in inflammatory bowel diseases: from pathogenesis to therapy. Protein Cell. 2021;12(5):331–45. https://doi.org/10.1007/s13238-020-00745-3.
36. Zheng J, Sun Q, Zhang J, Ng SC. The role of gut microbiome in inflammatory bowel disease diagnosis and prognosis. United Eur Gastroenterol J. 2022;10(10):1091–102. https://doi.org/10.1002/ueg2.12338.
37. Atreya R, Neurath MF. Biomarkers for personalizing IBD therapy: the quest continues. Clin Gastroenterol Hepatol. 2024;22(7):1353–64. https://doi.org/10.1016/j.cgh.2024.01.026.

Publisher's Note