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Efficacy and Safety of Fecal Microbiota Transplantation in Children with Refractory Functional Abdominal Pain Disorders: An Open-Label Pilot Trial with Exploratory Microbiome Analyses

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Abstract

Background

Fecal microbiota transplantation (FMT) rebuilds the gut microbiota, yet its efficacy in pediatric refractory functional abdominal pain disorders (FAPDs) is insufficiently described. The purpose of this study is to identify gut microbiota dysbiosis in pediatric FAPDs and to examine the effectiveness, safety, and microbial biomarkers of FMT in these patients.

Methods

In a two-phase study, gut microbiota (16S rRNA sequencing) were compared between 47 children with FAPDs and 39 healthy controls. Thereafter, a non-randomized, open-label prospective pilot trial (ClinicalTrials.gov: NCT05753774) then allocated the 47 refractory patients, according to their guardians'/and/or patients' preferences to receive FMT plus conventional therapy (n = 26) or conventional therapy only (n = 21). The trial was terminated early because of slow recruitment (47 of 50 planned participants). The primary endpoint was the composite response rate at weeks 4 and 8.

Results

At baseline, patients had significant gut microbiota dysbiosis: 3.5-fold Proteobacteria enrichment (20.6% vs. 5.8%; P < 0.001), 4.1-fold Escherichia–Shigella expansion (12.7% vs. 3.1%; P < 0.001), and reduced α-diversity (Shannon index 4.7 ± 1.1 vs. 5.4 ± 1.1; P = 0.002). The composite response rate was higher with FMT at week 4 (69.2% vs. 14.3%; RR 4.85, 95% CI 1.65–14.24; Fisher's exact P < 0.001) and week 8 (80.8% vs. 47.6%; RR 1.70, 95% CI 1.04–2.76; P = 0.029), with significantly better adjusted scores of symptoms in FMT group in both measurements. Post-FMT, Faecalibacterium and Blautia increased (+ 44.5% and + 99.1%) while Escherichia–Shigella declined (− 19.2%). Exploratory random forest models (seven genera for diagnosis; four genera for response prediction) yielded internally validated AUCs of 0.896 (95% CI 0.821–0.952) and 0.917 (95% CI 0.765–1.000), respectively. FMT was safe with transient constipation in 50.0% and no severe adverse events.

Conclusions

In this open-label pilot trial, FMT plus conventional therapy was associated with higher and more sustained response rates in children with refractory FAPDs. Exploratory microbial signatures further demonstrated potential for both diagnosis and treatment response prediction.

Trial registration

Registered at ClinicalTrials.gov on 11 February 2023 (registration number NCT05753774), before the enrolment of the first participant. https//clinicaltrials.gov/study/NCT05753774

1. Introduction

Chronic abdominal pain is one of the most common complaints in pediatric clinics, with approximately 90% of these cases attributed to functional abdominal pain disorders (FAPDs) [1]. According to the latest Rome IV criteria, FAPDs include several subtypes such as functional dyspepsia (FD), irritable bowel syndrome (IBS), and nonspecific functional abdominal pain (FAP-NOS), with a global prevalence of up to 11.7% [2]. Although defined as functional disorders driven by biopsychosocial factors, FAPDs significantly impact children's quality of life. Recurrent pain can lead to functional disability that disrupts their academic performance, physical activity, and daily routines [3] while also escalating healthcare costs [4]. It is important to notice, that anxiety and disease burden in children with FAPDs is similar to that of inflammatory bowel diseases [5].

Current clinical management of FAPDs faces two major challenges. Firstly, psychological therapies such as hypnotherapy and cognitive-behavioral therapy remain underutilized due to time limitations and availability issues. Secondly, medications, including antispasmodics, antihistamines, and tricyclic antidepressants, offer only short-term symptom relief. Their effectiveness is supported by low-quality evidence, varies between individuals, and is often followed by symptom relapse [6–7]. These difficulties appear because conventional therapies target isolated pathological pathways and fail to address the core mechanism of FAPDs—dysregulation of the microbiota–gut–brain axis [8]. Recent data indicate that gut dysbiosis triggers visceral hypersensitivity and intestinal barrier dysfunction through disrupted tryptophan metabolism and reduced short-chain fatty acid (SCFA) synthesis, playing a central role in FAPD pathogenesis [9]. While this evidence supports the rationale for microbiota-targeted therapies, existing probiotics and prebiotics provide only partial microbial modulation, which is insufficient for holistic ecosystem restoration [10].

Despite the proven efficacy of fecal microbiota transplantation (FMT) in recurrent C. difficile infections [11–12] and Crohn's disease [13], FMT in FAPDs is rarely explored. Currently, this field faces three major challenges: firstly, the extent of gut microbiota dysbiosis in children with FAPDs has not been clearly defined; secondly, there is a lack of standardized FMT protocols tailored for pediatric use, including age-appropriate formulation, dosage, delivery route, efficacy and safety assessments; thirdly, no specific biomarkers have been identified for diagnostic or therapeutic outcome prediction.

In order to solve these issues, we first conducted a cross-sectional case-control analysis to comprehensively characterize the gut microbial dysbiosis in children with FAPDs. Subsequently, we carried out a prospective interventional trial to evaluate the efficacy and safety of FMT in pediatric refractory FAPDs. Considering the fact, that FAP-NOS is used as residual diagnosis of FAPDs with different types of abdominal pain after excluding other subtypes, the interventional trial was conducted only in FD and IBS subtypes with more specific symptoms. Concurrently, we developed a lyophilized, enteric-coated FMT capsule specifically designed for children. The capsules were manufactured under a standardized encapsulation process to ensure safety and consistency in treatment delivery [14]. Furthermore, we developed exploratory predictive models using core microbial biomarkers (e.g., Faecalibacterium) to assess the feasibility of predicting treatment response. Collectively, this study aimed to provide novel strategies and practical tools for the precise intervention of FAPDs.

2. Materials and methods

2.1 Study design

This study was conducted in two phases. First, a cross-sectional case-control study was performed, involving 47 children diagnosed with FAPDs (Rome IV-confirmed FD or IBS subtypes) and 39 age- and sex-matched healthy controls (HCs). All participants underwent standardized baseline gut microbiota profiling via 16S rRNA sequencing targeting the V3–V4 hypervariable regions. Second, a non-randomized, open-label prospective trial was conducted from April 2023 to March 2026 (ClinicalTrials.gov ID: NCT05753774). All 47 participants with refractory FAPDs were allocated, based on guardian and/or patient preference, to the FMT plus conventional therapy group (FMT group, n = 26) or the conventional therapy-only group (control group, n = 21) (Fig. 1). The planned sample size of 50 assumed response rates of 30% with conventional therapy alone [6] and 70% with FMT plus conventional therapy [13, 14] (21 per group; two-sided $\alpha = 0.05$, power = 80%), inflated by approximately 15% for anticipated dropout. Enrolment was stopped at 47 participants because of slow recruitment, and no interim efficacy analysis was performed.

FMT was administered via two validated routes: orally using lyophilized capsules or via colonoscopic liquid infusion (total dose: $2.0–3.0 \times 10^{13}$ bacteria/course). Conventional therapy included first-line probiotics, cyproheptadine, and amitriptyline; proton pump inhibitors and other adjunctive agents were continued at stable doses in both groups (Table 1). At baseline (week 0) as well as at weeks 4 and 8, independent researchers who were blinded to group allocation conducted outcome evaluations of the two groups using validated scales, including the Gastrointestinal Symptom Rating Scale (GSRS), Faces Visual Analog Scale (F-VAS), revised Wong–Baker Faces Pain Scale (FPS-R), and Pittsburgh Sleep Quality Index (PSQI). Serial stool samples were collected aseptically at weeks 0 and 4 from the FMT group and processed within 2 hours.

Table 1
Baseline characteristics of the FMT and control groups

Parameters	FMT group (n = 26)	Control group (n = 21)	P
Age(year), M ± SD	8.9 ± 2.8	9.8 ± 3.1	0.334
Sex, male, n (%)	21 (80.8%)	15 (71.4%)	0.685
Disease subtype			0.821
IBS(n, %)	12 (46.2%)	9 (42.9%)	
FD(n, %)	14 (53.8%)	12 (57.1%)	
Concomitant treatments			
Probiotics,(n, %)	26 (100%)	21 (100%)	1
Amitriptyline(n, %)	6 (23.1%)	5 (23.8%)	1
Cyproheptadine(n, %)	10 (38.5%)	11 (52.4%)	0.34
PPIs(n, %)	22(84.6%)	21(100%)	0.176
L-glutamine and sodium gualenate, n (%)	10 (38.5%)	8 (38.1%)	0.98
Low-FODMAP (n, %)	4 (15.4%)	5 (23.8%)	0.721
GSRS score, M ± SD	33.42 ± 3.78	32.57 ± 4.91	0.518
PSQI score, M ± SD	6.6 ± 2.4	6.4 ± 1.5	0.652
FPS-R score, M ± SD	4.77 ± 1.14	4.76 ± 1.00	0.981
F-VAS score, M ± SD	48.38 ± 8.03	46.67 ± 5.91	0.403
Gastrointestinal symptoms			
Abdominal pain(n, %)	26 (100%)	21 (100%)	1
Diarrhea(n, %)	8 (30.8%)	6 (28.6%)	0.87
Constipation(n, %)	4 (15.4%)	3 (14.3%)	1
Nausea and vomiting (n, %)	6 (23.1%)	7 (33.3%)	0.435

IBS, irritable bowel syndrome; FD, functional dyspepsia; PPIs, proton pump inhibitors; GSRS, Gastrointestinal Symptom Rating Scale; PSQI, Pittsburgh Sleep Quality Index; FPS-R, revised Wong–Baker Faces Pain Scale; F-VAS, Faces Visual Analog Scale. Continuous variables were compared using independent t-tests or Mann–Whitney U tests and categorical variables using chi-square or Fisher's exact tests, as appropriate.

The primary endpoint was the composite response rate at weeks 4 and 8, defined as a ≥ 50% reduction in both the GSRS total score (or a final score ≤ 15) and either the F-VAS or FPS-R score. This composite measure summarised the co-primary outcomes registered at ClinicalTrials.gov (changes in GSRS and in self-reported pain at weeks 4 and 8). The secondary endpoints included α/β-diversity shifts, treatment-related adverse events, and microbial functional predictions. The study protocol was approved by the Ethics Committee of Tongji Hospital, Huazhong University of Science and Technology (TJ-IRB20220127), and is reported in accordance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines and their extension for non-randomized studies (TREND statement); the machine-learning analyses are reported in accordance with the TRIPOD statement, and the completed TREND and TRIPOD checklists are provided as supplementary files.

2.2 Inclusion and exclusion criteria of the FAPDs group

The inclusion criteria were as follows: (1) age 5–15 years; (2) refractory FAPD history ≥ 6 months, meeting the Rome IV criteria (inadequate response to ≥ 2 conventional therapies); (3) willingness to participate in the open-label prospective trial; and (4) provision of signed informed consent by guardians, completion of baseline microbiota testing, and attendance at ≥ 3 follow-up visits (weeks 0, 4, and 8).

The exclusion criteria were as follows: (1) evidence of organic disease (C-reactive protein [CRP] > 10 mg/L, erythrocyte sedimentation rate [ESR] > 20 mm/h, fecal calprotectin > 50 μg/g, or abnormal imaging/endoscopic findings); (2) severe psychiatric disorders or cognitive impairment; (3) contraindications to FMT (such as immunodeficiency or enterostomy); (4) incomplete data affecting efficacy evaluation; and (5) use of antibiotics within 8 weeks before baseline stool sampling.

2.3 Healthy control group and donor screening

Healthy controls were recruited from a pediatric health-screening program and had no current or historical gastrointestinal symptoms as verified by questionnaire and clinical interview. FMT donors were screened separately from healthy controls, although both followed a three-tier screening protocol established in previous studies [13, 14].

First, a preliminary questionnaire screened out individuals with allergies, metabolic syndrome, a history of gastrointestinal surgery, or other relevant medical histories.

Second, stool and blood samples were submitted for laboratory testing; participants were excluded upon detection of any of the following: enteric pathogens (e.g., C. difficile toxins, norovirus), antimicrobial-resistant organisms (e.g., extended-spectrum beta-lactamase-producing Enterobacteriaceae, carbapenem-resistant Enterobacteriaceae, vancomycin-resistant enterococci, and methicillin-resistant Staphylococcus aureus), and inflammatory biomarkers (e.g., fecal calprotectin, CRP, ESR).

Third, volunteers underwent a 3-week medical observation whereby daily symptom logs and biweekly clinical reviews were used to confirm an asymptomatic status. Donor–recipient matching followed age (± 2 years), gender, regional dietary habits, and absence of antibiotic/laxative use within the previous 3 months.

2.4 Standardized efficacy assessment

Several standardized assessments were conducted at weeks 0, 4, and 8. For the GSRS, a clinician-administered semi-structured interview evaluating five domains (reflux, abdominal pain, diarrhea, constipation, and dyspepsia; total score range: 15–105) was conducted. For F-VAS, patients rated their pain on an age-adapted 100-mm linear scale with cartoon facial gradients ("happy→neutral→distressed") for real-time pain intensity quantification (range: 0–100). For FPS-R, a six-level behaviorally anchored scale (0–10) combined with vital sign monitoring (heart rate/blood pressure correlations) was used as a visual pain assessment tool. For PSQI, a 30-day guardian-assisted sleep log assessing seven domains (total score range: 0–21) was used to evaluate overall sleep quality.

All concurrent therapies remained unchanged during the FMT intervention phase but could be tapered post-treatment. The primary endpoint was the composite response rate at weeks 4 and 8. A composite response, requiring a ≥ 50% reduction in GSRS score or a final score ≤ 15 AND a ≥ 50% reduction in either the F-VAS or FPS-R score, with no addition of any non-permitted medications, was considered treatment success. Treatment failure was defined as withdrawal before week 8, initiation of new medications, or the occurrence of severe adverse events. A second FMT course was offered based on guardian preference and logistical availability rather than on week-4 response status.

2.5 FMT preparation and protocol

Fecal microbiota suspensions and enteric-coated capsules were prepared using standardized protocols [13, 14], with the full preparation and quality-control procedures (donor-stool filtration, anaerobic centrifugation, lyophilization, encapsulation, and cryopreservation) detailed in the Supplementary Methods and Supplementary Tables 1–2.

FMT regimens included colonic infusion (daily administration for 5 consecutive days, with post-infusion supine positioning ≥ 2 h) or oral capsules (five capsules/dose, taken twice daily for 6 days), achieving a total bacterial dose of 2.0–3.0 × 10^{13} bacteria/course. Safety monitoring adhered to CTCAE v5.0, with adverse events, including transient fever (≥ 38.5°C) and abdominal pain (VAS ≥ 4), recorded throughout the study using active structured questioning at each visit and spontaneous reporting between visits.

2.6 Microbiota analysis

Sterile fecal samples were collected from patients with FAPDs and HCs at baseline and post-FMT (week 4), immediately frozen at − 80°C, and profiled by 16S rRNA gene sequencing of the V3–V4 region. Taxonomic profiling, diversity analyses, LEfSe, and PICRUSt2-based functional prediction (KEGG database) followed a standardized pipeline detailed in the Supplementary Methods; all predicted functional results are interpreted as exploratory inferences rather than direct metabolic measurements. Sequencing data were deposited in the Genome Sequence Archive (GSA: CRA025894; https://ngdc.cncb.ac.cn/gsa/browse/CRA025894).

2.7 Statistical analysis

The primary analysis followed the intention-to-treat principle, with all participants analysed in their allocated groups. Because all 47 enrolled participants completed the 8-week follow-up without withdrawal, loss to follow-up, or major protocol deviation, the intention-to-treat dataset was identical to the per-protocol set. Consequently, no imputation for missing efficacy data was required. Continuous variables are reported as mean ± SD and categorical variables as frequencies; normality was assessed with the Kolmogorov–Smirnov test. Response rates were compared between groups using Fisher's exact test, chosen a priori given the small expected cell counts, with relative risks (RRs) and 95% confidence intervals (CIs) reported. For continuous outcomes, between-group differences at weeks 4 and 8 were estimated by analysis of covariance (ANCOVA) adjusted for baseline scores; unadjusted comparisons used independent t-tests or Mann–Whitney U tests, and within-group changes were assessed with paired t-tests or Wilcoxon signed-rank tests. All tests were two-sided, with $P < 0.05$ considered significant.

β-diversity was compared using PERMANOVA (9,999 permutations). For multiple comparisons of microbial taxa, the false discovery rate was controlled with the Benjamini–Hochberg procedure ($q < 0.05$). Secondary and exploratory outcomes were not adjusted for multiplicity and are interpreted as hypothesis-generating.

Machine-learning model development and internal validation. Random forest classifiers were developed on the abundances of candidate genera and internally validated by resampling, as detailed in the Supplementary Methods. Briefly, performance was estimated by leave-one-out cross-validation (LOOCV; 95% CIs from 2,000 bootstrap resamples), repeated stratified five-fold cross-validation (10 repeats), and bootstrap optimism correction (Harrell's method). Sensitivity and specificity were derived at the Youden-optimal cutoff of the pooled cross-validated probabilities, and calibration and decision-curve analyses were performed. Analyses were performed in IBM SPSS Statistics (version 25) and R (version 4.6.1), and graphs were generated with GraphPad Prism (version 9).

3. Results

3.1 Study design and baseline characteristics

In phase I, a case-control analysis was performed on 86 stool samples (47 from children with FAPDs and 39 from healthy controls [HCs]) to characterize baseline dysbiosis and identify diagnostic biomarkers. No significant differences were observed in demographic variables between the two groups (Supplementary Table 3). In phase II, we conducted an open-label prospective trial enrolling 47 refractory FAPD patients. As noted above, the trial was terminated early because of slow recruitment, before reaching the planned sample size of 50. Based on guardian and/or patient preference, 26 patients received FMT combined with conventional therapy (the FMT group), while 21 received conventional therapy alone (control group). All 26 patients in the FMT group and all 21 patients in the control group completed the scheduled assessments at weeks 4 and 8; there were no dropouts, withdrawals, losses to follow-up, or major protocol deviations in either group. Both groups received comparable background regimens, including probiotics and adjunctive medications such as proton pump inhibitors and amitriptyline (Table 1). The two groups were well-matched at baseline in age, sex, medication use, and all symptom severity scores (all $P > 0.05$; Table 1). In the FMT group, transplantation was administered via oral capsules (n = 16) or fecal infusion (n = 10), with 15 patients undergoing a dual-course regimen (Table 2).

Table 2
Clinical data of the 26 patients in the FMT group

Patient ID	Disease subtype	Age (yr)	Sex	Patient–donor relationship	FMT course	Efficacy at week 4	Efficacy at week 8	AEs at week 4	AEs at week 8	Route of administration
P1	FD	7	Male	Nonrelative	Two courses	effective	effective	—	—	Colonic infusion
P2	FD	7	Female	Nonrelative	One course	effective	effective	Constipation	—	Oral capsules
P3	FD	13	Male	Nonrelative	One course	ineffective	ineffective	Constipation	—	Colonic infusion
P4	FD	14	Female	Nonrelative	Two courses	ineffective	effective	Constipation	Constipation	Colonic infusion
P5	FD	10	Female	Nonrelative	Two courses	effective	effective	Constipation, Abdominal distension	Constipation	Oral capsules
P6	FD	12	Male	Nonrelative	One course	effective	effective	—	—	Oral capsules
P7	FD	7	Male	Nonrelative	Two courses	effective	effective	Abdominal distension	—	Oral capsules
P8	FD	10	Male	Nonrelative	Two courses	ineffective	effective	—	—	Oral capsules
P9	FD	12	Female	Nonrelative	One course	effective	effective	—	—	Oral capsules
P10	FD	13	Male	Nonrelative	One course	ineffective	ineffective	—	—	Oral capsules
P11	FD	9	Male	Nonrelative	Two courses	effective	effective	Constipation, nausea	—	Oral capsules
P12	FD	11	Male	Nonrelative	One course	effective	effective	Constipation	—	Oral capsules
P13	FD	6	Male	Nonrelative	One course	ineffective	ineffective	—	—	Colonic infusion
P14	FD	9	Male	Nonrelative	One course	effective	effective	—	—	Colonic infusion
P15	IBS	13	Male	Nonrelative	Two courses	effective	effective	—	—	Colonic infusion
P16	IBS	10	Male	Nonrelative	Two courses	effective	effective	Constipation	—	Colonic infusion
P17	IBS	8	Male	Nonrelative	One course	ineffective	ineffective	Constipation	—	Oral capsules
P18	IBS	6	Male	Nonrelative	Two courses	ineffective	effective	Constipation	—	Oral capsules
P19	IBS	6	Male	Nonrelative	Two courses	effective	effective	Constipation, nausea	—	Colonic infusion
P20	IBS	12	Male	Nonrelative	One course	effective	effective	—	—	Oral capsules
P21	IBS	8	Female	Nonrelative	Two courses	effective	effective	—	—	Oral capsules
P22	IBS	6	Male	Nonrelative	Two courses	effective	effective	Constipation	Constipation	Oral capsules
P23	IBS	5	Male	Nonrelative	Two courses	ineffective	ineffective	—	—	Colonic infusion
P24	IBS	6	Male	Nonrelative	Two courses	effective	effective	Constipation, vomiting	—	Oral capsules

IBS, irritable bowel syndrome; FD, functional dyspepsia; AE, adverse event. Efficacy denotes composite responder status (≥ 50% reduction in GSRS or a final GSRS score ≤ 15, and ≥ 50% reduction in either pain scale).

Patient ID	Disease subtype	Age (yr)	Sex	Patient–donor relationship	FMT course	Efficacy at week 4	Efficacy at week 8	AEs at week 4	AEs at week 8	Route of administration
P25	IBS	6	Male	Nonrelative	Two courses	effective	effective	Constipation	—	Oral capsules
P26	IBS	6	Male	Nonrelative	One course	effective	effective	—	—	Colonic infusion

IBS, irritable bowel syndrome; FD, functional dyspepsia; AE, adverse event. Efficacy denotes composite responder status (≥ 50% reduction in GSRS or a final GSRS score ≤ 15, and ≥ 50% reduction in either pain scale).

3.2 Profound gut ecological collapse in pediatric FAPDs

Patients with FAPDs exhibited a state of profound gut ecological collapse compared to HCs (Figs. 2 and 3). This was characterized by a severe phylum-level imbalance, featuring significantly elevated Proteobacteria (20.6% vs. 5.8%; $P < 0.001$; $q < 0.001$ after FDR correction) and depleted Firmicutes (54.5% vs. 70.9%; $P < 0.001$; $q < 0.001$) (Supplementary Table 4).

This extended to the genus level, featuring a 4.1-fold enrichment of the pro-inflammatory Escherichia–Shigella (12.7% vs. 3.1%; $P < 0.001$) and depletion of beneficial genera including Faecalibacterium, Blautia, Agathobacter, and Dialister (all $P < 0.05$) (Supplementary Table 5, Fig. 2). This dysbiosis was systemic, reflected in reduced α-diversity (Shannon index: 4.7 ± 1.1 vs. 5.4 ± 1.1; $P = 0.002$), significant β-diversity separation (PERMANOVA $P = 0.001$) (Fig. 3B-C), and a pathogenic shift in microbial health indices (Gut Microbiome Health Index (GMHI): -0.15 vs. 1.94; Microbial Dysbiosis Index (MDI): -0.85 vs. -1.27; both $P < 0.001$, overall FAPD cohort [n = 47] vs. HCs [n = 39]) (Fig. 3E-F), where higher MDI values indicate greater dysbiosis.

3.3 Superior clinical efficacy and safety of FMT

FMT combination therapy was associated with rapid and sustained symptom improvement. At baseline, the two groups were well balanced across all key clinical scores (GSRS, PSQI, FPS-R, F-VAS; all $P > 0.05$) (Table 1).

By week 4, the composite response rate in the FMT group was 4.8-fold higher than that in controls (69.2% [18/26] vs. 14.3% [3/21]; RR 4.85, 95% CI 1.65–14.24; Fisher's exact $P < 0.001$). The FMT group achieved markedly greater reductions in pain scores (FPS-R: 2.4 ± 1.1 vs. 3.7 ± 1.3; mean difference − 1.33 [95% CI − 2.05 to − 0.61]; $P < 0.001$; F-VAS: 24.7 ± 12.2 vs. 35.5 ± 10.5; mean difference − 10.87 [95% CI − 17.65 to − 4.09]; $P = 0.002$) and gastrointestinal symptom burden (GSRS: 19.5 ± 5.7 vs. 25.4 ± 5.2; mean difference − 5.93 [95% CI − 9.18 to − 2.68]; $P < 0.001$). This therapeutic advantage persisted through week 8, with the sustained response rate remaining significantly higher in the FMT group (80.8% [21/26] vs. 47.6% [10/21]; RR 1.70, 95% CI 1.04–2.76; Fisher's exact $P = 0.029$). Significant between-group differences were maintained for GSRS and pain scores at week 8 (all $P < 0.05$), whereas PSQI scores did not differ between groups at either time point (Table 3, Fig. 4). Individual participant-level scale scores and responder status are provided in Supplementary Table 6.

Table 3
Comparison of validated scale scores between the two groups at weeks 4 and 8

Time	Scale	FMT group	Control group	Mean difference (95% CI)	P value	Adjusted difference (95% CI)	Adjusted P
4w	FPS-R	2.4 ± 1.1	3.7 ± 1.3	-1.33 (-2.05 to -0.61)	< 0.001	-1.33 (-1.97 to -0.70)	< 0.001
4w	F-VAS	24.7 ± 12.2	35.5 ± 10.5	-10.87 (-17.65 to -4.09)	0.002	-11.65 (-18.27 to -5.03)	< 0.001
4w	GSRS	19.5 ± 5.7	25.4 ± 5.2	-5.93 (-9.18 to -2.68)	< 0.001	-6.21 (-9.40 to -3.02)	< 0.001
4w	PSQI	4.6 ± 1.1	5.2 ± 1.1	−0.6 (− 1.23, 0.03)	0.08	—	—
8w	FPS-R	1.2 ± 1.3	2.1 ± 1.2	-0.86 (-1.59 to -0.14)	0.021	-0.86 (-1.60 to -0.13)	0.022
8w	F-VAS	16.9 ± 10.0	22.6 ± 6.9	−5.70 (− 10.85 to − 0.54)	0.031	−5.84 (− 11.09 to − 0.60)	0.030
8w	GSRS	16.8 ± 3.7	20.0 ± 3.4	-3.23 (-5.32 to -1.14)	0.003	-3.24 (-5.37 to -1.12)	0.004
8w	PSQI	4.5 ± 1.1	4.7 ± 1.0	−0.2 (− 0.80, 0.40)	0.504	—	—

GSRS, Gastrointestinal Symptom Rating Scale; PSQI, Pittsburgh Sleep Quality Index; FPS-R, revised Wong–Baker Faces Pain Scale; F-VAS, Faces Visual Analog Scale. Mean differences are unadjusted between-group differences (FMT minus control); adjusted differences were estimated by ANCOVA with the baseline score as covariate. PSQI was not adjusted because the unadjusted between-group difference was not significant.

Safety was favorable: adverse events (AEs) in the FMT group were predominantly mild and transient (initial incidence: 53.8% [14/26]), primarily transient constipation (13 patients, 50.0%). The incidence of AEs declined substantially to 11.5% (3/26) by week 8. No severe AEs or treatment-

related serious adverse events (SAEs) were reported.

In an exploratory subgroup analysis stratified by route of administration, oral capsules and colonic instillation showed comparable efficacy (week 4: 75.0% vs. 60.0%, Fisher's exact P = 0.664; week 8: 87.5% vs. 70.0%, P = 0.340). Constipation was numerically more common with oral capsules than with colonic instillation (56.3% [9/16] vs. 40.0% [4/10], P = 0.688). Similarly, although no significant difference was observed between single-course and dual-course regimens, the dual-course regimen showed numerically superior efficacy at week 8 (93.3% vs. 63.6%, P = 0.128).

3.4 FMT-driven restoration of gut microbial ecology

A total of 75 fecal samples were collected to evaluate the efficacy of FMT in ameliorating gut microbiota dysbiosis in children with FAPDs. These samples included 26 pre-treatment fecal samples, 26 post-treatment fecal samples collected at week 4, of which one was excluded because of contamination, leaving 25 paired pre–post samples, and 24 donor-matched fecal samples. Among the donors, 22 donors were individually matched to 22 recipients based on age and sex. The remaining two donors were each matched with two recipients who were also age- and sex-matched.

FMT induced robust structural restoration of the gut microbiota, increasing beneficial genera (e.g., Faecalibacterium + 44.5%, Blautia + 99.1%) and decreasing pathobionts (Escherichia–Shigella − 19.2%) (Supplementary Table 7, Fig. 5A). This restoration was accompanied by significantly increased α-diversity (Shannon index: 4.59 to 5.33; P = 0.015) and a near-complete normalization of the GMHI (P < 0.001) (Fig. 5B-C). In the FMT subgroup (n = 26), the MDI (− 0.50) was higher than the mean of the overall FAPD cohort (− 0.85), indicating more pronounced dysbiosis among patients who opted for FMT; MDI decreased significantly to − 0.78 at week 4 (P < 0.05), approaching the level of healthy controls (− 1.27) and indicating partial ecological restoration (Fig. 5D).

Post-FMT constipation was associated with a 1.86-fold increase in the Firmicutes/Bacteroidetes (F/B) ratio compared with patients without constipation, suggesting a link between donor-community structure and gut transit after FMT.

After FMT treatment, disease subtype-specific predicted metabolic changes were observed: in IBS-subtype patients, an increase in Faecalibacterium genus abundance (Supplementary Table 8) was accompanied by predicted activation of the p53 pathway and apoptosis pathway; whereas in FD patients, an increase in Blautia genus abundance (Supplementary Table 9) was associated with predicted suppression of tryptophan metabolism (Fig. 5E–F).

3.5 Distinct microbial–metabolic signatures define FMT response

Responders (n = 17) and non-responders (n = 8) with qualified post-FMT stool samples exhibited starkly divergent post-FMT profiles (one responder's week-4 sample was excluded because of contamination, leaving 25 of 26 samples for post-FMT microbiota comparisons).

Responders were characterized by a 3.6-fold higher abundance of Bacteroidota (21.5% vs. 6.0%) and enrichment of SCFA-producing genera (e.g., Bacteroides, Faecalibacterium). Conversely, non-responders showed persistent dominance of Proteobacteria (25.4% vs. 9.1% in responders) and elevated pro-inflammatory taxa (Escherichia–Shigella: 4.7-fold increase; two-sided Mann–Whitney U test on raw read counts (8 non-responders vs. 17 responders), P = 0.028) (Supplementary Tables 10–11 and 14, Fig. 6). This was reflected in significantly higher α-diversity (P < 0.001) and distinct β-diversity (P = 0.004) in responders compared to non-responders (Fig. 7A-B).

Metabolically, FMT responders showed predicted activation of energy metabolism pathways (AMPK signaling [ko04152, FC = + 1.43, P = 0.014], oxidative phosphorylation [ko00190, FC = + 1.2, P = 0.001], and D-glutamine and D-glutamate metabolism [ko00471, FC = + 1.22, P = 0.014]). Non-responders showed predicted dysregulation of tryptophan metabolism (ko00380, FC = + 1.40, P = 0.012) (Fig. 7C), indicative of an inflammatory metabolic profile.

3.6 Exploratory predictive modelling for diagnosis and treatment guidance

A random forest model based on seven key genera (Faecalibacterium, Escherichia–Shigella, Ruminococcus, Blautia, Agathobacter, Dialister, and Coprococcus) yielded an apparent (in-sample) AUC of 0.906 for distinguishing patients with FAPDs from HCs, with an apparent out-of-bag (OOB) AUC of 0.890 (Fig. 2H, Supplementary Figure S1A, Supplementary Table 12). Discrimination was essentially unchanged under internal validation: leave-one-out cross-validation (LOOCV) gave an AUC of 0.896 (95% CI 0.821–0.952); repeated stratified five-fold cross-validation (10 repeats) gave a mean AUC of 0.895 ± 0.066 (range 0.708–0.986); and bootstrap optimism correction gave a corrected AUC of 0.894, indicating limited overfitting. At the Youden-optimal cutoff of the pooled cross-validated probabilities, sensitivity was 83.0% (39/47) and specificity was 79.5% (31/39) (calibration intercept/slope − 0.182/1.355; Brier score 0.134). The genus-level abundance data underlying this diagnostic model are provided in Supplementary Table 13.

The response-prediction model was developed on baseline (pre-FMT) samples, all 26 of which passed quality control; the single contaminated week-4 sample therefore did not affect this analysis. A second random forest model incorporating Faecalibacterium, Escherichia–Shigella, Coprococcus, and Bacteroides yielded an apparent (in-sample) AUC of 0.971 (apparent OOB AUC 0.917) for identifying future FMT responders before treatment; internal validation produced more conservative estimates (Fig. 7D, Supplementary Figures S1B and S2, Supplementary

Table 12): an LOOCV AUC of 0.917 (95% CI 0.765–1.000) and a bootstrap-corrected AUC of 0.933, with a sensitivity of 83.3% (15/18) and a specificity of 100.0% (8/8) at the optimal cutoff. The genus-level read-count data underlying this response-prediction model are provided in Supplementary Table 14. Estimates were nevertheless unstable across resampling folds (repeated five-fold mean AUC 0.950, range 0.500–1.000), reflecting the small number of non-responders (n = 8). These exploratory models suggest that FMT success may depend on the restoration of SCFA producers and concomitant suppression of pathobionts, providing a hypothesis-generating, dual-purpose precision framework for diagnosis and therapeutic prognosis.

4. Discussion

4.1 Major findings and pathophysiological basis

This study delineates a state of marked gut dysbiosis in children with FAPDs, characterized by a 3.5-fold increase in Proteobacteria, significant depletion of Firmicutes, and a 4.1-fold expansion of Escherichia–Shigella compared to healthy controls. These features established the microbiological background for the subsequent FMT intervention. Subsequent FMT combined with conventional therapy was associated with a higher composite response rate at week 4, with sustained between-group differences through week 8. FMT was accompanied by partial ecological restoration, significantly increasing beneficial taxa such as Faecalibacterium (+ 44.5%) and enhancing α-diversity, which correlated strongly with improved clinical outcomes. Furthermore, internally validated machine learning models support the translational potential of these findings, with a 7-genus diagnostic panel (cross-validated AUC = 0.896) and a 4-genus predictive model (cross-validated AUC = 0.917) providing exploratory signals for stratifying patients and forecasting therapeutic response.

4.2 Ecological collapse in pediatric FAPDs and age-specificity

Current evidence on gut microbiota in pediatric patients with FAPDs remains limited. Our study identified an age-specific ecological collapse, marked by a 3.5-fold increase in Proteobacteria, significant depletion of butyrate-producing genera (Faecalibacterium, Agathobacter, and Blautia reduced by 3.2-, 2.4-, and 1.7-fold, respectively), and a reduction in α-diversity (ΔShannon = − 0.7, P = 0.002). This dysbiosis pattern partially overlapped with that observed in adult IBS [15], though pediatric patients exhibited a more pronounced expansion of Escherichia–Shigella (4.1-fold compared with HCs), suggesting age-dependent pathogenic mechanisms.

These findings extend previous observations that gut microbiota dysbiosis contributes to the pathogenesis of FAPDs [16–17], provide a pediatric-specific characterization of that dysbiosis, and offer a rationale for microbiota-directed therapeutic strategies such as FMT.

4.3 Rapid onset with sustained benefit: mechanism of FMT-driven ecological reset

FMT combination therapy showed a biphasic efficacy pattern of rapid initiation and sustained reinforcement. At week 4, the composite response rate of the FMT group was 4.8 times that of the control group (69.2% vs. 14.3%). Analysis of 16S rRNA sequencing data suggests that this early clinical benefit may be mediated by FMT-driven ecological niche resetting. The key mechanism proposed involves the direct supplementation of short-chain fatty acid-producing bacteria (e.g., Faecalibacterium), which may help suppress the proliferation of pro-inflammatory microbiota, thereby promoting mucosal repair and modulating neurotransmitter balance—a mechanism corroborated by prior research [18–20]. These findings further illustrate the strong link between gut–brain axis dysfunction and microbiota–metabolome imbalance in FAPDs [21], while highlighting the therapeutic potential of FMT in reestablishing microbial homeostasis [22]. Notably, while drug-treated patients exhibited delayed improvement by week 8, the FMT group maintained significant clinical superiority (80.8% vs. 47.6%; Fisher's exact P = 0.029), suggesting that sustained symptom relief may result from FMT-driven modulation of microbial metabolism [23], potentially acting synergistically with concomitant drugs. This effect is likely driven by gut metabolic remodeling through the engraftment of butyrate-producing bacteria and by synergistic interactions that enhance drug efficacy beyond monotherapy levels. Because the trial was open-label without a placebo control, expectation effects—which are substantial in pediatric functional abdominal pain—may have contributed to the observed benefit and are considered further in the Limitations section.

4.4 Predicted metabolic reprogramming and subtype-specific mechanisms

Exploratory functional inference based on 16S rRNA data (PICRUSt2) suggested distinct predicted metabolic profiles between FMT responders and non-responders: responders were enriched in predicted energy-metabolism pathways (e.g., AMPK signaling and oxidative phosphorylation), whereas non-responders showed predicted tryptophan-metabolism dysregulation—a pattern consistent with hypotheses linking microbial tryptophan metabolism to visceral hypersensitivity [24]. Predicted pathway profiles also differed between the IBS and FD subtypes (Fig. 5E–F): the IBS subtype was associated with predicted increases in p53- and apoptosis-related functions alongside increased Faecalibacterium abundance, whereas the FD subtype was linked to enrichment of Blautia and predicted suppression of tryptophan metabolism [25–27]. The biological meaning of these predicted pathway shifts—particularly the p53/apoptosis signal in the IBS subtype—is uncertain and may reflect community-level compositional effects rather than host-relevant metabolic change; direct metagenomic and metabolomic validation is required.

4.5 Toward precision medicine: diagnostic and predictive modelling

To address diagnostic and therapeutic challenges in pediatric patients with FAPDs—including the lack of reliable biomarkers (Rome IV exclusion-based diagnosis) and therapeutic heterogeneity (drug response rate < 50% and lack of a specific pharmacologic agent) [28, 29]—we developed a 7-genus diagnostic model that retained good discrimination under internal cross-validation (LOOCV AUC = 0.896, 95% CI 0.821–0.952). This model featured Faecalibacterium and Escherichia–Shigella as the highest-ranked core non-invasive biomarkers by random forest variable importance. A 4-genus response-prediction model (LOOCV AUC = 0.917, 95% CI 0.765–1.000) was also developed to monitor ecological restoration by tracking Faecalibacterium abundance changes and Proteobacteria suppression. If validated externally, these models could form a closed-loop dysbiosis diagnosis–ecological repair monitoring system that enables implementation of a functional consortium transplantation (FCT) strategy, a concept to be tested prospectively.

4.6 Safety profile and strategy optimization

FMT-related adverse events were observed in 53.8% of patients (n = 14), predominantly self-limiting constipation (n = 13), which is consistent with safety profiles reported in adult populations [30].

Notably, post-FMT constipation correlated with a higher Firmicutes/Bacteroidetes (F/B) ratio, potentially linked to delayed gut transit. To mitigate this, we propose a "graded transplantation" strategy: initially using low-F/B donor microbiota enriched with Bacteroidetes, followed by gradual ecological rebalancing and supplementation with prebiotics (5–10 g/day fructooligosaccharides; 15–20 g/day soluble fiber) to support microbial adaptation.

Lyophilized oral capsules showed comparable efficacy (75.0% vs. 60.0%, P = 0.664) and a comparable constipation incidence (56.3% vs. 40.0%, P = 0.688) to traditional liquid FMT. Their non-invasive delivery, standardized production, and superior pediatric compliance position them as a first-line option, particularly for repeat interventions [31, 32]. While prior studies have reported 88–92% efficacy in recurrent C. difficile infection [33, 34], to our knowledge this is the first prospective study to validate lyophilized FMT capsules in children with FAPDs. The 8-week safety follow-up is short for pediatric FMT, and longer-term surveillance (≥ 12 months) is warranted.

4.7 Limitations and future directions

Several limitations warrant consideration. First, group allocation was based on patient/guardian preference rather than randomization, and the open-label design without a sham or placebo control permits selection bias and expectation effects—which are substantial in pediatric functional abdominal pain—and may have inflated the estimated benefit of FMT. Second, as an exploratory pilot trial that was terminated early because of slow recruitment, the sample size (n = 47) limited statistical power, particularly for the week-8 comparison and the subgroup analyses, and the findings should be interpreted together with their confidence intervals. Third, our machine-learning models were trained and internally validated on limited sample sizes (n = 86 for diagnostic modeling and n = 26 for response prediction, including merely 8 non-responders). Moreover, because candidate genera were identified using the entire dataset before model training, the cross-validated performance estimates are likely inflated and warrant external validation. Finally, functional inferences relied on PICRUSt2 prediction without metagenomic or metabolomic confirmation, and the single-center design in a Chinese pediatric population may limit generalizability. Adequately powered, multicenter, randomized, double-blind, placebo-controlled trials with prespecified external validation of the predictive models are warranted.

5. Conclusions

This study delineated marked gut dysbiosis in children with FAPDs. In this open-label pilot trial, FMT combined with conventional therapy was associated with a higher response rate at week 4 and sustained benefits through week 8, accompanied by partial restoration of gut microbial ecology that correlated with clinical improvement. Internally validated exploratory microbial signatures showed potential for diagnosis and response prediction, although these models remain hypothesis-generating pending external validation. Further studies should confirm these findings in multicenter, randomized, double-blind, placebo-controlled trials and explore subtype-specific strategies to optimize FMT for refractory FAPDs.

Declarations

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Ethics approval and consent to participate

The study was approved by the Medical Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (TJ-IRB20220127). This approval covers the institutional FMT program for pediatric inflammatory and functional gastrointestinal disorders, under which our previous trials of FMT in children with Crohn's disease [13, 14] and the present trial in children with functional

abdominal pain disorders were conducted. The present trial was additionally registered at ClinicalTrials.gov (NCT05753774) before enrolment of the first participant, and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from the guardians of all participants, and age-appropriate assent was obtained from the children.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Author Contribution

Conceptualization: B.Z., Y.Q.W., S.N.S.; methodology: B.Z., S.N.S., S.X.L.; formal analysis: C.D., J.Y.H.; investigation: X.S.L., H.Z.H., M.L.R.; data curation: B.Z., S.H., S.X.L.; writing—original draft: B.Z., Y.Q.W.; writing—review and editing: B.Z., Y.Q.W., S.N.S., S.X.L.; supervision: Y.Q.W. All authors have read and approved the final manuscript. All authors reviewed the manuscript.

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Data Availability

16S rRNA gene sequencing data were deposited into the Genome Sequence Archive under accession number CRA025894 and are available at the following URL: https://ngdc.cncb.ac.cn/gsa/browse/CRA025894

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Figures

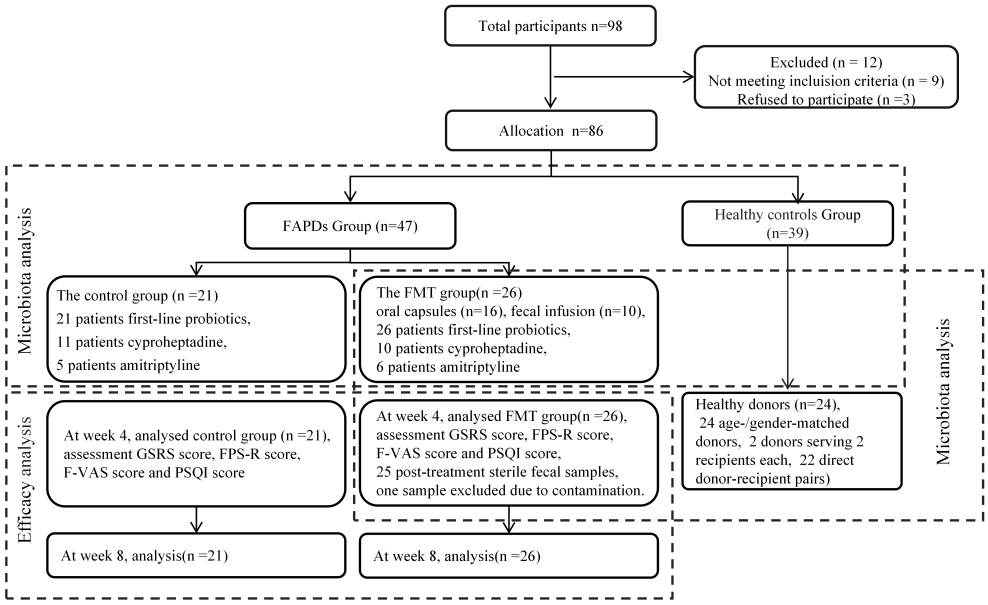


Figure 1

Study profile of patient enrollment. Of the 98 participants assessed for eligibility, 12 were excluded, leaving 86 participants allocated to the FAPDs group (n = 47) and the healthy control group (n = 39). In the FMT group, 25 of 26 patients provided paired pre- and post-treatment stool samples for microbiota analysis; all 47 allocated patients were included in the intention-to-treat clinical analysis. FAPDs, functional abdominal pain disorders; FMT, fecal microbiota transplantation; FPS-R, revised Wong–Baker Faces Pain Scale; F-VAS, Faces Visual Analog Scale; GSRS, Gastrointestinal Symptom Rating Scale; PSQI, Pittsburgh Sleep Quality Index.

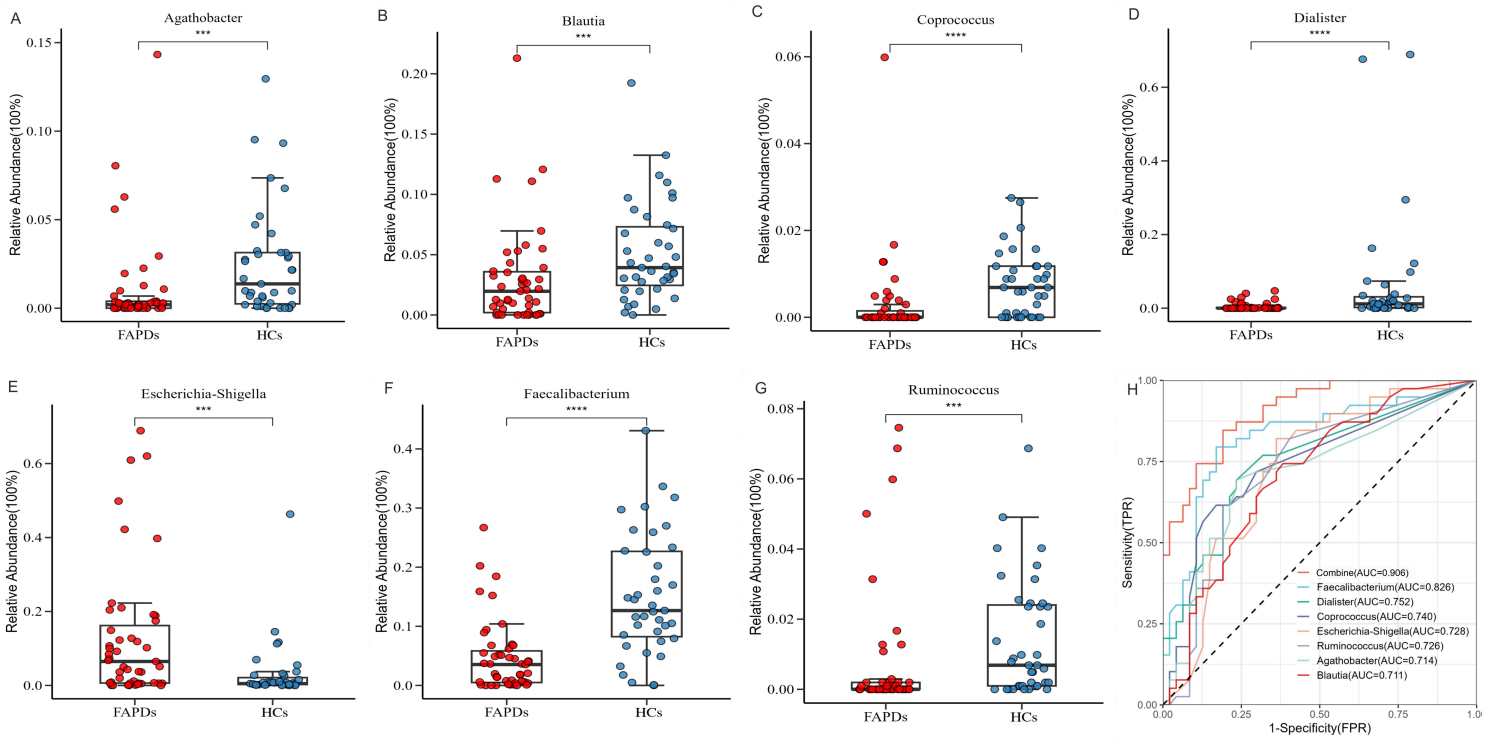


Figure 2

A diagnostic model incorporating seven key genera. (A–G) Comparison of seven core bacterial genera (Agathobacter, Blautia, Coprococcus, Dialister, Escherichia–Shigella, Faecalibacterium, and Ruminococcus) between HCs and FAPDs at baseline (Mann–Whitney U tests, ***P < 0.001, ****P < 0.0001). (H) The seven core genera were assessed as potential biomarkers to distinguish patients with FAPDs from HCs (apparent AUC = 0.906; leave-one-out cross-validated AUC = 0.896, 95% CI 0.821–0.952; sensitivity 83.0% [39/47], specificity 79.5% [31/39]). Internal validation curves are provided in Supplementary Figure S1. The ROC curves in panel H represent apparent (in-sample) fits of single genera and of the combined 7-genus classifier; internally validated performance is reported in Supplementary Figure S1 and Supplementary Table 12.

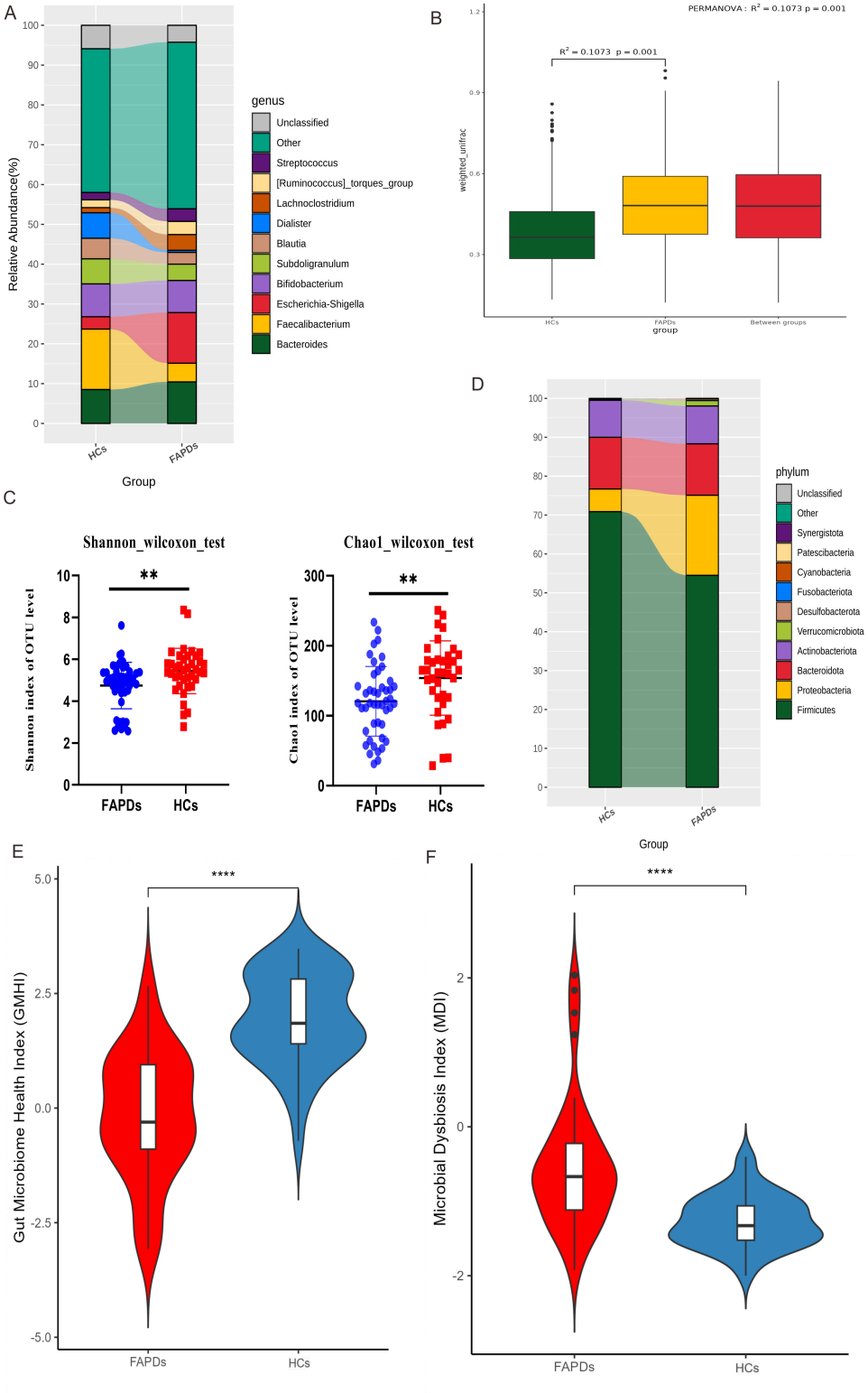


Figure 3

Compared with HCs, pediatric patients with FAPDs exhibited characteristic features of dysbiosis. (A) Genus-level differences between HCs and FAPDs at baseline. (B) Boxplot of β-diversity based on weighted UniFrac distances, showing differences in microbial communities between HCs and FAPDs at baseline (PERMANOVA). (C) Alpha diversity indices (Chao1 and Shannon) between HCs and FAPDs at baseline. Alpha diversity was significantly reduced in FAPDs compared with HCs (Mann–Whitney U tests, **P < 0.01). (D) Phylum-level differences between HCs and FAPDs at baseline. (E) Comparison of Gut Microbiome Health Indices (GMHIs) between HCs and FAPDs. GMHIs were significantly lower in FAPDs than in HCs (Wilcoxon rank-sum test, ****P < 0.0001). (F) Comparison of the microbial dysbiosis index (MDI) between HCs and FAPDs at baseline. MDI was significantly lower in HCs than in FAPDs (Wilcoxon rank-sum test, ****P < 0.0001). FAPDs, functional abdominal pain disorders; HCs, healthy controls.

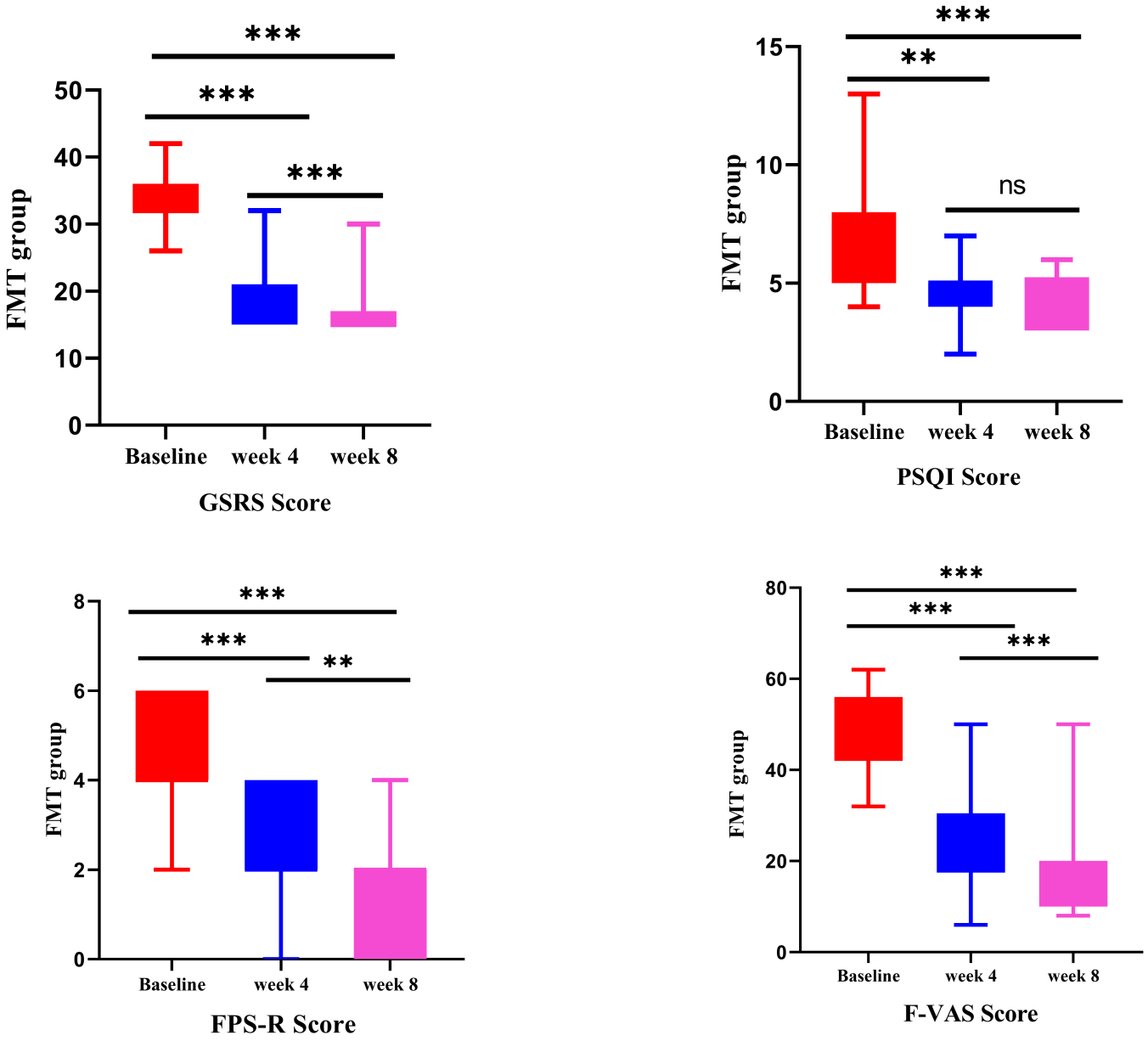


Figure 4

Changes in validated scale scores before and after treatment in the FMT group. Scores of the GSRS, FPS-R, F-VAS, and PSQI at baseline, week 4, and week 8 post-treatment are shown. (Paired Wilcoxon tests were used to evaluate within-group differences; *P < 0.05, **P < 0.01, ***P < 0.001).

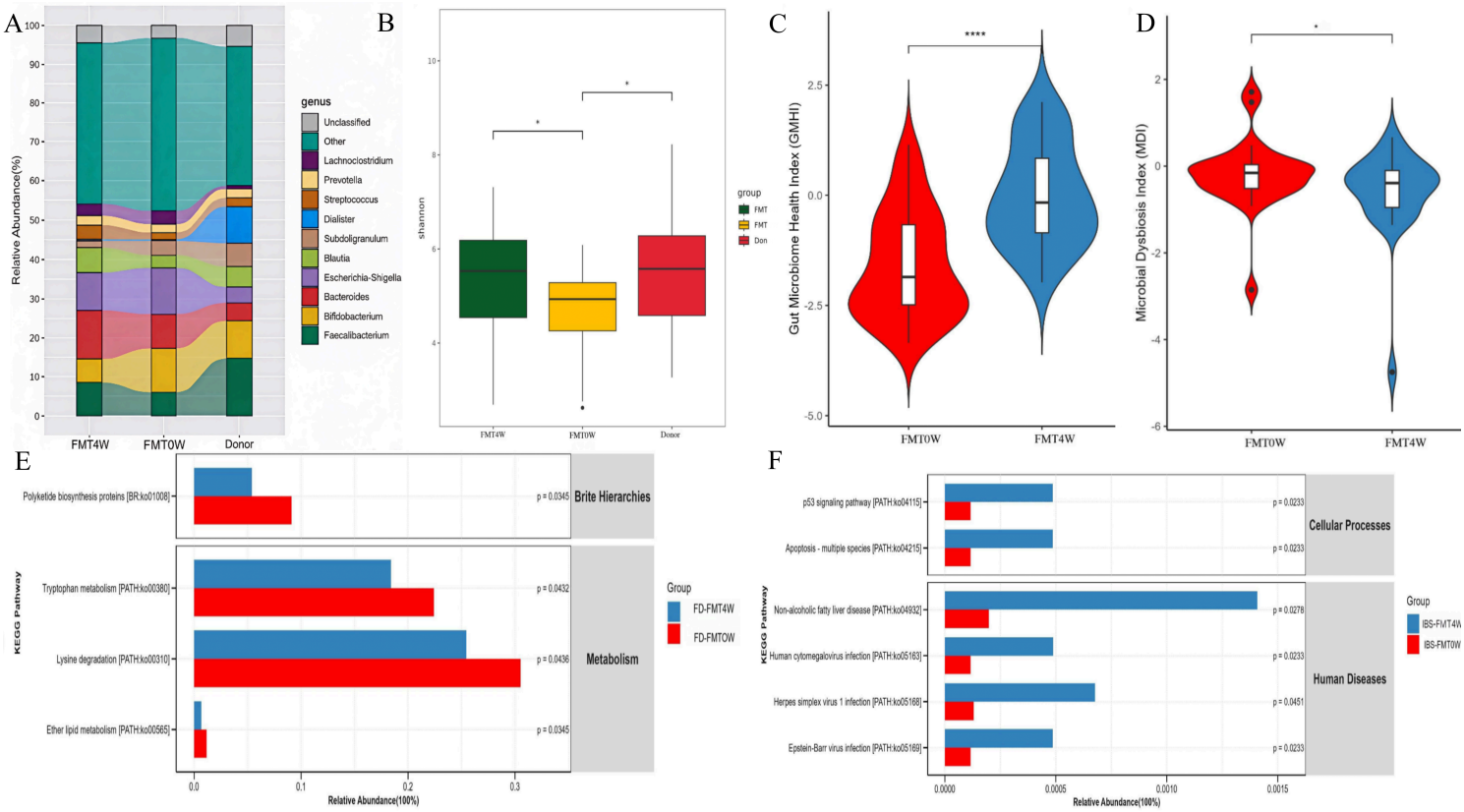


Figure 5

Dynamic changes in the gut microbiota before (baseline, FMT0W) and after treatment (FMT4W) in the FMT group. (A) Genus-level differences between donors and patients with FAPDs at different time points. (B) Changes in the alpha diversity index (Shannon) among donors and patients with FAPDs at different time points. Alpha diversity was significantly lower in the FMT0W group than in donors and the FMT4W group (Kruskal–Wallis H test, *P < 0.05). (C) Comparison of GMHIs between the FMT0W and FMT4W groups. GMHIs were significantly lower in the FMT0W group than in healthy donors and the FMT4W group (Wilcoxon rank-sum test, ****P < 0.0001). (D) Comparison of MDI between the FMT0W and FMT4W groups. MDI was significantly lower in the FMT4W group than in the FMT0W group (Wilcoxon rank-sum test, *P < 0.05). (E) Differential relative functional abundance before and after FMT treatment in the FD subtype (based on the KEGG database). Tryptophan metabolism was inhibited, with its relative abundance significantly reduced at FMT4W compared with FMT0W. (F) Differential relative functional abundance before and after FMT treatment in the IBS subtype (based on the KEGG database). The relative abundance of p53 signaling (ko04115) and apoptosis-related functions increased significantly at FMT4W compared with FMT0W.

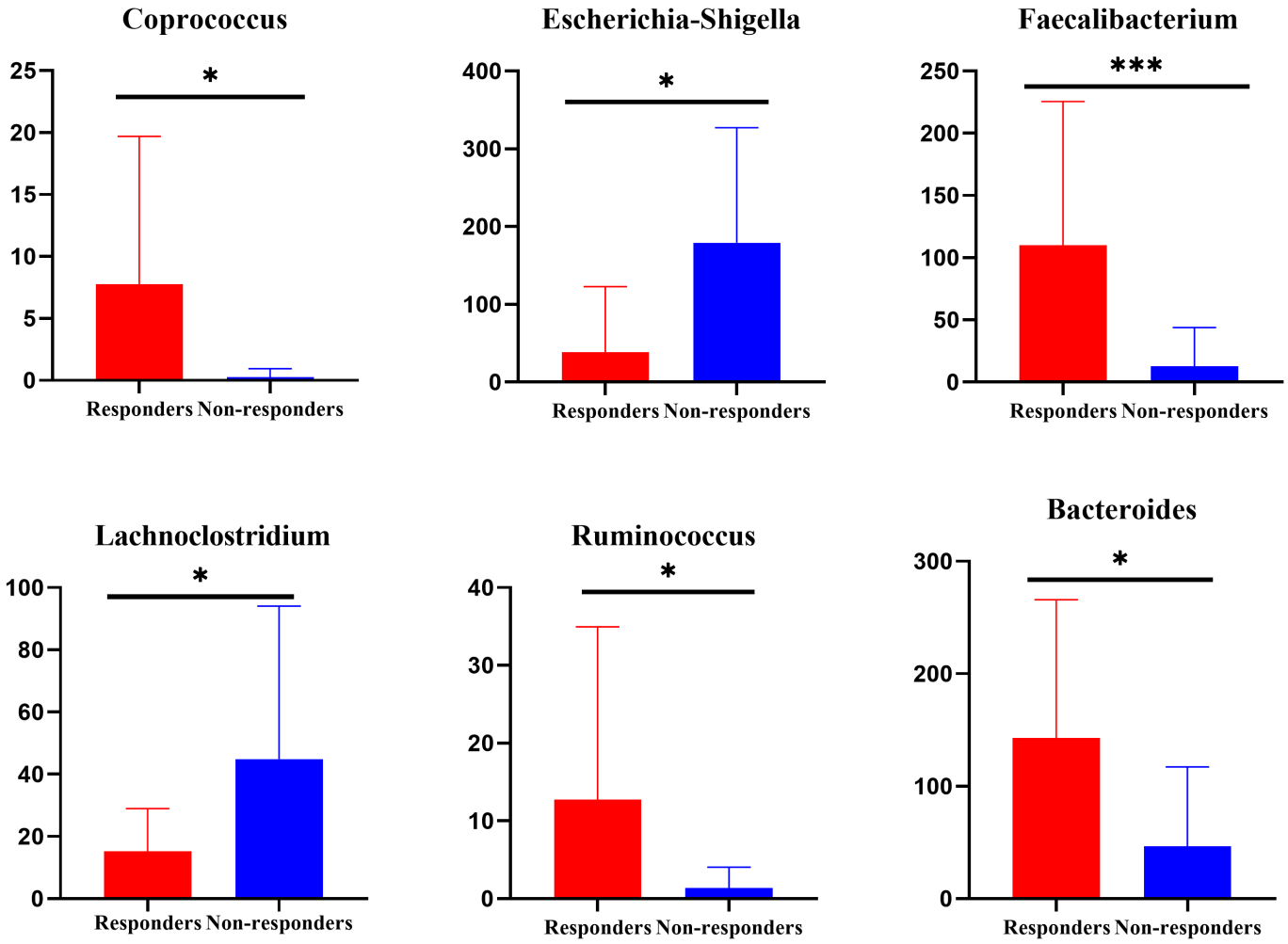


Figure 6

Comparison of six core bacterial genera (Bacteroides, Faecalibacterium, Ruminococcus, Coprococcus, Escherichia–Shigella, and Lachnoclostridium) between FMT responders (n=17) and non-responders (n=8) with qualified post-FMT samples (Mann–Whitney U tests; *P < 0.05, **P < 0.01, ***P < 0.001).

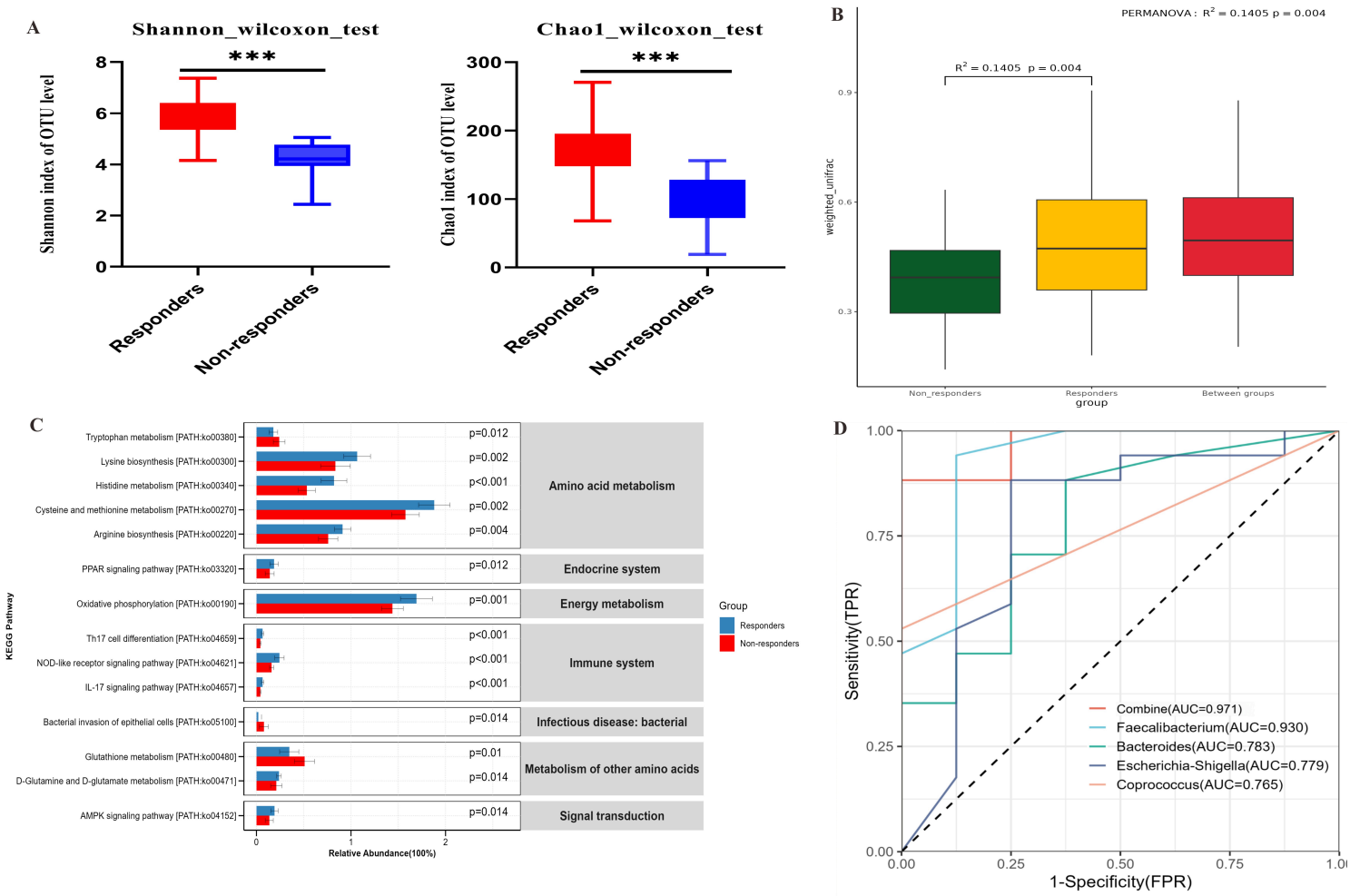


Figure 7

Dynamic changes in the gut microbiota between responders and non-responders. (A) Alpha diversity indices (Chao1 and Shannon) in responders (n=17) and non-responders (n=8) after FMT treatment. Alpha diversity was significantly lower in non-responders than in responders (Mann–Whitney U tests, ***P < 0.001). (B) Boxplot of β-diversity based on weighted UniFrac distances, showing differences in microbial communities between responders and non-responders (PERMANOVA). (C) Differential relative predicted functional abundance between responders and non-responders after FMT treatment (based on the KEGG database). Compared with non-responders, responders at FMT4W showed a significant increase in the relative abundance of the AMPK signaling pathway and oxidative phosphorylation, while tryptophan metabolism was significantly inhibited. (D) Four core genera were assessed as potential gut biomarkers to predict the efficacy of FMT therapy (apparent AUC = 0.971; leave-one-out cross-validated AUC = 0.917, 95% CI 0.765–1.000; sensitivity 83.3% [15/18], specificity 100.0% [8/8]). Internal validation, calibration, and decision-curve analyses are provided in Supplementary Figures S1–S2. The ROC curves in panel D represent apparent (in-sample) fits; internally validated performance is reported in Supplementary Figures S1–S2 and Supplementary Table 12.

Supplementary Files

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