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Efficacy of Fecal Microbiota Transplantation for Patients With Irritable Bowel Syndrome at 3 Years After Transplantation

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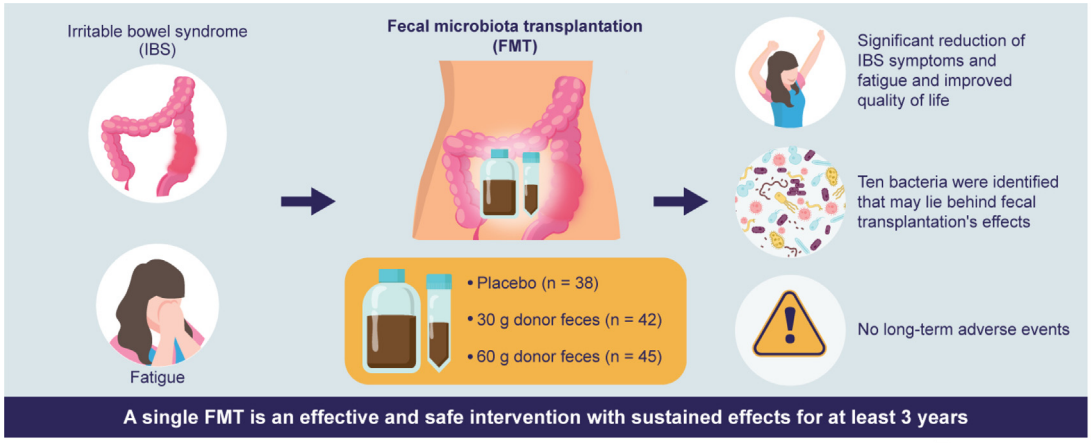

See editorial on page 815.

BACKGROUND & AIMS: The long-term efficacy and possible adverse events of fecal microbiota transplantation (FMT) for irritable bowel syndrome (IBS) are unknown. This study performed a 3-year follow-up of the patients in our previous clinical trial to clarify these aspects. **METHODS:** This study included 125 patients (104 females, and 21 males): 38 in a placebo group, 42 who received 30 g of donor feces, and 45 who received 60 g of donor feces. Feces was administered to the duodenum. The patients provided a fecal sample and completed 5 questionnaires at baseline and at 2 and 3 years after FMT. Fecal bacteria and dysbiosis index were analyzed using 16S ribosomal RNA gene polymerase chain reaction DNA amplification/probe hybridization covering the V3 to V9 regions. **RESULTS:** Response rates were 26.3%, 69.1%, and 77.8% in the placebo, 30-g, and 60-g groups, respectively, at 2 years after FMT, and 27.0%, 64.9%, and 71.8%, respectively, at 3 years after FMT. The response rates were significantly higher in the 30-g and 60-g groups than in the placebo group. Patients in the 30-g and 60-g groups had significantly fewer IBS symptoms and fatigue, and a greater quality of life both at 2 and 3 years after FMT. The dysbiosis index decreased only in the active treatment groups at 2 and 3 years after FMT. Fluorescent signals of 10 bacteria had significant correlations with IBS symptoms and fatigue after FMT in the 30-g and 60-g groups. No long-term adverse events were recorded. **CONCLUSIONS:** FMT performed according to our protocol resulted in high response rates and long-standing effects with only few mild self-limited adverse events. This study was registered at www.clinicaltrials.gov (NCT03822299)

Keywords: *Alistipes*; *Eubacterium biforme*; *Faecalibacterium prausnitzii*; Fatigue; Retransplantation.

The intestinal bacterial composition is considered to play a pivotal role in irritable bowel syndrome (IBS) pathophysiology.[1] Fecal microbiota transplantation (FMT) might be a promising treatment for IBS, and this has been investigated in 7 randomized controlled trials (RCTs).[2] In 4

Abbreviations used in this paper: **DI, dysbiosis index; FAS, Fatigue Assessment Scale; FMT, fecal microbiota transplantation; IBS, irritable bowel syndrome; IBS-C, constipation-predominant IBS; IBS-D, diarrhea-predominant IBS; IBS-M, mixed-diarrhea-and-constipation IBS; IBS-QOL, IBS-Quality of Life; IBS-SSS, IBS Severity Scoring System; RCT, randomized controlled trial; SF-NDI, Short-Form Nepean Dyspepsia Index.**

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WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

Fecal microbiota transplantation is a promising treatment for patients with irritable bowel syndrome with unknown long-term effects and adverse events.

NEW FINDINGS

This study applied a safe fecal microbiota transplantation protocol to irritable bowel syndrome with a high response rate and long-standing effects. The study identified 10 bacteria that are associated with irritable bowel syndrome symptoms and fatigue.

LIMITATIONS

The study investigated a targeted selection of the intestinal bacterial contents, and did not include the IBS-U irritable bowel syndrome subtype.

IMPACT

A successful fecal microbiota transplantation protocol for irritable bowel syndrome and identifying the bacteria that may lie behind its effects facilitates the clinical use of the fecal microbiota transplantation, and the identified bacteria as probiotics.

of these, FMT reduced symptoms and improved the quality of life of patients with IBS, whereas no effects were indicated in the other 3.[2] The difference in these results was likely because of differences in the protocols used, the selected donors, the cohort of treated patients, the fecal transplant dose, and the route by which the transplant was administratered.[2]

The longest observation time of the previously mentioned RCTs was 1 year, and hence the long-term efficacy and long-term adverse events of FMT for IBS are unknown.[2] Its long-term safety raised some concerns after patients who received FMT for a *Clostridium difficile* infection were followed up, bringing about concerns of weight gain, development or worsening of inflammatory bowel diseases, cancer, autoimmune diseases, allergies, and neurological diseases.[3]

The present study was a 3-year follow-up of the patients with IBS who received FMT in our previous RCT, aiming to determine the long-term efficacy and possible long-term side effects of FMT.[4] Both female and male patients with IBS were included in our previous RCT.[4]

Material and Methods

Study Design

The study design has previously been described in detail.[4] To summarize, patients provided a fecal sample and completed 5 questionnaires to assess their symptoms and quality of life at baseline and at 2 and 3 years after FMT.

Patients

This study initially included 165 patients (Figure 1).These patients were randomized into placebo (own feces), 30 g of donor feces, or 60 g of donor feces groups as described in detail for our previous study.[4] In the placebo group, 10 patients were excluded, as they received a second FMT with a donor transplant, and 7 patients dropped out 2 years after FMT. In the 30-g group, 11 were excluded due to pregnancy (n = 1) and due to retransplantation with 60 g of donor feces at 3 to 4 months after the first FMT (n = 10), and 2 patients dropped out at 2 years after FMT. In the 60-g group, 6 patients were excluded due to pregnancy (n = 2), breast cancer diagnosis (n = 1), pyelonephritis (n = 1), gastroenteritis caught overseas (n = 1), and after receiving abdominal surgery (n = 1), and 4 patients dropped out 2 years after FMT (Figure 1). Therefore, 125 patients were finally investigated in this study: 38 in the placebo group, 42 in the 30-g group, and 45 in the 60-g group. The characteristics of included patients at baseline are listed in Table 1.

The patient inclusion and exclusion criteria have previously been presented in detail.[4] To summarize, the inclusion criteria were being 18 to 85 years old and having a total score on the IBS Severity Scoring System (IBS-SSS) of $\geq$175. The exclusion criteria were the presence of a systemic disease; immune deficiency; being treated by immune-modulating medication such as Methotrexate, Azathioprine, Ciclosporin, tumor necrosis factor α inhibitors, and steroids; pregnancy or planning pregnancy; lactating; severe psychiatric disorders; alcohol or drug abuse; or use of probiotics, antibiotics, or IBS medications within 8 weeks before the start of the study.

Donor

The donor has previously been described in detail.[4] To summarize, he was a healthy male aged 36 years with a normal body mass index who was born via vaginal delivery, breastfed, a nonsmoker, was not taking any medication, was only treated a few times with antibiotics, exercised regularly, and consumed a sport-specific diet that was richer in protein, fiber, minerals, and vitamins than the average diet.[4] He was also not a first-degree relative of any recipient. He had high microbial diversity (normobiosis), and his fecal bacteria composition was different from that of 254 healthy subjects for 14 of the 48 tested bacterial markers.[4] His fecal bacteria composition was tested every 3 months and appeared to be stable over the period in which he donated his feces.[4] Fecal samples were taken from the donor at the baseline, and 2 and 3 years.

Fecal Sample Collection, Preparation, and Administration

The collection, preparation, and administration of feces has previously been described.[4] To summarize, fecal samples from the donor and patients were immediately frozen and kept at −20°C until they were delivered to the laboratory, where they were stored at −80°C. The patients were randomized into 3 groups: 30 g of own feces (placebo group), 30 g of donor feces, and 60 g of donor feces. The feces were thawed for 2 days at 4°C, manually mixed with 40 mL of sterile saline, filtered through a 110 cm × 10 cm nonwoven swab (OneMed, Helsinki, Finland), and administered to the distal duodenum via the working channel of a gastroscope.

Symptoms and Quality-of-Life Assessment

Symptoms were assessed using the IBS-SSS and the Birmingham IBS Symptom Questionnaire, and fatigue was

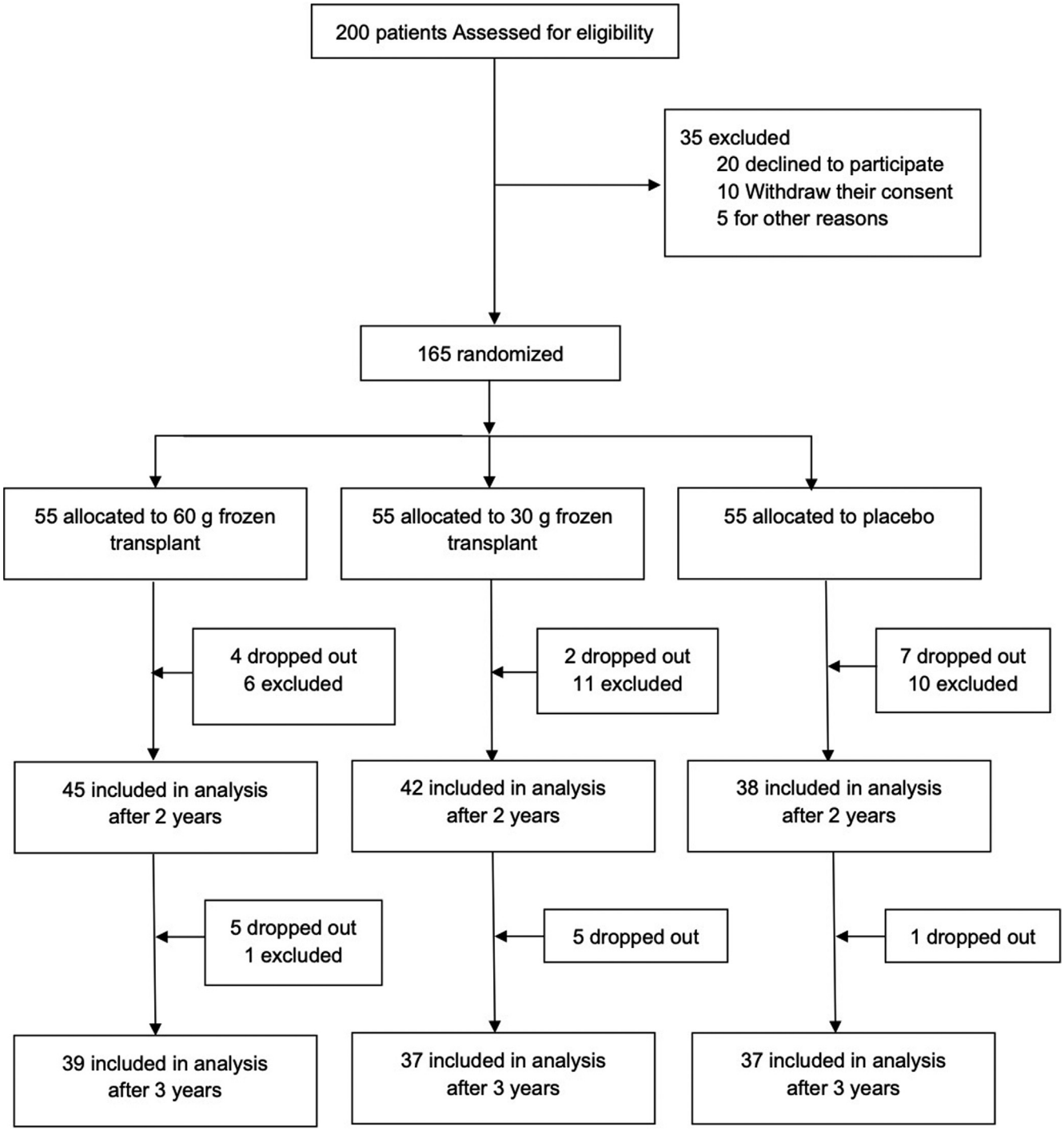

Figure 1. Enrollment flowchart and randomization of the patients.

measured using the Fatigue Assessment Scale (FAS) questionnaire.[5–7] Responders were patients who exhibited a reduction of $\geq$50 points on the IBS-SSS after FMT, whereas those with an IBS-SSS total score of $\leq$75 were considered in complete remissions.[5] Quality of life was measured using the IBS-Quality of Life (IBS-QOL) and Short-Form Nepean Dyspepsia Index (SF-NDI) questionnaires.[8,9] An increase in IBS-QOL, and a decrease in SF-NDI score indicated a quality-of-life improvement.

Bacterial Analysis

The fecal bacteria composition were analyzed using the GA-map Dysbiosis Test, a method using a predetermined targets approach, as described in detail previously.[10] To summarize, this test uses 16S ribosomal RNA polymerase chain reaction DNA amplification covering the variable regions V3 to V9 , followed by DNA probe hybridization of the predetermined 48 bacterial markers covering >300 bacteria at different taxonomic levels. The dysbiosis index (DI) was measured on a 5-

Table 1. Patient Characteristics at Baseline

	Overall	Placebo	30-g FMT	60-g FMT	*P*
Number	125	38	42	45	
Age, *y*	40.3 ± 13.2	42.3 ± 14.7	38.5 ± 12.4	39.7 ± 13.2	.6
Sex, female/male	104/21	34/4	31/9	37/8	.2
IBS-D	47	12	17	18	
IBS-C	46	16	15	15	.9
IBS-M	32	10	10	12	
Patients with severe IBS symptoms,[a] %	62	55	67	62	.6
IBS-SSS score	314.9 ± 76.3	300.3 ± 81.6	322.8 ± 64.1	315.3 ± 81.1	.5
Birmingham IBS-S score	25.1 ± 8.1	23.3 ± 8.1	26.7 ± 5.1	25.4 ± 6.8	.050
FAS score	31.1 ± 4.9	31.5 ± 4.5	30.8 ± 5.0	31.2 ± 4.5	.634
IBS-QOL score	113.5 ± 21.8	117.8 ± 20.2	109.8 ± 22.3	113.4 ± 22.4	.117
SF-NDI score	29.9 ± 7.2	29.1 ± 6.2	30.1 ± 7.2	30.5 ± 8.3	.728
DI	3.2 ± 1.1	3.1 ± 1.2	3.4 ± 1.0	3.2 ± 1.1	.743
Patients with dysbiosis	77.3%	74.4%	78.8%	78.6%	.735
PPI medication	58 (46.4)	21 (38.2)	19 (45.2)	18 (32.7)	.810
Birth control medication	81 (64.8)	25 (45.5)	26 (61.9)	30 (54.5)	.601
Antimigraine medication	12 (9.6)	3 (5.5)	5 (11.9)	4 (7.3)	.764
Medication against asthma/allergies	18 (14.4)	6 (10.9)	7 (16.7)	5 (9.1)	.829
Medication with levothyroxine	3 (2.4)	1 (1.8)	0 (0)	2 (3.6)	.361
Medication with heart/vascular drugs	6 (4.8)	3 (5.5)	2 (4.8)	1 (1.8)	.595

NOTE. Data are mean ± SD or n (%) values except where indicated otherwise.
PPI, proton-pump inhibitor.
[a]IBS-SSS total score of ≥300.

point scale from 1 (normal) to 5 (severe dysbiosis), where values of 1 and 2 indicate normobiosis, and those of 3 to 5 indicate dysbiosis.[10]

Retransplantation of Relapsed Patients

Ten patients who responded to FMT at 3 months after transplantation and relapsed 3 years after FMT, 5 from the 30-g group and 5 from the 60-g group received another transplant of 90 g of donor feces using the same methods. These patients comprised 7 women and 3 men aged 41.0 ± 17.7 years (mean ± SD). Diarrhea-predominant IBS (IBS-D), constipation-predominant IBS (IBS-C), and mixed-diarrhea-and-constipation IBS (IBS-M) were classified in 3, 4, and 3 patients, respectively. These patients completed the IBS-SSS, Birmingham IBS Symptom Questionnaire, FAS, IBS-QOL, and SF-NDI, and delivered a fecal sample both before and 3 months after retransplantation.

Outcomes

The primary endpoint was a reduction in the IBS-SSS total score of ≥50 points at 3 months following transplantation. The secondary endpoint was a change in the DI and intestinal bacterial profile.

Adverse Events and Medication

The patients were asked to record bowel habits and register any adverse events in a diary. They were also asked to continue use and record their consumption of rescue medications (polyethylene glycol and loperamide), which was permitted during the previous randomized, double-blind, and placebo-controlled study. They were also asked to record the use of other IBS medications. The patients continued using their medications, which are listed in Table 1.

Statistical Analysis

The differences in response, complete remission, sex, IBS subtypes, dysbiosis, and medication use were assessed using Fisher's exact test. Differences between patients at baseline and at 2 and 3 years after transplantation in IBS-SSS, Birmingham IBS Symptom Questionnaire, FAS, IBS-QOL, and SF-NDI scores, DI, bacterial fluorescence signals, and IBS subtypes were analyzed using the Kruskal-Wallis test and a posttest of Dunn's multiple comparisons. The nonparametric Mann-Whitney test was used to identify differences between responders and nonresponders, patients with moderate and severe IBS symptoms, and women and men. Scaled principal-components analysis of log-transformed bacterial fluorescence signals was

performed, with the results visualized using a different color for each group, together with ellipses that included 80% of the samples within each group. Targeted Maximum Likelihood Estimation was performed to test the effect of the dropouts and excluded patients on the outcome.

Ethics

The study was approved by the West Regional Committee for Medical and Health Research Ethics, Bergen, Norway (approval no. 2017/1197/REK vest). All subjects provided both oral and written consent before participating. This study was registered at www.clinicaltrials.gov (NCT03822299).

Results

Patients, Donor, and FMT Response

Three years after FMT, 11 patients had dropped out: 1 in the placebo group, 5 in the 30-g group, and 5 in the 60-g group. One patient from the 60-g group was excluded because of pregnancy (Figure 1). Targeted Maximum Likelihood Estimation showed that the dropouts and excluded patients did not affect the outcome of the study regarding symptoms, quality of life and DI (Supplementary Material).

The donor had a DI of 1 at baseline and at 2 and 3 years after FMT, indicating that bacterial abundance remained healthy (normal) over the follow-up period. The bacterial profile of the donor was stable throughout the observation period (Supplementary Figure 1). The abundance of 15 bacterial markers deviated from that of healthy subjects during this period (Supplementary Figure 2).

The response rates of the 30-g and 60-g groups were significantly higher than in the placebo group at 2 and 3 years after FMT (Figure 2). Figure 2 illustrates the complete remission rates of patients at 2 and 3 years after FMT for all groups.

Symptoms and Quality of Life

The total IBS-SSS scores in the 30-g and 60-g groups were significantly lower than those in the placebo group at both 2 and 3 years after FMT (Figure 3 and Supplementary Table 1). The scores for the 4 items in the IBS-SSS—abdominal pain, abdominal distension, bowel-habits dissatisfaction, and quality-of-life interference—were also significantly reduced compared with the placebo group at 2 and 3 years after FMT (Supplementary Table 1). The total scores on the Birmingham IBS Symptom Questionnaire in the 30-g and 60-g groups were significantly lower than those in the placebo group at 2 and 3 years after FMT (Figure 3 and Supplementary Table 2). The total FAS scores in the 30-g and 60-g groups were significantly lower than those in the placebo group at 2 and 3 years after FMT (Figure 3 and Supplementary Table 3).

The total IBS-QOL scores were significantly higher in the 30-g and 60-g groups than in the placebo group at 2 and 3 years after FMT (Figure 3 and Supplementary Table 4). The total SF-NDI scores were significantly lower in the 30-g and 60-g groups than in the placebo group at 2 and 3 years after FMT (Figure 3 and Supplementary Table 5).

Bacterial Analysis

DIs decreased significantly 2 and 3 years after FMT in the 30-g and 60-g groups, but not in the placebo group (Supplementary Figure 3). DI was positively correlated with the total IBS-SSS and FAS scores (Supplementary Figure 4). The proportion of patients with dysbiosis decreased significantly at 2 and 3 years after FMT in all groups (Supplementary Figure 5).

The fecal bacterial profiles of the placebo, 30-g, and 60-g groups changed over time (Figure 4). Although the fecal bacterial profiles were similar in all groups at baseline, it differed significantly at 2 and 3 years after FMT (Figure 4).

The fluorescence signals of 19 bacterial markers changed between baseline and 2 years after FMT in the placebo group (Supplementary Table 6). The fluorescence signals of 9 bacterial markers decreased, whereas the remaining 10 increased. The fluorescence signals of 22 bacterial markers changed between baseline and 3 years after FMT. The fluorescence signals of 10 bacterial markers decreased and those of the remaining 12 increased (Supplementary Table 6).

The fluorescence signals of 26 bacteria markers changed at both 2 and 3 years after FMT in the 30-g group (Supplementary Table 7). The fluorescence signals of 15 and 18 bacteria markers increased at 2 and 3 years after FMT, respectively, and those of 11 and 8 bacteria markers decreased at 2 and 3 years after FMT, respectively. Among these bacteria markers, 11 and 10 changed and did not change in the placebo group at 2 and 3 years after FMT, respectively (Supplementary Table 7).

The fluorescence signals of 27 and 24 bacteria markers changed at 2 and 3 years after FMT, respectively, in the 60-g group (Supplementary Table 8). Of these, the fluorescence signals of 15 and 13 increased at 2 and 3 years after FMT, respectively, and those of 12 and 11 decreased at 2 and 3 years after FMT, respectively. Of the bacteria markers that changed at 2 and 3 years after FMT, 12 and 11 did not change in the placebo group. The fluorescence signals of 7 bacteria markers increased at both 2 and 3 years after FMT. The fluorescence signals of 5 and 4 bacteria markers decreased at 2 and 3 years after FMT, respectively (Supplementary Table 8).

Correlations

Of the bacteria markers whose fluorescence signals changed in the 30-g and 60-g groups but not in the placebo group at both 2 and 3 years after FMT, 9 were significantly correlated with the total IBS-SSS scores (Figure 5). Six of these bacteria markers whose fluorescence signals increased after FMT were negatively correlated with the total IBS-SSS scores: *Alistipes*, *Bacteroides* spp, *Prevotella* spp, *Parabacteroides johnsonii*, *Firmicutes*, *Eubacterium biforme*, and *Faecalibacterium prausnitzii*. The remaining 3 bacteria markers, whose fluorescence signals decreased after FMT, were correlated positively with the total IBS-SSS scores: *Coprobacillus cateniformis*, *Streptococcus salivarius* spp *thermophilus*, and Enterobacteriaceae (Figure 5).

Ten bacteria markers whose fluorescence signals changed in the 30-g and 60-g groups after FMT, but not

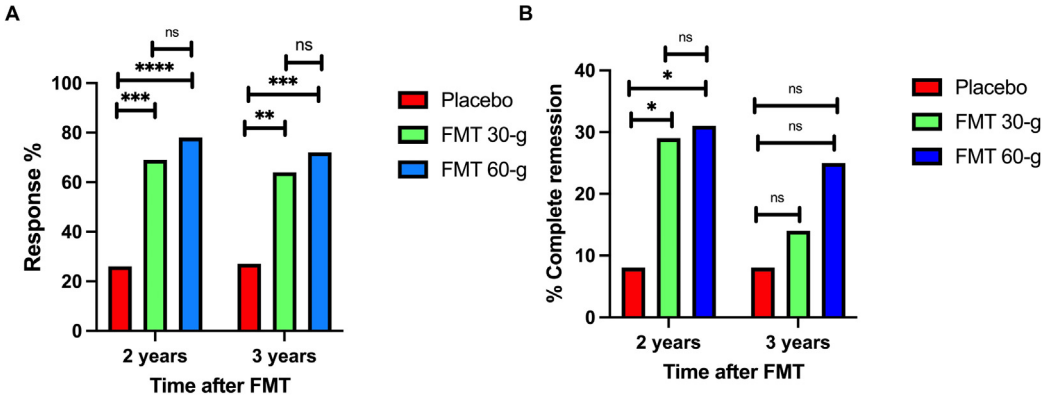

Figure 2. Response rates (*A*) and complete remission rates (*B*) in the placebo, 30-g, and 60-g groups at 2 and 3 years after FMT. ns, not significant; *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$.

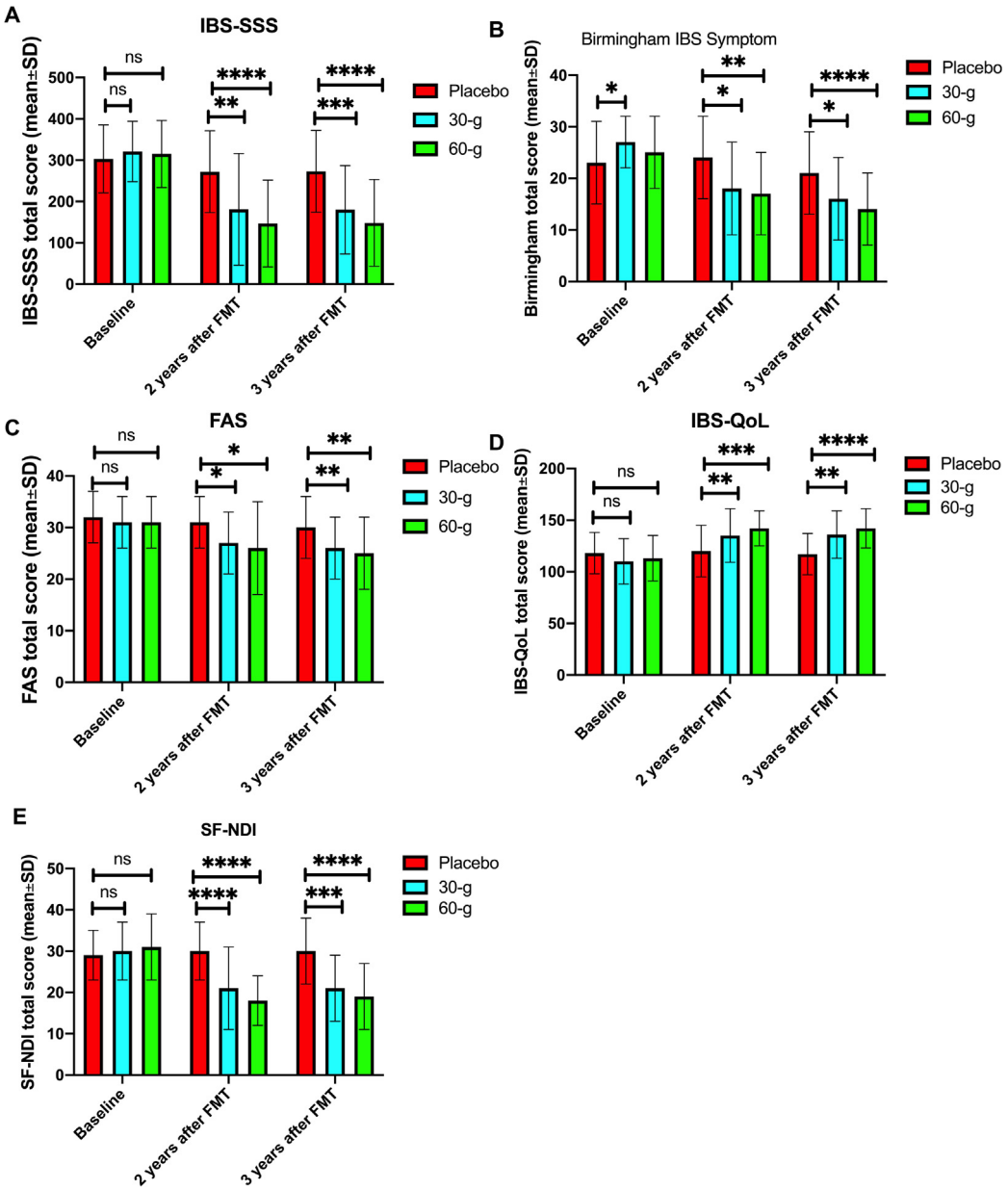

Figure 3. Total scores on the IBS-SSS (*A*), Birmingham IBS Symptom Questionnaire (*B*), FAS (*C*), IBS-QOL (*D*), and SF-NDI (*E*) in the placebo, 30-g, and 60-g groups at baseline and at 2 and 3 years after FMT. ns, not significant; *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$.

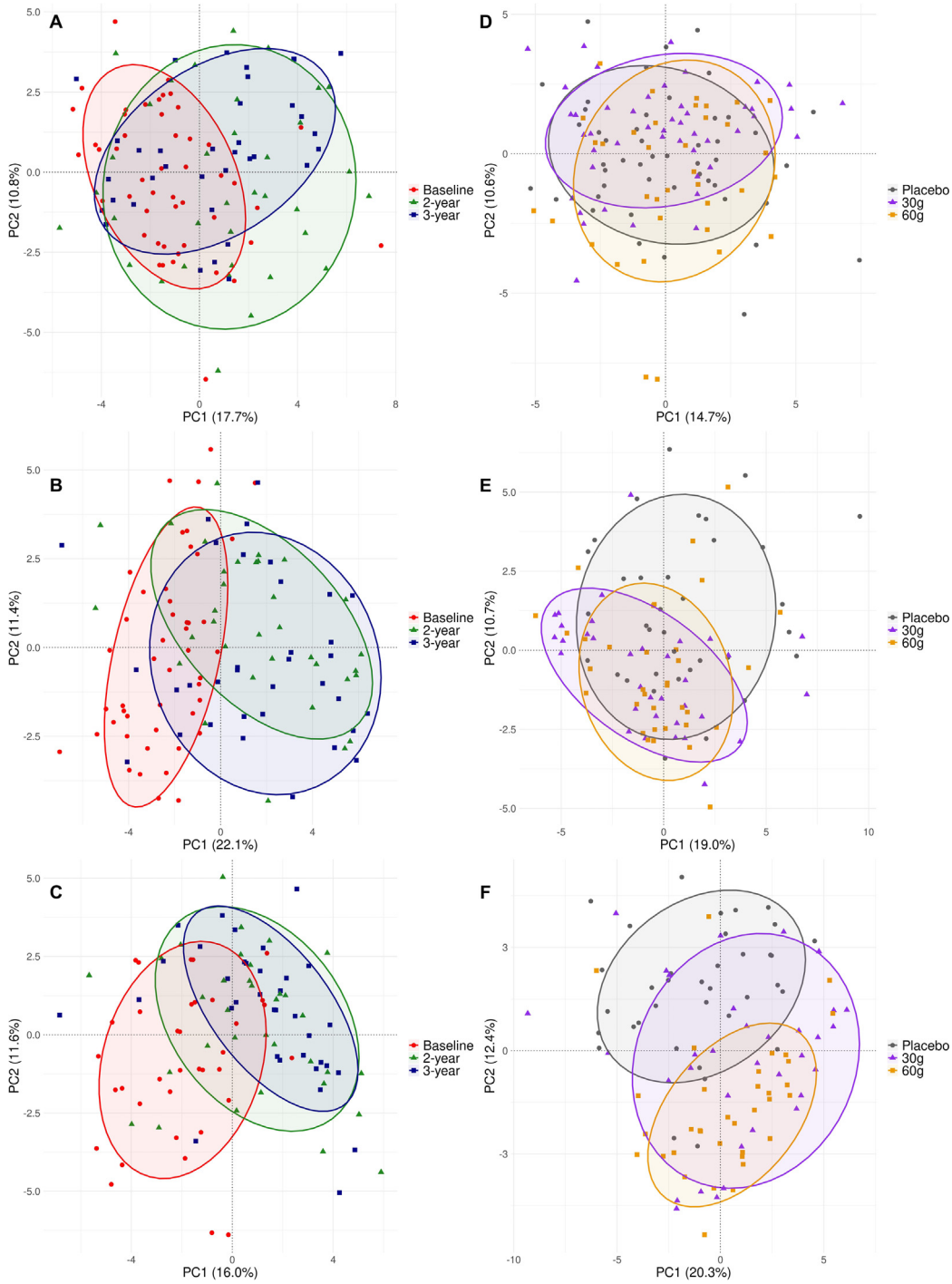

Figure 4. Results of the scaled principal-components analysis of the fecal bacterial profiles. The bacterial profiles of the placebo group (*A*), 30-g group (*B*), and 60-g group (*C*) changed over time. The changes in the 30-g and 60-g groups differed from those in the placebo group. At baseline, the bacterial profiles of the placebo, 30-g, and 60-g groups overlapped (*D*). Two (*E*) and 3 (*F*) years after FMT, the bacterial profiles of the 30-g and 60-g groups differed markedly from those of the placebo group.

in the placebo group, were significantly correlated with the total FAS scores (Figure 6). Seven bacteria markers whose fluorescence signals increased after FMT were correlated negatively with the total FAS scores. The other 3 whose fluorescence signals decreased after FMT were correlated positively with the total FAS scores (Figure 6).

Differences Between Responders and Nonresponders

Responders had significantly lower total scores on the IBS-SSS, Birmingham IBS Symptom Questionnaire, FAS, and SF-NDI, and higher total IBS-QOL scores than did non-responders at both 2 and 3 years after FMT (Supplementary Figure 6).

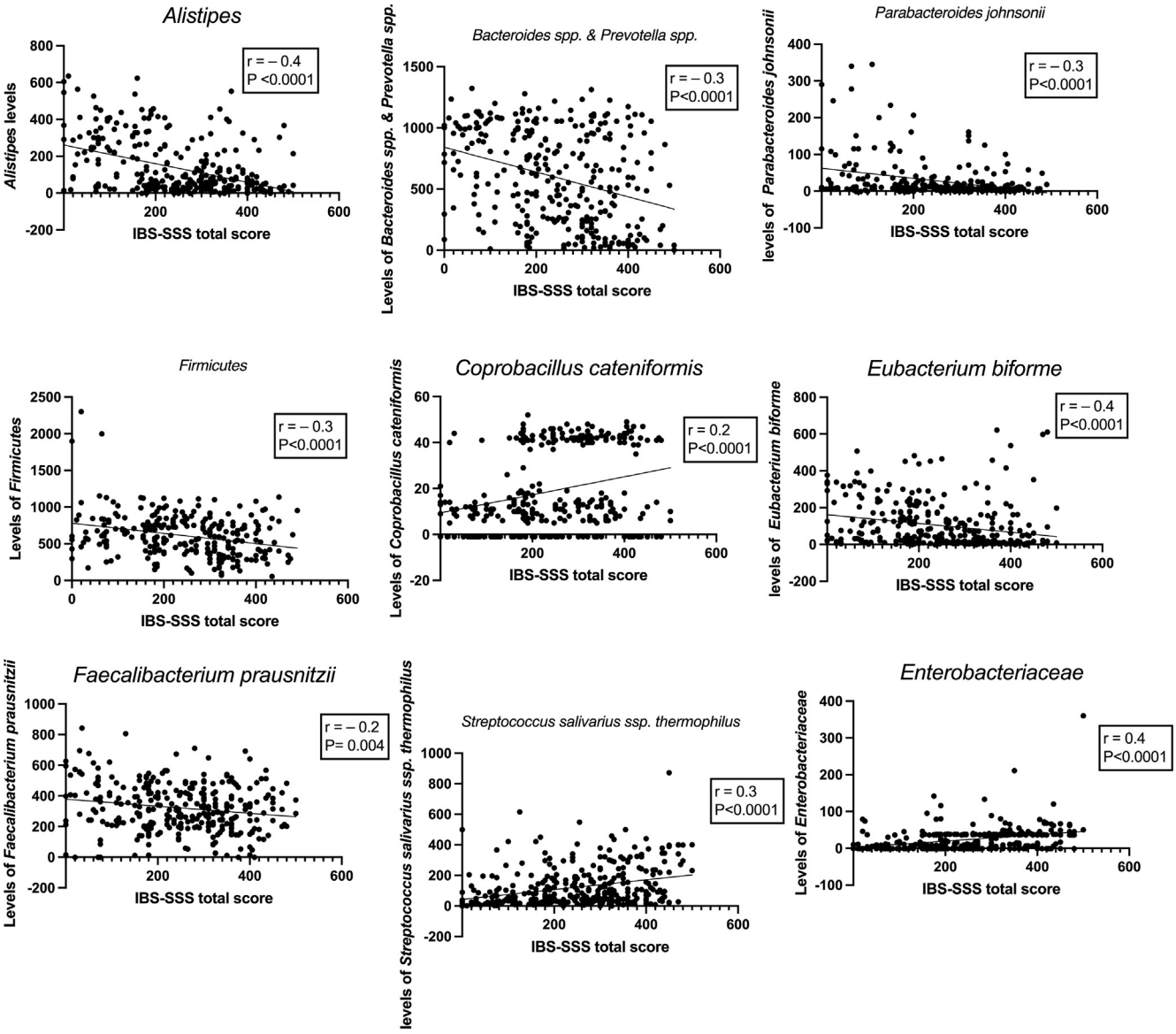

Figure 5. Fluorescence signals of 9 bacteria that changed significantly in both the 30-g and 60-g groups at 2 and 3 years after FMT correlated with the total IBS-SSS score.

The proportions of patients with dysbiosis among responders were 84%, 30%, and 37% at baseline and at 2 and 3 years after FMT, respectively; the corresponding values among nonresponders were 72%, 41%, and 39%. There was no significant difference between the proportion of patients with dysbiosis among responders and nonresponders at baseline and at 2 and 3 years after FMT (P = .2, .2, and >.999, respectively). There were no differences in DIs between responders and nonresponders at baseline, but the DI of responders was significantly lower than that of nonresponders at 2 and 3 years after FMT (Supplementary Figure 7).

The bacterial profiles of responders and nonresponders were similar at baseline but differed at 2 and 3 years after FMT (Supplementary Figure 8). At baseline, the fluorescence signals of *Lactobacillus* spp were 45.8 ± 87.6 and 60.4 ± 95.2 among responders and nonresponders, respectively. The fluorescence signal of *Lactobacillus* spp was significantly lower in responders than in nonresponders (P = .04). The fluorescence signals of 10 and 7 bacteria were significantly higher in responders than in nonresponders at 2 and 3 years after FMT, respectively (Supplementary Tables 9 and 10).

Differences Between Patients With Moderate and Severe IBS Symptoms

The proportion of patients with severe IBS symptoms in the 30-g and 60-g groups, but not in the placebo group, was significantly reduced at 2 and 3 years after FMT (Supplementary Table 11). The response rates of patients with severe and moderate IBS symptoms were 74% and 56%, respectively, at 2 years after FMT; the corresponding values for 3 years after FMT were 72% and 50%. Patients

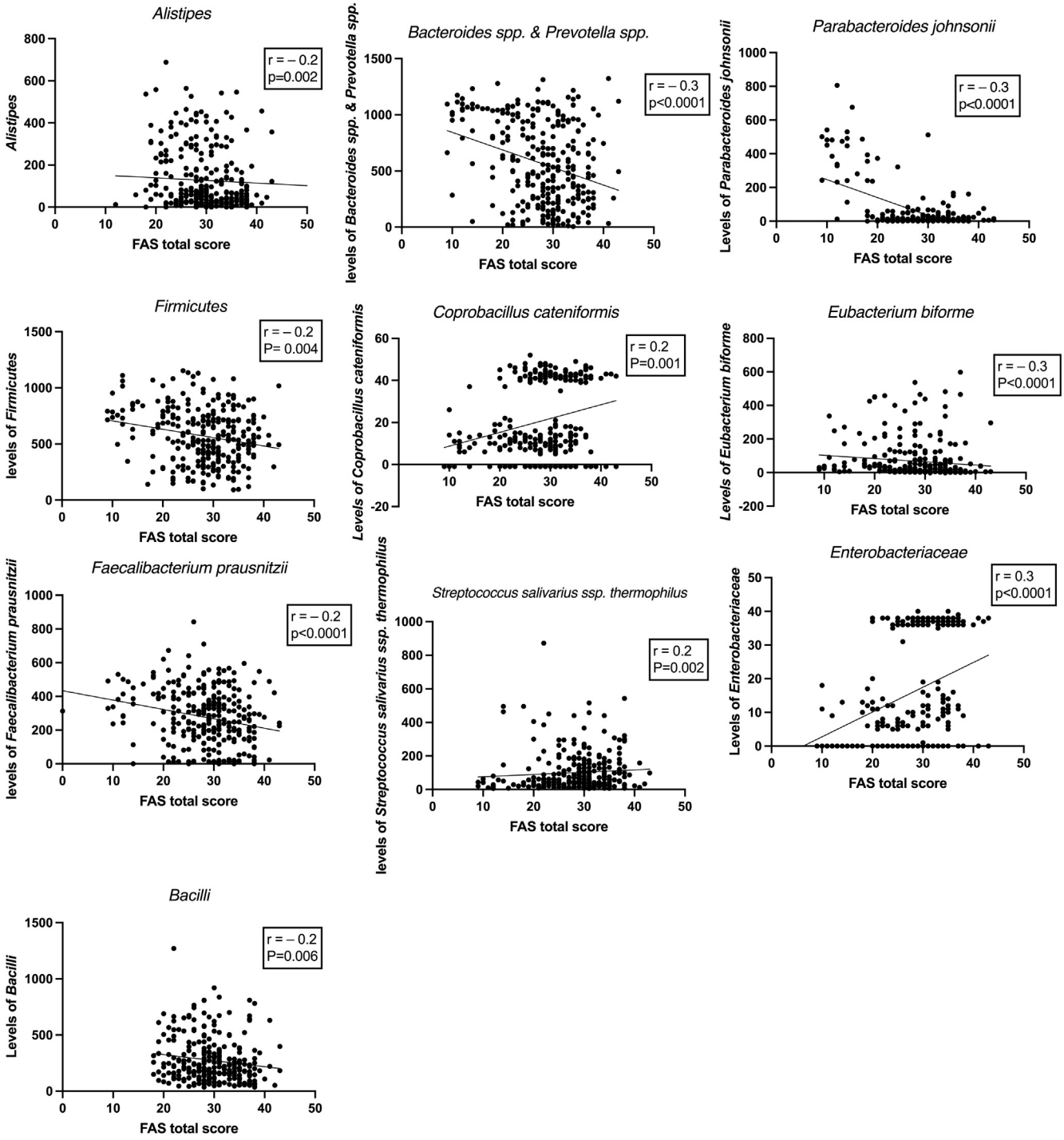

Figure 6. Among the bacteria whose fluorescence signals changed significantly at 2 and 3 years after FMT in the 30-g and 60-g groups, 10 were correlated with the total FAS score.

with severe IBS symptoms had significantly higher response rates both 2 and 3 years after FMT (P = .02 and .01, respectively).

The total IBS-SSS and Birmingham IBS Symptom Questionnaire scores were higher in patients with severe IBS symptoms than in those with moderate IBS symptoms at baseline. The difference between these scores decreased over time between 2 and 3 years after FMT, and did not differ at 3 years after FMT (Supplementary Figure 9). The total scores on the IBS-QOL and SF-NDI were lower and higher, respectively, in patients with severe IBS symptoms than in those with moderate IBS symptoms at baseline. However, there was no difference between the scores of patients with severe and moderate symptoms at 2 and 3 years after FMT (Supplementary Figure 9). Total FAS scores did not differ between

patients with severe and moderate IBS symptoms at all observation intervals.

Although the DIs of patients with severe and moderate IBS symptoms did not differ at baseline, that of patients with severe IBS symptoms was lower than that of patients with moderate IBS symptoms at 2 and 3 years after FMT (Supplementary Figure 10).

The fecal bacterial profiles of patients with moderate and severe IBS symptoms exhibited a varied overlap over time after FMT (Supplementary Figure 11). At baseline, the fluorescence signals of *Clostridia* in patients with moderate and severe IBS symptoms were 411.6 ± 56.9 and 385.6 ± 41.4, respectively. The fluorescence signals of *Clostridia* were significantly higher in patients with moderate IBS symptoms than in patients with severe IBS symptoms ($P = .01$). The fluorescence signals of *Firmicutes* in patients with moderate and severe IBS symptoms were 576.1 ± 267.6 and 735.0 ± 268.2, respectively. Patients with severe IBS symptoms had a significantly higher fluorescence signal for *Firmicutes* than did patients with moderate IBS symptoms ($P = .01$). The fluorescence signals of *Eubacterium siraeum* and *Lactobacillus* spp were 310.1 ± 153.8 and 20.8 ± 24.3 in patients with moderate IBS symptoms, respectively; the corresponding values for patients with severe IBS symptoms were 402.6 ± 146.3 and 11.1 ± 15.9. Patients with severe IBS symptoms had a significantly higher fluorescence signal for *Eubacterium siraeum*, and lower signals of *Lactobacillus* spp than did patients with moderate IBS symptoms ($P = .01$, and .03, respectively). There were no significant differences in the remaining tested bacteria between patients with moderate and severe IBS symptoms at baseline and at 2 and 3 years after FMT.

Sex Differences

Women had higher response rates than men at both 2 and 3 years after FMT (Supplementary Figure 12). There were no differences between the complete remission rates of women and men at both 2 and 3 years after FMT (Supplementary Figure 12).

The total IBS-SSS, Birmingham IBS Symptom Questionnaire, FAS, IBS-QOL, and SF-NDI scores did not differ between women and men at baseline and at 2 and 3 years after FMT (Supplementary Figure 13).

There was no significant difference between the DIs of women and men at baseline and at 2 and 3 years after FMT (Supplementary Figure 14). The fecal bacterial profile of women and men overlapped at all time points after FMT (Supplementary Figure 15). The fluorescence signals of 11 bacterial markers differed significantly between female and male patients with IBS at baseline (Supplementary Table 12). The fluorescence signals of 4 bacteria differed significantly between female and male patients with IBS at 2 years after FMT (Supplementary Table 13). The levels of 5 bacteria differed significantly between female and male patients with IBS at 3 years after FMT, including bacteria that had already changed from baseline (Supplementary Table 14).

Differences Between IBS Subtypes

The response rates in patients with IBS-D and IBS-M were significantly higher than in those with IBS-C at 2 years after FMT (Supplementary Figure 16). The response rates of patients with IBS-D did not differ significantly from those of patients with IBS-C and patients with IBS-M at 3 years after FMT. The complete remission rate of IBS-D did not differ significantly from those of IBS-C and IBS-M at 2 and 3 years after FMT (Supplementary Figure 16).

The total IBS-SSS, Birmingham IBS Symptom Questionnaire, FAS, IBS-QOL, and SF-NDI scores did not differ between patients with IBS-D, IBS-C, and IBS-M at baseline and at 2 and 3 years after FMT (Supplementary Figure 17).

DIs did not differ between patients with IBS-D, IBS-C, and IBS-M at baseline and at 2 and 3 years after FMT (Supplementary Figure 18)

The fecal bacteria profiles of the IBS subtypes changed over time and exhibited different overlaps at baseline and at 2 and 3 years after FMT (Supplementary Figure 19). The fluorescence signals of *Streptococcus* spp at baseline were 30.1 ± 38.4, 62.3 ± 15.7, and 69.4 ± 24.6 in IBS-D, IBS-C, and IBS-M, respectively, and here were significantly lower in IBS-D than in IBS-C and IBS-M ($P = .0002$ and $<.0001$, respectively). The fluorescence signals of *Veillonella* spp were 325.6 ± 81.3, 297.5 ± 66.1, and 260.7 ± 74.1 in IBS-D, IBS-C, and IBS-M, respectively, and hence were significantly lower in IBS-M than in IBS-D ($P = .01$). With the exception of *Alistipes*, there was no significant difference between the IBS subtypes regarding the fluorescence signals of all tested bacteria at 2 and 3 years after FMT. At 3 years after FMT, the fluorescence signals of *Alistipes* were 279.7 ± 173.9, 298.9 ± 153.5, and 158.5 ± 106.3 in IBS-D, IBS-C, and IBS-M, respectively, and hence were significantly lower in IBS-M than in IBS-D and IBS-C ($P = .02$ and .01, respectively).

Retransplantation of Relapsed Patients

Eight of the 10 retransplanted patients (80%) responded to FMT at 3 months after retransplantation. IBS symptoms were significantly reduced at 3 months after retransplantation as assessed by IBS-SSS and the Birmingham IBS Symptom Questionnaire (Supplementary Figure 20). However, the total FAS, IBS-QOL, and SF-NDI scores did not differ significantly between before and after retransplantation (Supplementary Figure 20).

The DIs before and 3 months after retransplantation were 2.4 ± 0.9 and 1.4 ± 0.7, respectively, and hence were significantly lower at 3 months after FMT than before retransplantation ($P = .03$). The proportions of patients with IBS with dysbiosis were 50% and 10% before and 3 months after retransplantation, respectively, with no significant difference between before and after retransplantation. Fecal bacterial profiles changed considerably at 3 months after retransplantation (Supplementary Figure 21). The levels of 5 bacteria changed significantly 3 months after retransplantation (Supplementary Table 15).

Adverse Events Diet and Medication

Mild intermittent abdominal pain, diarrhea, and constipation were reported within the first 2 days after FMT (Supplementary Table 16), but no other adverse events were reported during the follow-up period. Two patients who developed diverticulitis at 2 and 3 months after FMT did not experience any new diverticulitis attacks during the follow-up period.[4] The patients consumed average Norwegian diet before and after FMT.

Among the patients in the placebo group, 32 regularly used medication to counteract IBS symptoms. These included loperamide, polyethylene glycol, and bulking agents (psyllium or ispaghula). The remaining 6 patients used linaclotide (n = 4) or prucalopride (n = 2).

In the 30-g group, 2 patients used loperamide once and 1 used polyethylene glycol twice. Three patients used loperamide, another 3 used polyethylene glycol, and 6 used loperamide and polyethylene glycol regularly.

In the 60-g group, 4 patients used loperamide, 2 used polyethylene glycol, and another 4 used both loperamide and polyethylene glycol regularly.

Discussion

The present study found that most patients with IBS maintained their response at 3 years after receiving FMT. Furthermore, those who received FMT had significantly reduced abdominal symptoms and fatigue, and an increased quality of life at 2 and 3 years after FMT. Except for the placebo group, the proportion of FMT-treated patients with severe IBS symptoms was also significantly lower at 2 and 3 years after FMT. It showed further that patients who relapsed within 3 years subsequently responded to FMT on retransplantation, which is consistent with earlier observations.[11] It is therefore reasonable to conclude that the FMT protocol for IBS as used in the present study resulted in high response rates and had durable long-term effects.[4]

The protocol used in our study differs in several aspects from those used in the 6 other RCTs of FMT on patients with IBS.[2] In the present study, donor selection was based on clinical criteria and a specific fecal microbial signature.[4] The clinical criteria were based on factors that are considered to positively or negatively affect intestinal microbiota and on the intestinal bacterial profile and diversity of the donor, which were compared with those of healthy subjects to identify possible deviations.[2] Moreover, the bacterial profile of the donor was stable over time, a criterium that was found to be associated with an effective donor.[4,11] In our protocol, fecal samples from both the donor and patients were immediately frozen, delivered frozen, and were kept frozen until transplantation was performed. Fecal samples were thawed at 4°C, mixed, and filtered manually. The human intestine is dominated by strictly anaerobic bacteria, and freezing and keeping them at 4°C for a short period protects their viability and growth.[12,13] Freezing the transplant from the donor and the fecal samples from the patients could have preserved the bacteria within the fecal samples, while keeping and transporting fecal samples in normal air could damage these bacteria. The findings of the present study support this assumption, in that 6 of the 10 bacteria that appeared to affect IBS symptoms and fatigue are anaerobic: *Alistipes*, *Parabacteroides johnsonii*, *Bacteroides* spp, *Prevotella* spp, *Eubacterium biforme*, *Faecalibacterium prausnitzii*, and *Coprobacillus cateniformis*.[14–18] Mixing the transplant from the donor manually without using mixing devices may have improved the preservation of its bacterial content. Finally, the transplant was administered into the small intestine. Whether administering the fecal transplant to the small or large intestine is more effective is yet to be determined, but in the 2 RCTs with the most-positive outcomes, the fecal transplant was administered to the small intestine.[4,11]

The findings that the bacterial profiles and proportions of patients with dysbiosis in both the placebo and FMT-treated groups changed over time indicate that intestinal microbiota is a dynamic time-varying process. Microbiota dysbiosis is believed to be an important contributing factor to IBS etiology.[19] However, its relevance in IBS has recently been questioned.[20,21] It seems that the degree of dysbiosis, rather than its presence or absence, is the clinically relevant factor. This assumption is supported by our finding that DIs decreased in FMT-treated patients but not in placebo patients, and that the proportion of patients with dysbiosis did not differ between the placebo and FMT groups. This assumption is also supported by the observation that DI was positively correlated with the total IBS-SSS and FAS scores.

In the active treatment groups, 9 bacteria whose fluorescence signals changed after FMT were correlated with the total IBS-SSS and FAS scores. One bacterium whose fluorescence signals changed in the active treatment group was also correlated with total FAS scores. The 7 bacteria with increased fluorescence signals were negatively correlated with the total IBS-SSS and FAS scores. The remaining 3 bacteria with decreased fluorescence signals were positively correlated with the total IBS-SSS and FAS scores. The importance of these 10 bacteria in improving IBS symptoms and fatigue is supported by the observation that fluorescence signals of 6 of these bacteria were higher in responders than in nonresponders. Furthermore, in patients with IBS, *Alistipes*, *Faecalibacterium prausnitzii*, and *Parabacteroides* levels have been reported to decrease, whereas Enterobacteriaceae levels increase.[22–25]

The roles that the previously mentioned bacteria play in IBS symptom manifestation and fatigue are yet to be determined. However, *Alistipes* appears to play a significant role in several diseases, such as depression, anxiety, chronic fatigue syndrome, autism, cirrhosis, and aging.[14] *Faecalibacterium prausnitzii* is the most important butyrate producer in the human colon, and *Eubacterium biforme* produces large amounts of butyric acid.[16,17,26–28] Notably, within the same cohort, fecal butyric acid levels were significantly increased at 1 month and 1 year after FMT in both the 30-g and 60-g groups.[29,30] Butyrate provides colonic epithelial cells with energy, affects the immune response, modulates colonic hypersensitivity, and decreases intestine-cell permeability and intestinal motility.[2]

The authors of a recently published RCT on FMT for patients with refractory IBS concluded that the successful

application of FMT is subset-dependent.[11] The present study found that all categories of patients with IBS benefited from FMT, but differences were detected between these categories. Female patients have been reported to respond better than male patients at 3 months after FMT.[11,31] The present study found that at 2 and 3 years after FMT, women still responded better than men. It is difficult to determine whether this difference is caused by psychosocial or biological factors. Patients with severe IBS symptoms had higher FMT response rates and lower DIs than those with moderate IBS symptoms in the present study. Our previous RCT found no differences in FMT response between the IBS subtypes at 3 months after FMT.[4] The observation that patients with IBS-C had a lower FMT response rate than those with IBS-D and IBS-M at 2 years after FMT is difficult to interpret because there were no differences between the IBS subtypes at 3 years after FMT.

Concerns have been raised about the safety of FMT for IBS, considering that IBS is a benign condition.[32–34] Discussion on the safety issues of FMT as an intervention for gastrointestinal and nongastrointestinal diseases was prompted by a report of 2 patients who experienced serious adverse events after FMT for indications other than IBS, which resulted in 1 fatality.[35,36] Moreover, some concerns on the long-term side effects in patients who received FMT to treat *Clostridium difficile* infection have also been discussed.[3] The short-term adverse events observed after FMT in patients with IBS were mild and self-limiting gastrointestinal symptoms.[2] The present finding that the FMT-treated patients with IBS had no further adverse events after a 3-year follow-up period indicates that FMT is a safe intervention. However, it should be remembered that the patients included in this study did not have a systemic disease or immune deficiency.

Major strengths of this study are that it included a relatively large IBS patient cohort comprising 3 IBS subtypes and used a single well-defined donor. However, limitations of this study are that it did not include the fourth IBS subtype, IBS-U, and it only investigated a part of the intestinal bacterial contents.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at https://doi.org/10.1053/j.gastro.2022.06.020.

References

1. El-Salhy M, Hatlebakk JG, Hausken T. Diet in irritable bowel syndrome (IBS): interaction with gut microbiota and gut hormones. Nutrients 2019;11:1824.
2. El-Salhy M, Hausken T, Hatlebakk JG. Current status of fecal microbiota transplantation for irritable bowel syndrome. Neurogastroenterol Motil 2021;33:e14157.
3. Perler BK, Chen B, Phelps E, et al. Long-term efficacy and safety of fecal microbiota transplantation for treatment of recurrent clostridioides difficile infection. J Clin Gastroenterol 2020;54:701–706.
4. El-Salhy M, Hatlebakk JG, Gilja OH, et al. Efficacy of faecal microbiota transplantation for patients with irritable bowel syndrome in a randomised, double-blind, placebo-controlled study. Gut 2020;69:859–867.
5. Francis CY, Morris J, Whorwell PJ. The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. Aliment Pharmacol Ther 1997;11:395–402.
6. Roalfe AK, Roberts LM, Wilson S. Evaluation of the Birmingham IBS Symptom Questionnaire. BMC Gastroenterol 2008;8:30.
7. Hendriks C, Drent M, Elfferich M, et al. The Fatigue Assessment Scale: quality and availability in sarcoidosis and other diseases. Curr Opin Pulm Med 2018;24:495–503.
8. Drossman DA, Patrick DL, Whitehead WE, et al. Further validation of the IBS-QOL: a disease-specific quality-of-life questionnaire. Am J Gastroenterol 2000;95:999–1007.
9. Drossman DA, Camilleri M, Mayer EA, et al. AGA technical review on irritable bowel syndrome. Gastroenterology 2002;123:2108–2131.
10. Casén C, Vebø HC, Sekelja M, et al. Deviations in human gut microbiota: a novel diagnostic test for determining dysbiosis in patients with IBS or IBD. Aliment Pharmacol Ther 2015;42:71–83.
11. Holvoet T, Joossens M, Vázquez-Castellanos JF, et al. Fecal microbiota transplantation reduces symptoms in some patients with irritable bowel syndrome with predominant abdominal bloating: short- and long-term results from a placebo-controlled randomized trial. Gastroenterology 2021;160:145–157.e8.
12. Thomson P, Núñez P, Quera R, et al. Gastrointestinal microbiome, what is behind fecal microbiota transplantation? New Microbes and New Infections 2021;42:100898.
13. Bircher L, Geirnaert A, Hammes F, et al. Effect of cryopreservation and lyophilization on viability and growth of strict anaerobic human gut microbes. Microb Biotechnol 2018;11:721–733.
14. Parker BJ, Wearsch PA, Veloo ACM, et al. The genus Alistipes: gut bacteria with emerging implications to inflammation, cancer, and mental health. Front Immunol 2020;11:906.
15. Sakamoto M, Kitahara M, Benno Y. *Parabacteroides johnsonii sp. nov*., isolated from human faeces. Int J Syst Evol Microbiol 2007;57:293–296.
16. Feng Y, Stams AJM, Sánchez-Andrea I, de Vos WM. *Eubacterium maltosivorans* sp. nov., a novel human intestinal acetogenic and butyrogenic bacterium with a versatile metabolism. Int J Syst Evol Microbiol 2018;68:3546–3550.
17. Louis P, Flint HJ. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. FEMS Microbiol Lett 2009;294:1–8.

18. Kageyama A, Benno Y. *Coprobacillus catenaformis gen. nov., sp. nov*., a new genus and species isolated from human feces. Microbiol Immunol 2000;44:23–28.
19. El-Salhy M, Mazzawi T. Fecal microbiota transplantation for managing irritable bowel syndrome. Expert Rev Gastroenterol Hepatol 2018;12:439–445.
20. Enck P, Mazurak N. Dysbiosis in functional bowel disorders. Ann Nutr Metab 2018;72:296–306.
21. Lloyd-Price J, Mahurkar A, Rahnavard G, et al. Erratum: Strains, functions and dynamics in the expanded Human Microbiome Project. Nature 2017;551:256.
22. Jeffery IB, O'Toole PW, Ohman L, et al. An irritable bowel syndrome subtype defined by species-specific alterations in faecal microbiota. Gut 2012;61:997–1006.
23. Pittayanon R, Lau JT, Yuan Y, et al. Gut microbiota in patients with irritable bowel syndrome-a systematic review. Gastroenterology 2019;157:97–108.
24. Liu HN, Wu H, Chen YZ, et al. Altered molecular signature of intestinal microbiota in irritable bowel syndrome patients compared with healthy controls: a systematic review and meta-analysis. Dig Liver Dis 2017;49:331–337.
25. Vich Vila A, Imhann F, Collij V, et al. Gut microbiota composition and functional changes in inflammatory bowel disease and irritable bowel syndrome. Sci Transl Med 2018;10:eaap8914.
26. Ferreira-Halder CV, Faria AVS, Andrade SS. Action and function of Faecalibacterium prausnitzii in health and disease. Best Pract Res Clin Gastroenterol 2017;31:643–648.
27. Sokol H, Pigneur B, Watterlot L, et al. *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci U S A 2008;105:16731–16736.
28. Duncan SH, Hold GL, Harmsen HJM, et al. Growth requirements and fermentation products of *Fusobacterium prausnitzii*, and a proposal to reclassify it as *Faecalibacterium prausnitzii gen. nov., comb. nov*. Int J Syst Evol Microbiol 2002;52:2141–2146.
29. El-Salhy M, Valeur J, Hausken T, et al. Changes in fecal short-chain fatty acids following fecal microbiota transplantation in patients with irritable bowel syndrome. Neurogastroenterol Motil 2021;33:e13983.
30. El-Salhy M, Kristoffersen AB, Valeur J, et al. Long-term effects of fecal microbiota transplantation (FMT) in patients with irritable bowel syndrome. Neurogastroenterol Motil 2022;34:e14200.
31. El-Salhy M, Casen C, Valeur J, et al. Responses to faecal microbiota transplantation in female and male patients with irritable bowel syndrome. World J Gastroenterol 2021;27:2219–2237.
32. Barbara G, Ianiro G. Faecal microbial transplantation in IBS: ready for prime time? Gut 2020;69:795–796.
33. Camilleri M. FMT in IBS: a call for caution. Gut 2021;70:431.
34. El-Salhy M. FMT in IBS: how cautious should we be? Gut 2021;70:626–628.
35. DeFilipp Z, Bloom PP, Torres Soto M, et al. Drug-resistant *E. coli* bacteremia transmitted by fecal microbiota transplant. N Engl J Med 2019;381:2043–2050.
36. Blaser MJ. Fecal microbiota transplantation for dysbiosis - predictable risks. N Engl J Med 2019;381:2064–2066.

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Renate Winkel, MD (Formal analysis: Supporting; Methodology: Supporting; Writing – review & editing: Equal).
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Conflict of Interest
The authors disclose no conflicts.

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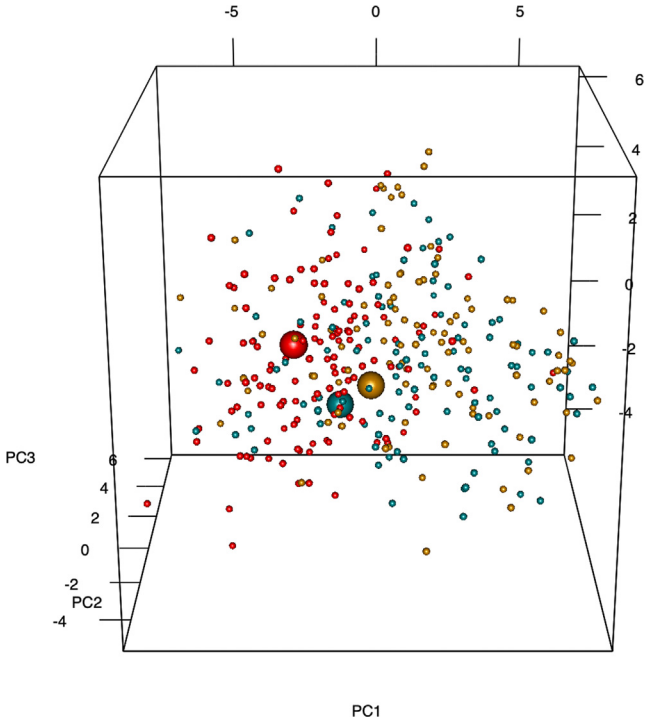
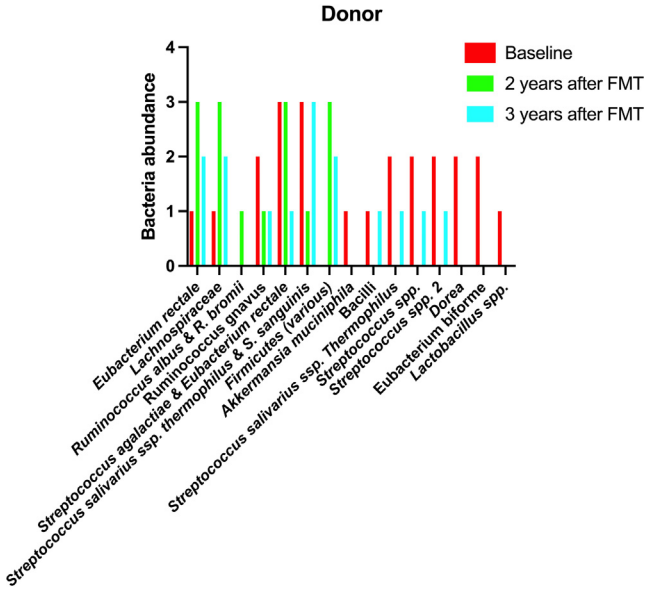
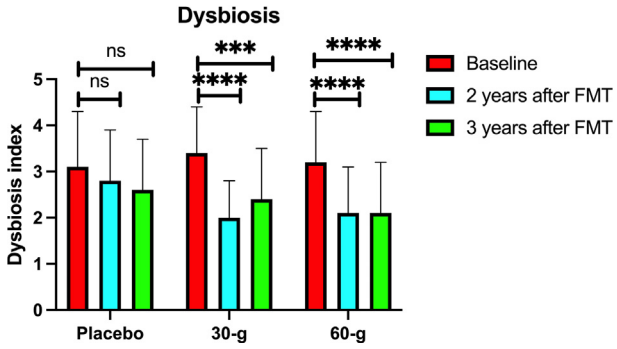
Data Availability
Data presented in this study are available from the corresponding author on request.



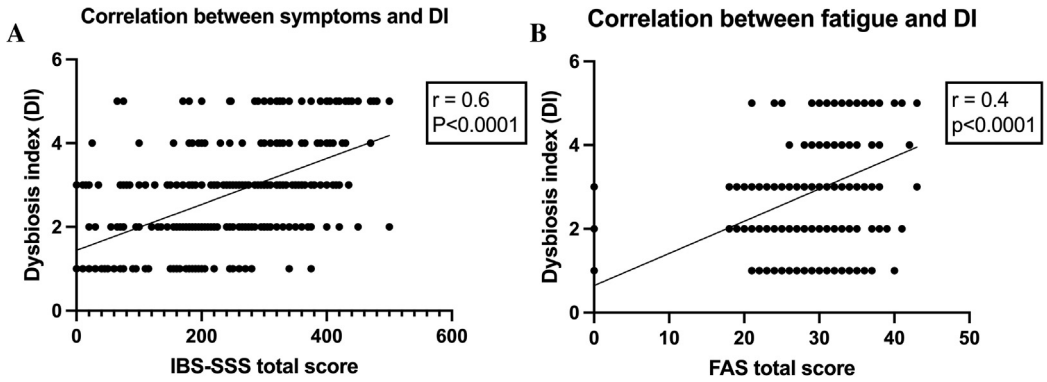
Supplementary Figure 1. Scaled principal-components analysis plot of fecal samples from the donor and patients at baseline (*red*) and at 2 years (*yellow*) and 3 years (*turquoise*) after FMT. The donor and patient samples are represented by *large* and *small circles*, respectively. The donor samples are closely grouped and were very similar over the follow-up period to those at baseline.



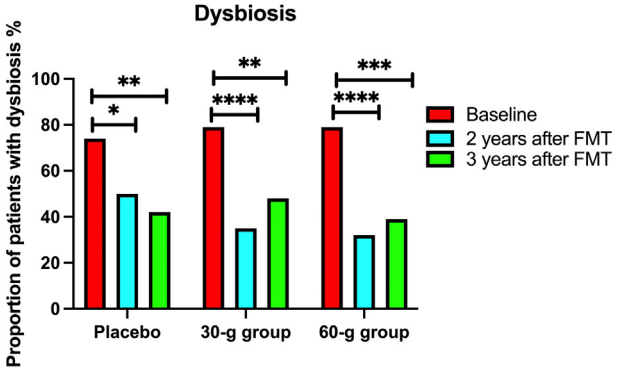
Supplementary Figure 2. Fecal bacterial profiles of the donor at baseline and at 2 and 3 years after FMT. The fecal bacterial profile of the donor deviated from that of healthy subjects for 15 of the 48 bacterial markers. The level of a healthy subject was 0.



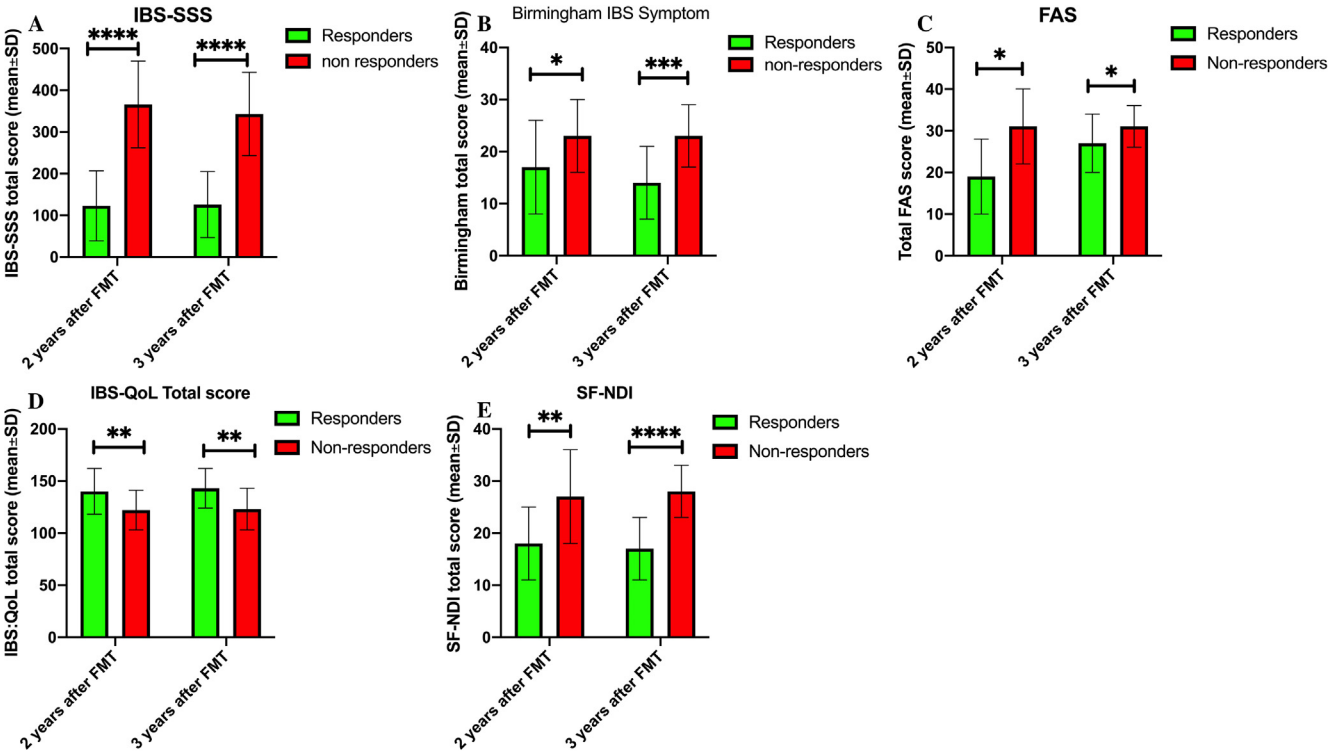
Supplementary Figure 3. DIs of the placebo, 30-g, and 60-g groups at baseline and at 2 and 3 years after FMT. ns, not significant; ***$P < .001$; ****$P < .0001$.



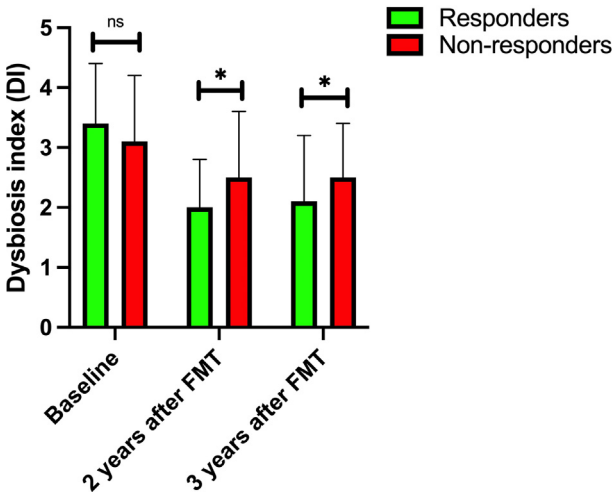
Supplementary Figure 4. Correlations between DI and total IBS-SSS (*A*) and FAS scores (*B*).



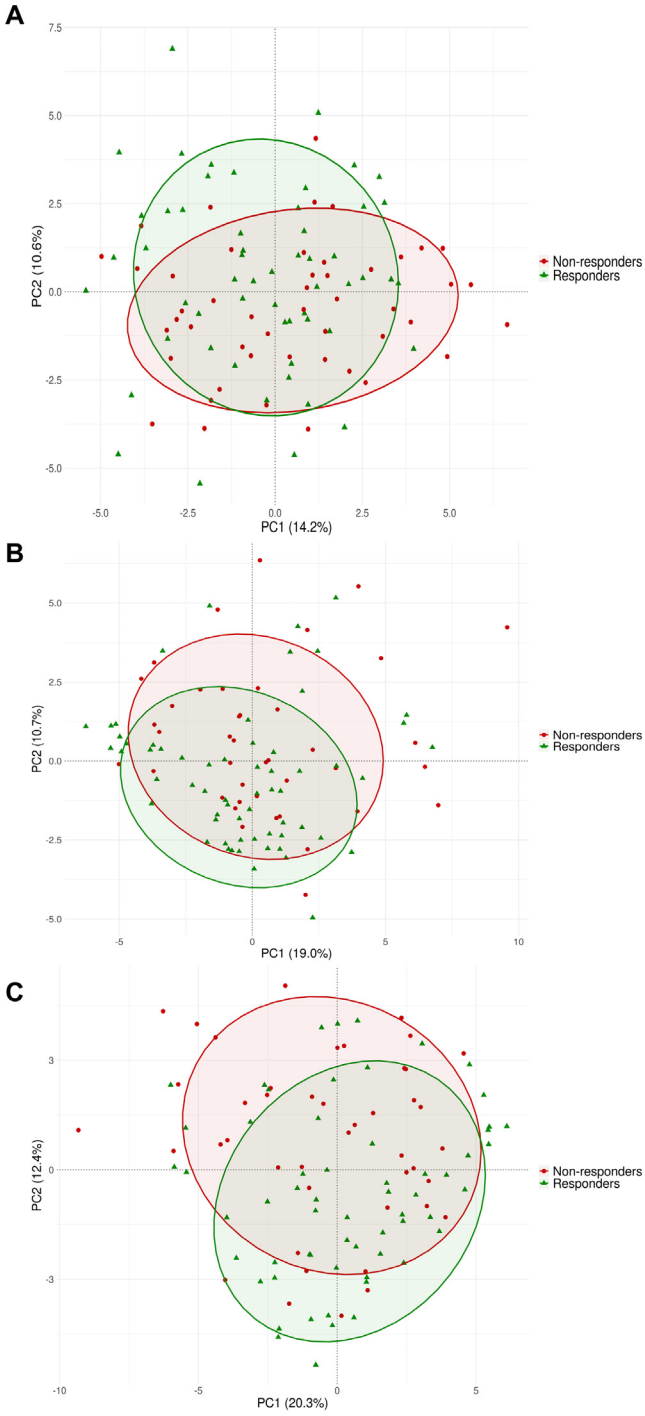
Supplementary Figure 5. Proportions of patients with dysbiosis in the placebo, 30-g, and 60-g groups at baseline and at 2 and 3 years after FMT. *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$.



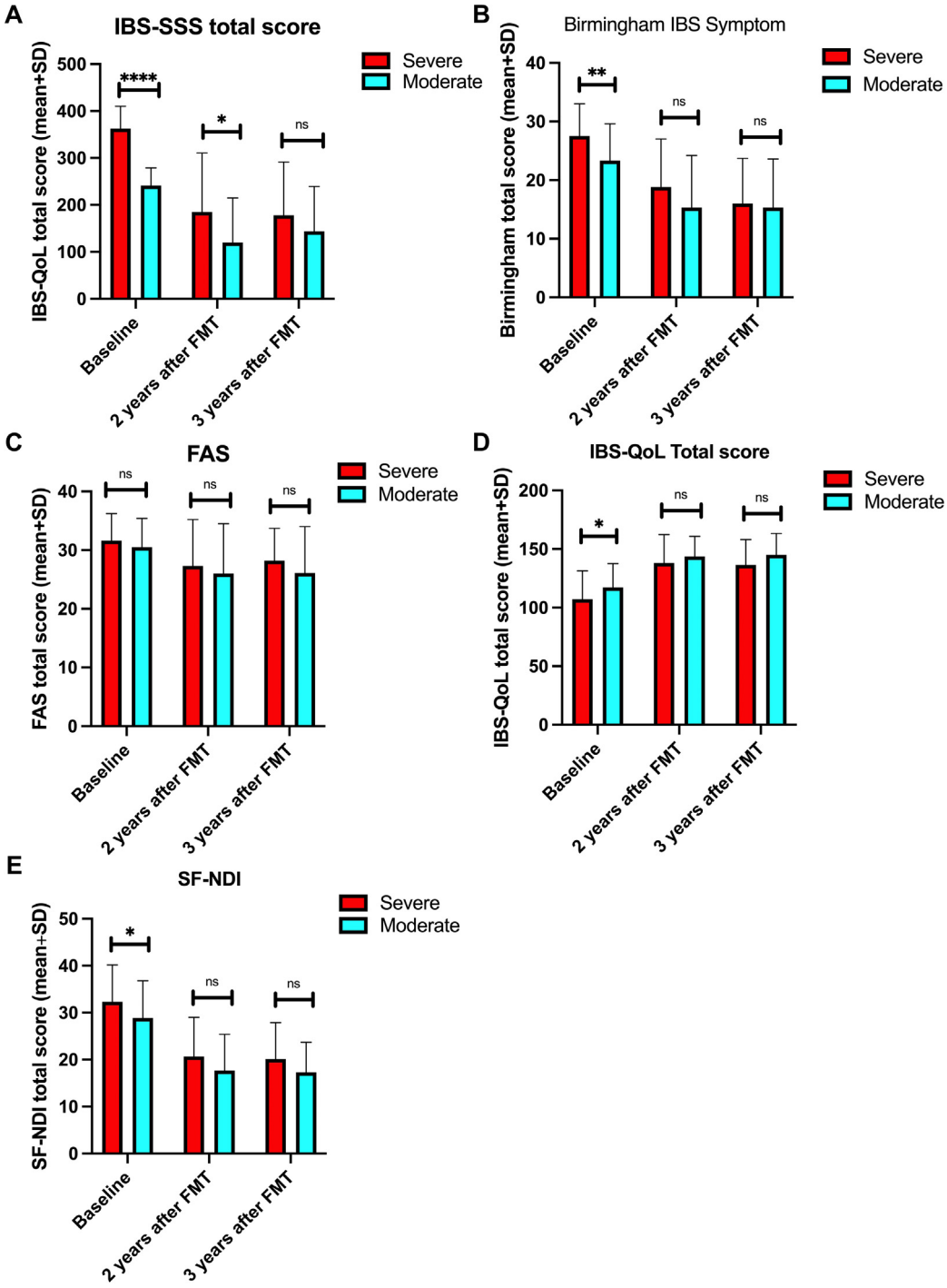
Supplementary Figure 6. Differences between responders and nonresponders in total scores on the IBS-SSS (*A*), Birmingham IBS Symptom Questionnaire (*B*), FAS (*C*), IBS-QOL (*D*), and SF-NDI (*E*). *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$.



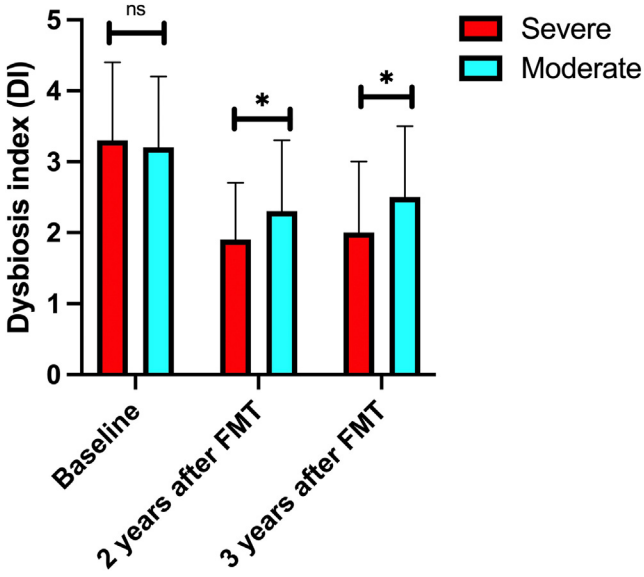
Supplementary Figure 7. DIs of responders and non-responders at baseline and at 2 and 3 years after FMT. ns, not significant; *$P < .05$.



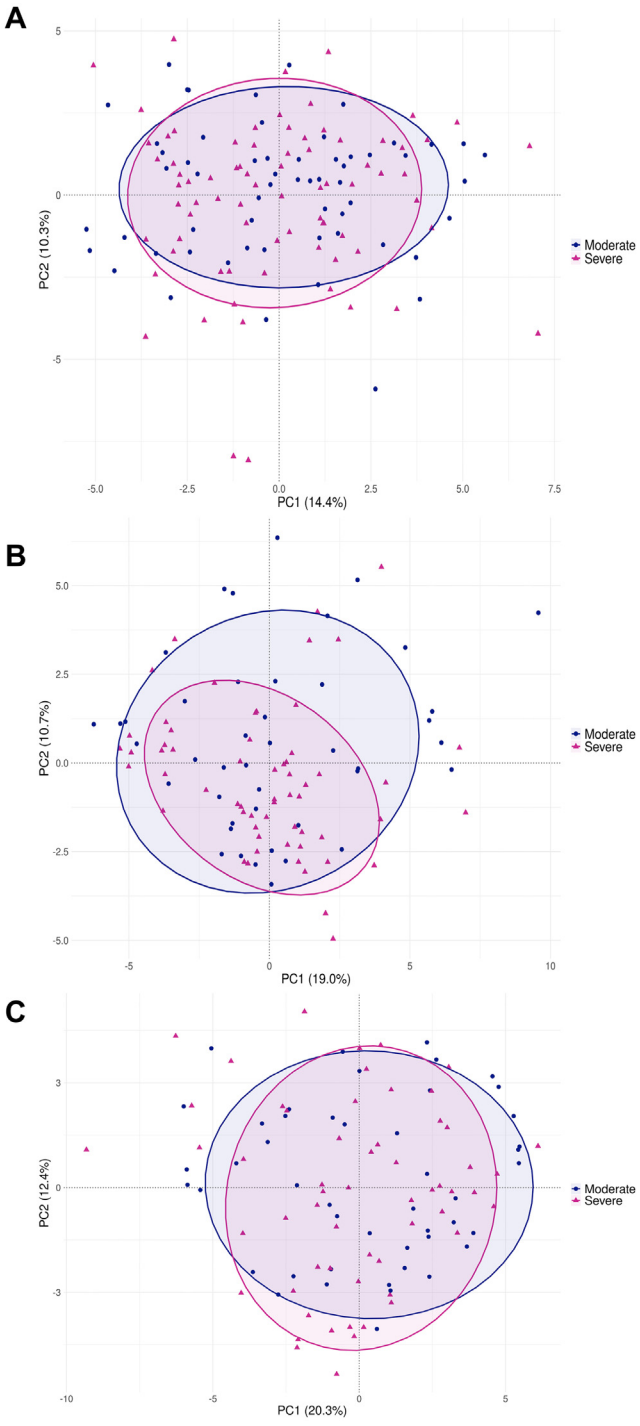
Supplementary Figure 8. Scaled PCA plot of the fecal bacterial profiles of the responders and nonresponders at baseline (*A*) and at 2 years (*B*) and 3 years (*C*) after FMT. The overlap between responders and nonresponders changed at 2 and 3 years after FMT.



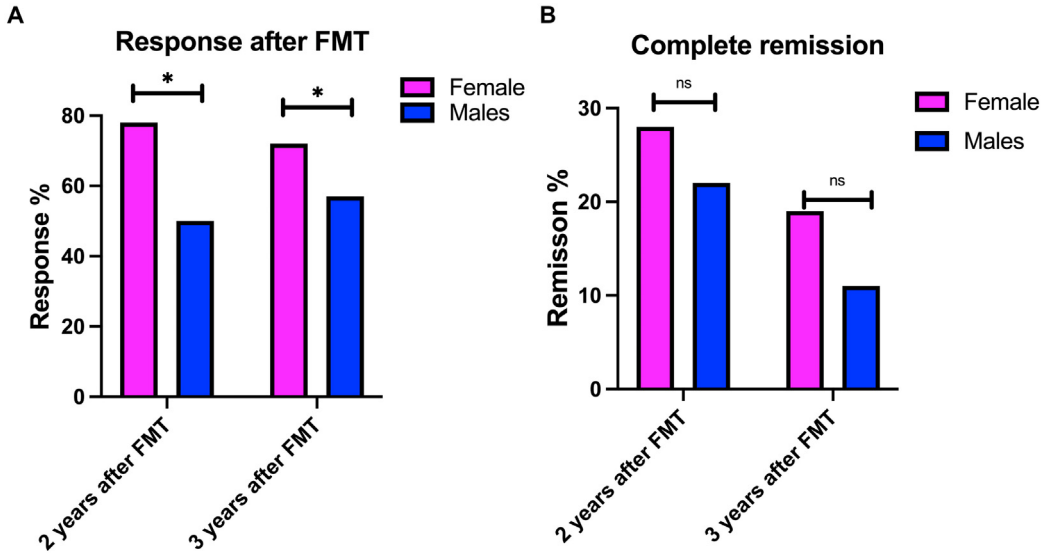
Supplementary Figure 9. Total scores on the IBS-SSS (*A*), Birmingham IBS Symptom Questionnaire (*B*), FAS (*C*), IBS-QOL (*D*), and SF-NDI (*E*). ns, not significant; *$P < .05$; **$P < .001$; ****$P < .0001$.



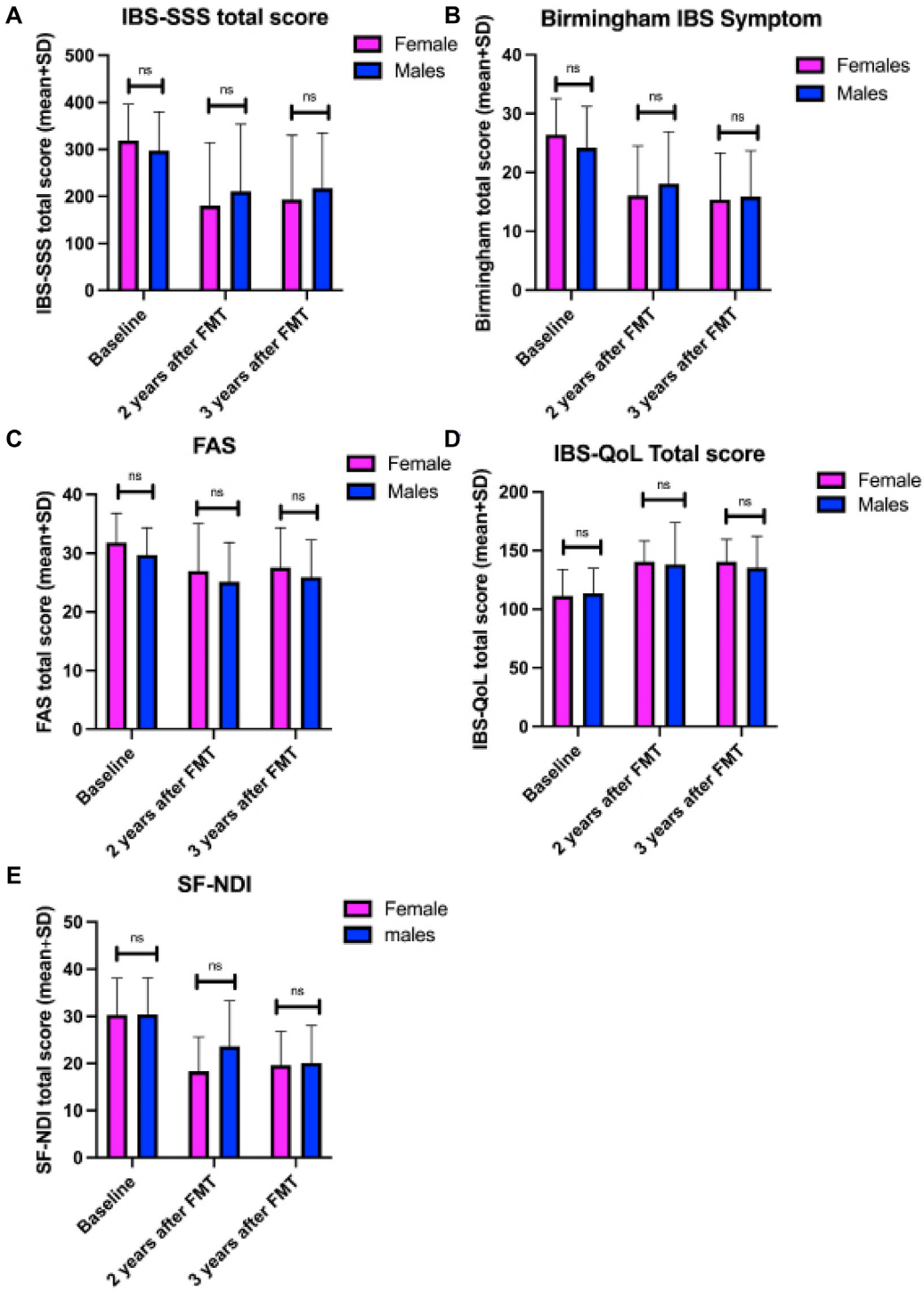
Supplementary Figure 10. DIs of patients with moderate (IBS-SSS total score of ≥175) and severe (IBS-SSS total score of ≥300) IBS symptoms at baseline and at 2 and 3 years after FMT. ns, not significant; *$P < .05$.



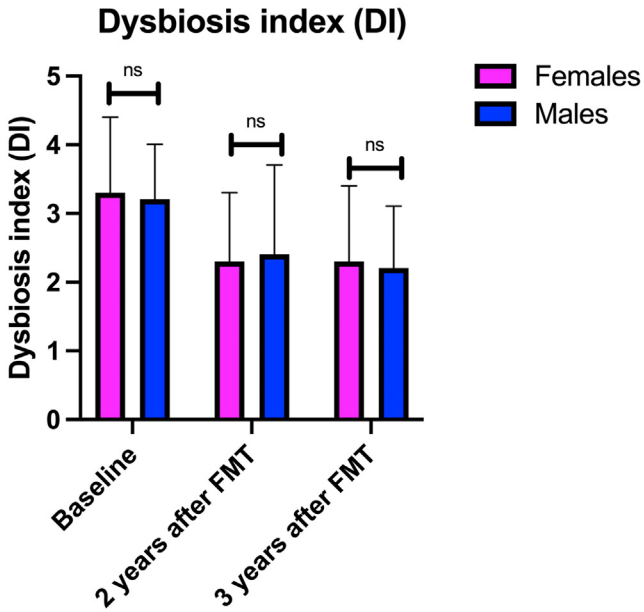
Supplementary Figure 11. Scaled principal-components analysis plot of patients with IBS with moderate and severe symptoms at baseline (*A*) and at 2 years (*B*) and 3 years (*C*) after FMT.



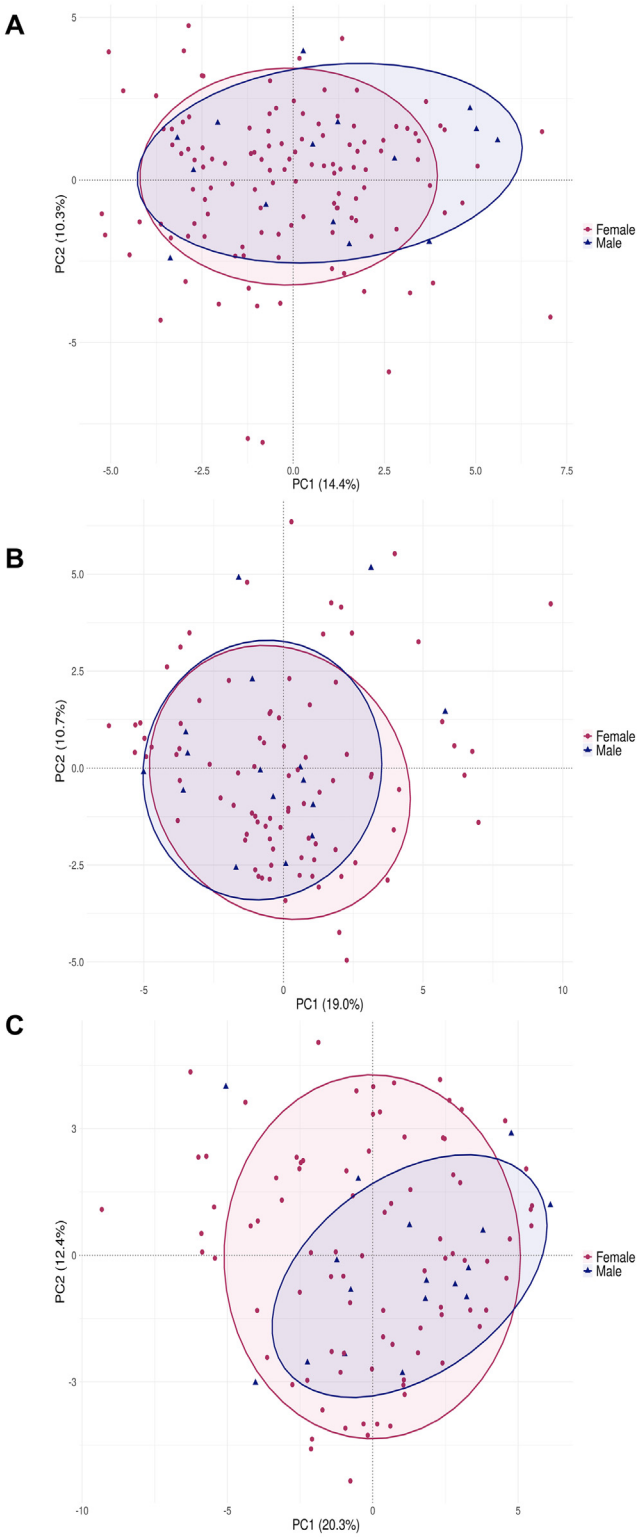
Supplementary Figure 12. FMT response rates (*A*) and complete remission rates (*B*) of female and male patients with IBS. ns, not significant; *$P < .05$.



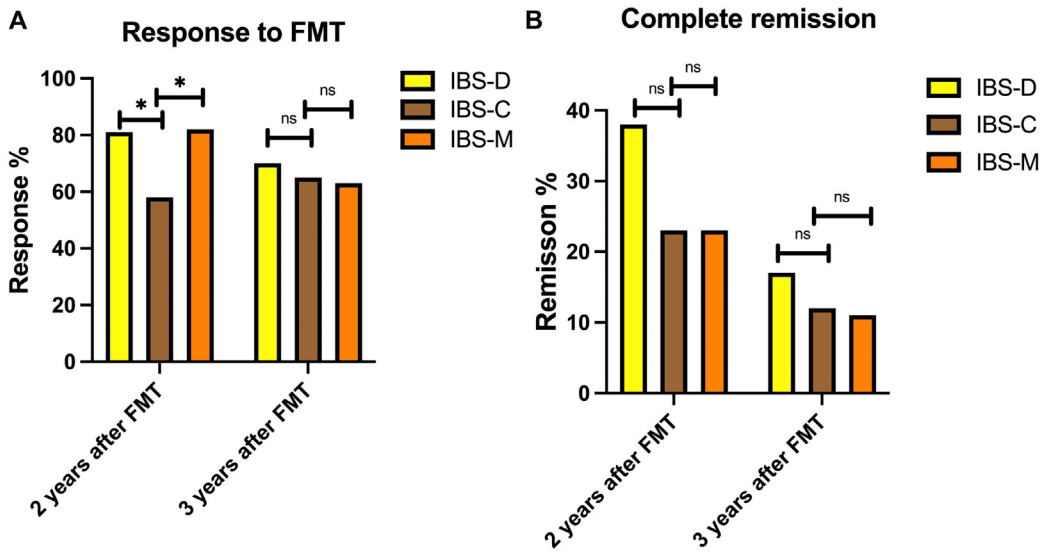
Supplementary Figure 13. Total scores on the IBS-SSS (*A*), Birmingham IBS Symptom Questionnaire (*B*), FAS (*C*), IBS-QOL (*D*), and SF-NDI (*E*) for female and male patients with IBS at baseline and at 2 and 3 years after FMT. ns, not significant.



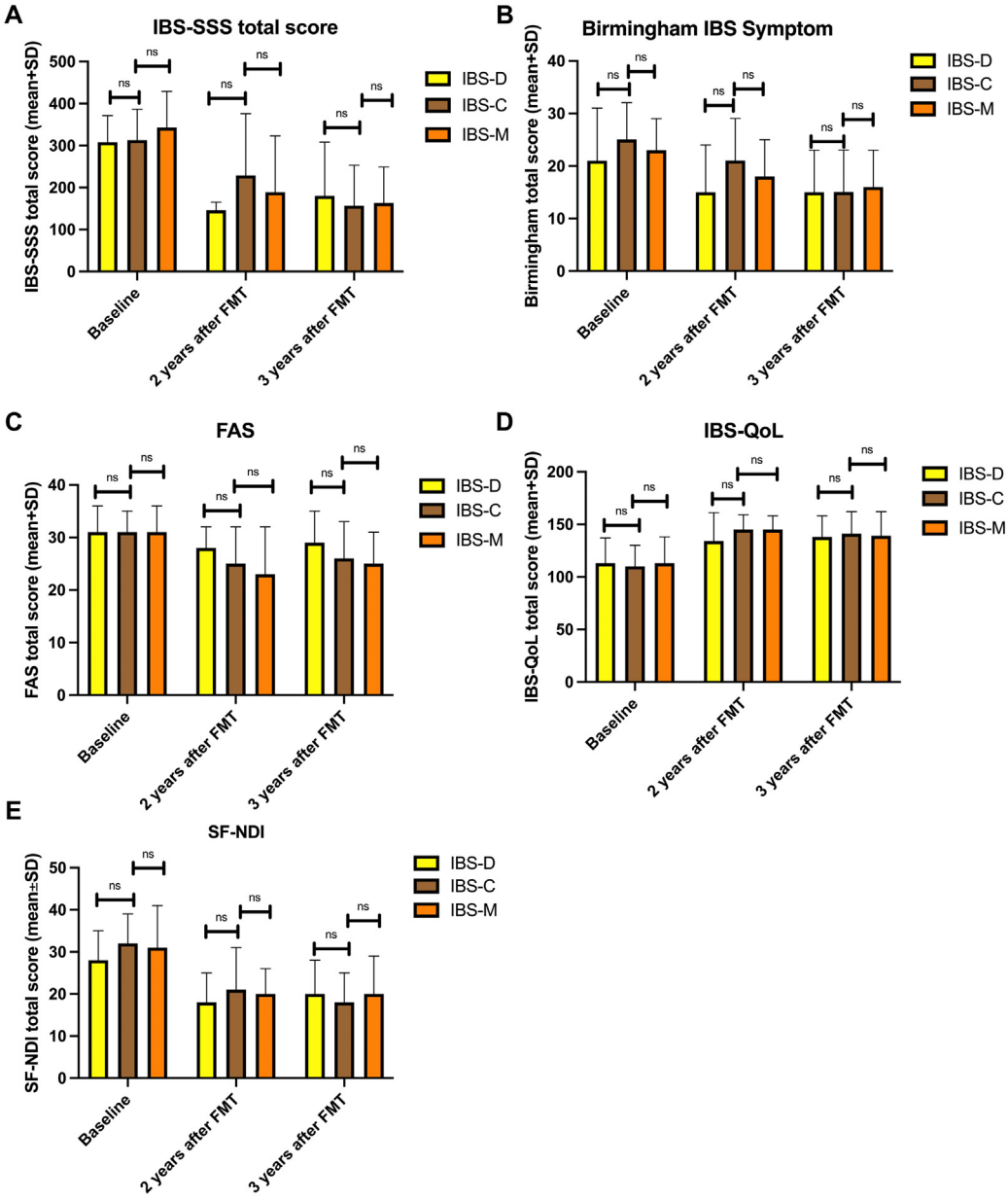
Supplementary Figure 14. DIs of female and male patients at baseline and at 2 and 3 years after FMT. ns, not significant.



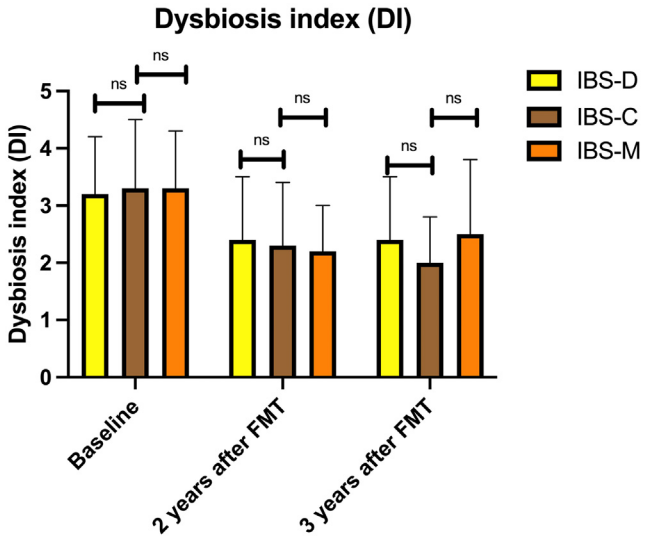
Supplementary Figure 15. Scaled principal-components analysis plot of the fecal bacteria profiles of female and male patients at baseline (*A*) and at 2 years (*B*) and 3 years (*C*) after FMT. Although the bacterial profile of female and male patients changed over time, they mostly overlapped.



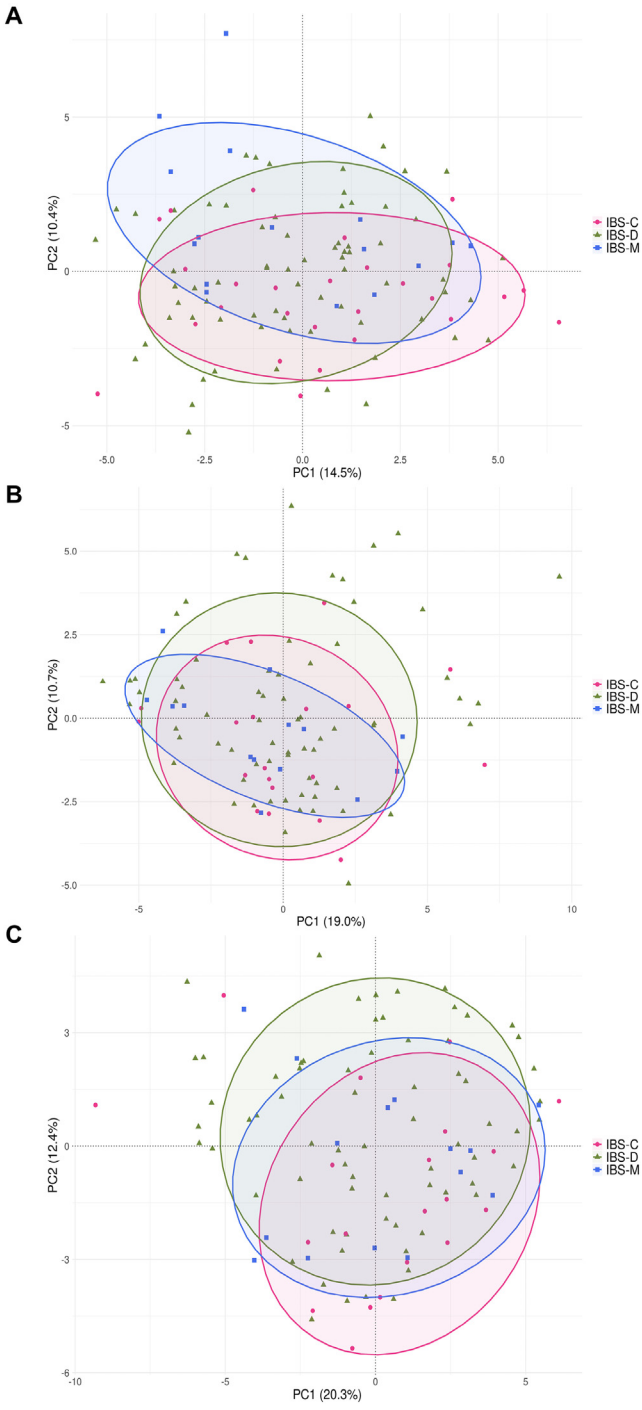
Supplementary Figure 16. FMT response rates (*A*) and complete remission rates (*B*) of patients with IBS-D, IBS-C, and IBS-M at 2 and 3 years after FMT. ns, not significant; *$P < .05$.



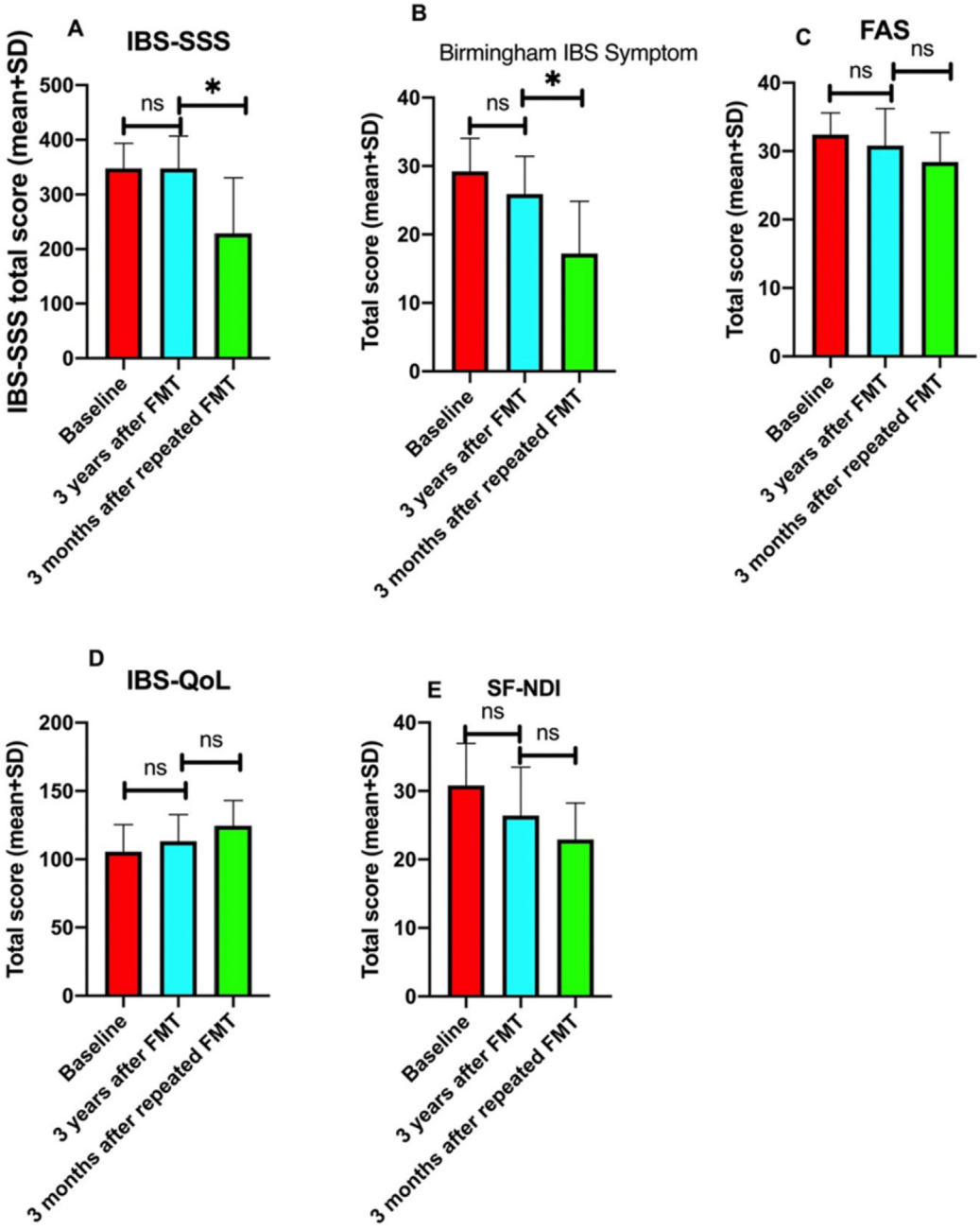
Supplementary Figure 17. Total scores on the IBS-SSS (*A*), Birmingham IBS Symptom Questionnaire (*B*), FAS (*C*), IBS-QOL (*D*), and SF-NDI (*E*) for IBS subtypes at baseline and at 2 and 3 years after FMT. ns, not significant.



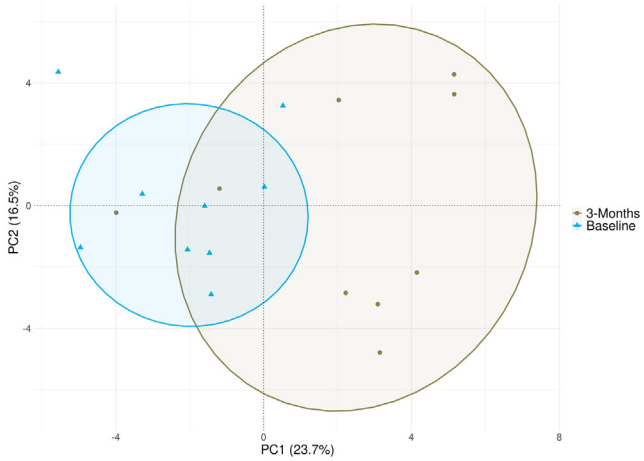
Supplementary Figure 18. DIs for IBS subtypes at baseline and at 2 and 3 years after FMT. ns, not significant.



Supplementary Figure 19. Scaled PCA plot of fecal bacteria profiles of IBS-C, IBS-D, and IBS-M at baseline (*A*) and at 2 years (*B*) and 3 years (*C*) after FMT.



Supplementary Figure 20. Effects of retransplantation on patients with IBS who responded to FMT at 3 months, but relapsed after 3 years on the total scores on the IBS-SSS (*A*), Birmingham IBS Symptom Questionnaire (*B*), FAS (*C*), IBS-QOL (*D*), and SF-NDI (*E*). ns, not significant; *$P < .05$.



Supplementary Figure 21. Scaled principal-components analysis plot of the fecal bacteria profile before and after retransplantation of the patients with IBS who responded to FMT after 3 months, but relapsed after 3 years.