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Factors affecting the outcome of fecal microbiota transplantation for patients with irritable bowel syndrome

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Abstract

Background: A previous study that introduced a Fecal microbiota transplantation (FMT) protocol with a high efficacy applied a combination of favorable factors.

Aims: The present study aimed to evaluate some of these factors.

Methods: This study included 186 patients with IBS randomized 1:1:1 into transplant administered to the colon (single LI), to the duodenum (single SI), or to the duodenum twice with a 1-week interval (repeated SI). The patients provided a fecal sample and were asked to complete five questionnaires at baseline and at 3, 6, and 12 months after FMT. The fecal bacteria composition and dysbiosis index (DI) were analyzed using 16S rRNA gene PCR DNA amplification/probe hybridization covering regions V3–V9.

Results: The response rate was significantly higher in single SI than in single LI at 12 months after FMT. Symptoms and quality of life improved in all the treated groups at all time intervals after FMT. The abdominal symptoms were significantly reduced and the quality of life improved for repeated SI compared with for single SI. DI significantly decreased in all the treated groups at all observation times after FMT. The bacterial profiles changed in all groups at all observation intervals. However, these changes differed between single LI and single SI/repeated SI.

Conclusion: Administrating transplant to the small intestine had a long-term higher response rate than that administrated to the large intestine, and led to long-term colonization of beneficial bacteria. Repeating FMT had more effect on symptoms and quality of life than a single FMT. (www.clinicaltrials.gov: NCT04236843).

KEYWORDS
dysbiosis, fatigue, *Holdemanella biformis*, *Lactobacillus* spp.

1 | INTRODUCTION

Fecal microbiota transplantation (FMT) as a treatment for irritable bowel syndrome (IBS) has been tested in seven randomized controlled trials (RCTs).[1] The outcomes of these RCTs have varied considerably, probably due to differences in the FMT protocols used.[1] One of these RCTs found that FMT had a high efficacy according to both the IBS–symptom severity system (IBS-SSS) and the rigorous requirements of the European Medicines Agency (EMA), and the Food and Drug Administration (FDA) using a composite responder endpoint.[2] Patients in that RCT experienced long-standing effects up to 3 years after FMT with only few mild self-limited adverse events.[2–4] Such excellent

This study was previously presented as an abstract at UEGW 2022, at IBS days meeting 2022 and at EHMSG Workshop 2022.

results could be explained by a combination of favorable factors such as a high dose of donor feces, careful donor selection, and feces transplant being handled in such a way that preserved both aerobic and nonaerobic bacteria.[1] However, it is not clear whether the route of administrating the donor's feces is one of these favorable factors. In the RCTs that the donor's transplant administrated to the large intestine resulted no effect or a moderate and short-term improvement were reported.[1,5,6] On the other hand, transplanting the donor's feces into the small intestine had a high efficacy and durable effects.[2–4,7]

The present study aimed to determine the effects of increasing the fecal dose of the transplant, repeating FMT, and the route of administering the fecal transplant using the same protocol that we applied in our previous RCT.[2]

Key points

- Administrating donor's transplant to the small intestine had a higher efficacy, and led to long-term colonization of beneficial bacteria than administrating the donor's transplant to the large intestine.
- Repeating FMT had more effect on symptoms and quality of life than a single FMT.

2 | METHODS

2.1 | Study design

The patients provided a fecal sample and were asked to complete five questionnaires to assess their abdominal symptoms, fatigue, and quality of life. The patients also provided a fecal sample and completed similar sets of questionnaires the baseline and at 3, 6, and 12 months after FMT. Polyethylene glycol and loperamide were allowed during the intervention as rescue medication.

2.2 | Enrollment and randomization of patients

2.2.1 | Randomization

The patients were randomized 1:1:1 in blocks of six to receiving 90 g of donor feces administered to the cecum of the colon (single LI), to the distal duodenum (single SI), or to the distal duodenum twice with a 1-week interval (repeated SI). Randomization was done by a nurse who was not involved in the trial (Figure 1).

2.2.2 | Patients

This study recruited 200 patients who fulfilled Rome IV criteria, with 186 being included in the study (Figure 1). The medical history was obtained for all patients, and a complete physical examination was done; moreover, blood tests for full blood count, electrolytes, and inflammatory markers including fecal calprotectin, liver tests, and thyroid function tests. They also underwent a gastroscopy with duodenal biopsies and a colonoscopy with biopsies to exclude other gastrointestinal diseases. The baseline characteristics of included patients are listed in Table 1.

The inclusion criteria were,

1. older than 18 years,
2. having a moderate-to-severe IBS symptoms (total IBS-SSS score of ≥175).

The exclusion criteria were,

1. the presence of a systemic disease,
2. pregnancy or planning pregnancy, or lactating,
3. abdominal surgery, with the exception of appendectomy, cholecystectomy, caesarean section, and hysterectomy.
4. Having immune deficiency, or being treated by immune-modulating medication such as methotrexate, azathioprine, ciclosporin, TNFα inhibitors, and steroids.
5. Severe psychiatric disorders, alcohol, or drug abuse,
6. use of probiotic, antibiotic, or IBS medication within 8 weeks prior to the start of the study.

2.3 | Donor

The super-donor used in the study was the same as that used in our previous study.[2] Briefly, he was a healthy Caucasian 40-year-old male, nonsmoker, not taking any medication regularly and had a normal BMI. He had been born via a vaginal delivery, breastfed, and had taken only a few courses of antibiotics during his life. He exercised regularly and took sport-specific dietary supplements, which made his diet richer than average in protein, fiber, minerals, and vitamins. He was screened according to the European guidelines for FMT donors.[8,9] He was vaccinated against COVID-19 and tested weekly for COVID-19 during the period in which he donated his feces. His fecal bacteria composition was tested at the baseline and every 3 months during the trial period. The donor had a dysbiosis index (DI) of 1 for all tested fecal samples and had a stable bacterial profile (Supplementary Figures S1, S2 and Supplementary Table S1).

2.4 | Fecal sample collection, preparation, and administration

The collection and preparation of feces, and administration of the fecal transplant have previously been described in detail.[2] Briefly, 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. Faces were thawed for 2 days at 4°C, and then a 90-g sample was mixed manually with 90 mL of sterile saline, and filtered through a 110 cm × 10 cm nonwoven swab (OneMed,

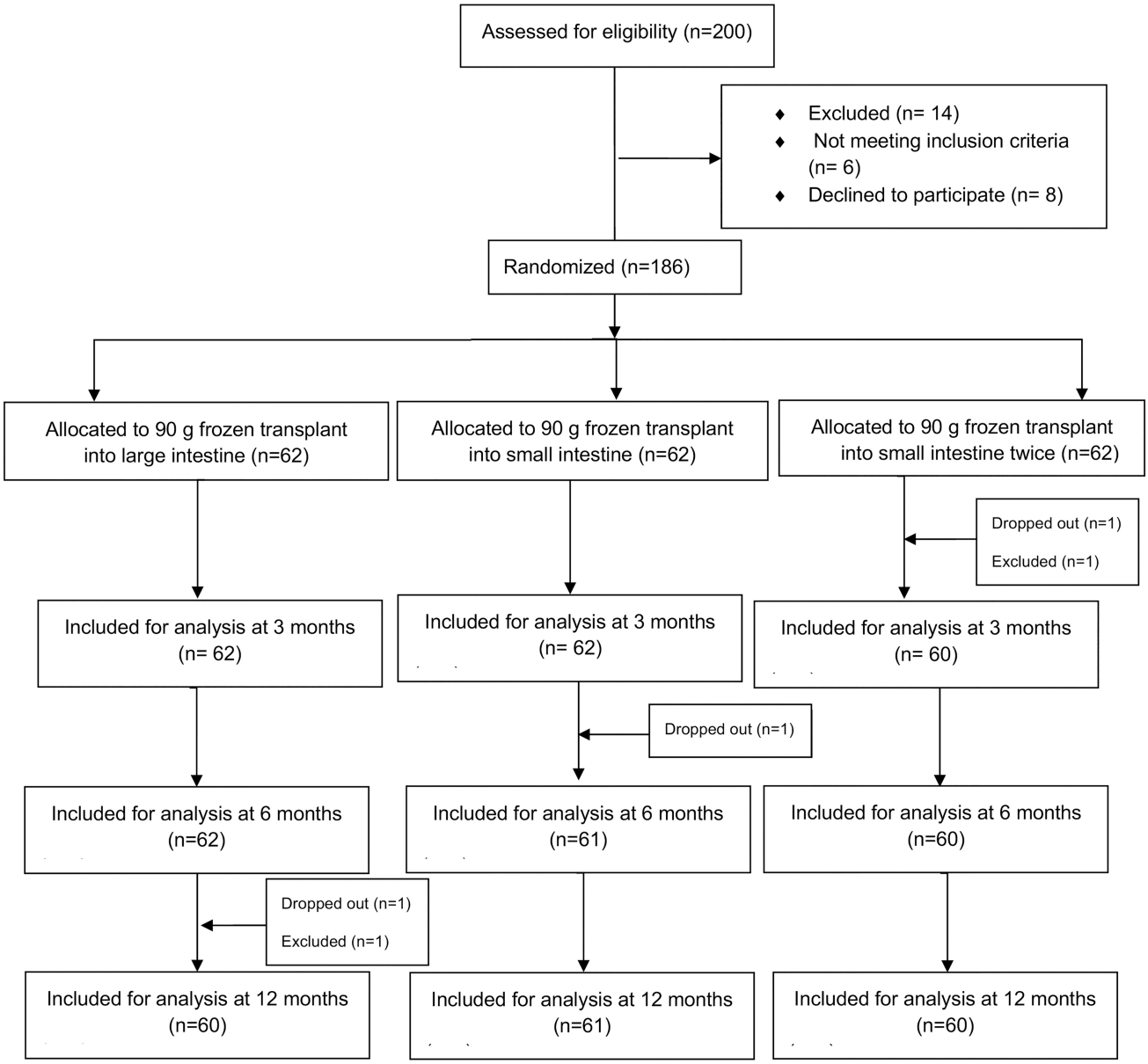

FIGURE 1 CONSORT (Consolidated Standards of Reporting Trials) diagram demonstrating the enrollment and randomization of IBS patients.

Helsinki, Finland). The fecal transplant was administered to the colon cecum after bowel preparation via the working channel of a colonoscope in the single LI group, and to the distal duodenum after overnight fast via the working channel of a gastroscope in the single SI group and repeated SI group.

2.5 | Symptoms and quality-of-life assessment

Symptoms were assessed using the IBS-SSS, the Birmingham IBS Symptom Questionnaire (BSQ), and the Fatigue Assessment Scale (FAS).[10–12] The responders were patients whose total IBS-SSS score decreased by ≥50 points after FMT, while those with an total score of ≤75 were considered to be in complete remission.[10] Quality of life was measured using the IBS Quality of Life Instrument (IBS-QoL) and Short-Form Nepean Dyspepsia Index (SF-NDI) questionnaires.[13,14] Higher IBS-QoL and lower SF-NDI scores indicate a better quality of life.

2.6 | Bacterial analysis

Fecal bacteria compositions and DIs were analyzed using the GA-map® Dysbiosis Test. The fecal samples were homogenized and bacterial cells were mechanically disrupted. This test uses 16S rRNA gene PCR DNA amplification/probe hybridization covering regions V3–V9 followed by DNA probe hybridization of 48 bacterial markers as described previously in detail.[15] The predetermined bacterial markers detect bacteria within 5 phyla (Firmicutes, Proteobacteria, Bacteroidetes, Tenericutes, and Verrucomicrobia), and cover 10 bacterial classes, 36 genera, and 32 species. Thus, the test cover >300 bacteria at different

TABLE 1 Characteristics of patients at the trial baseline.

	Overall	Single large intestine FMT	Single small intestine FMT	Repeated small intestine FMT	*p* values
Number	186	62	62	62	
Age, years	37.2 ± 12.7	40.0 ± 13.6	36.7 ± 12.6	34.3 ± 11.2	0.08[c]
Sex, female/male	131/55	46/16	40/22	45/17	0.2[d]
Body mass index (BMI)	24.6 ± 5.0	25.3 ± 5.7	24.9 ± 5.4	23.7 ± 2.5	0.6[c]
IBS-D	77	26	25	26	
IBS-C	61	20	21	20	0.8[d]
IBS-M	48	16	16	16	
Duration of IBS, years	22.8 ± 13.5	22.3 ± 13.9	24.3 ± 14.3	21.8 ± 13.5	0.7[c]
IBS-SSS total score	349.3 ± 75.9	349.2 ± 67.5	352.8 ± 80.7	346.3 ± 80.6	0.9[c]
Moderate symptom severity[a]	40 (23%)	14 (23%)	13 (22%)	13 (25%)	0.9[d]
Severe symptoms[b]	132 (77%)	46 (77%)	47 (78%)	39 (75%)	
Birmingham IBS symptom	26.3 ± 6.1	26.2 ± 5.5	26.0 ± 6.2	26.6 ± 6.7	0.6[c]
FAS	34.2 ± 5.2	34.5 ± 4.7	34.4 ± 5.9	33.6 ± 4.9	0.4[c]
IBS-QoL	97.9 ± 20.9	98.1 ± 19.6	95.3 ± 22.2	100.0 ± 20.9	0.4[c]
SF-NDI	34.7 ± 7.2	35.2 ± 6.5	35.8 ± 7.4	33.0 ± 7.6	0.1[c]
Dysbiosis index (ID)	2.3 ± 1.0	2.4 ± 1.0	2.3 ± 1.0	2.1 ± 1.0	0.3[c]
Born by caesarean section	20 (10.8%)	5 (8.1%)	9 (14.5%)	6 (9.7%)	0.5[d]
Formula-fed	32 (17.2%)	13 (20.9%)	8 (12.9%)	11 (17.7%)	0.4[d]
Smoker	17 (9.1%)	6 (9.7%)	7 (11.3%)	4 (6.5%)	0.8[d]
Ceased smoking	57 (30.6%)	21 (33.9%)	18 (29.0%)	18 (29.0%)	0.5[d]
Tried FODMAP	147 (79.0%)	51 (82.3%)	52 (83.9%)	44 (71.0%)	0.9[d]
PPI medication	23 (12.4%)	7 (11.3%)	10 (16.1%)	6 (9.7%)	0.7[d]
Birth control medication	51 (27.4%)	14 (22.6%)	14 (22.6%)	23 (37.1%)	0.02[d]
Painkiller medication	20 (10.8%)	8 (12.9%)	9 (14.5%)	3 (4.8%)	0.3[d]
Medication against asthma/allergies	43(23.1%)	13 (21.0%)	14 (22.6%)	16 (25.8%)	0.5[d]
Medication with laevothyroxine	3 (1.6%)	0 (0%)	2 (3.2%)	1 (1.6%)	0.4[d]
Anti-depression medication	28 (15.1%)	7 (11.3%)	9 (14.5%)	12 (9.4%)	0.3[d]
Medication with heart/vascular drugs	11(5.9%)	2 (3.2%)	4 (6.5%)	5 (8.1%)	0.4[d]

Note: Data are mean ± SD, *n* (%), except where indicated otherwise.

[a]IBS-SSS total score between 175 and 300;

[b]IBS-SSS total score of ≥300. PPI, proton-pump inhibitor.

[c]Statistical test: Kruskal–Wallis's test and a posttest of Dunn's multiple comparisons;

[d]Statistical test: Fisher's exact test.

taxonomic levels.[15,16] The DI was measured on a 5-point scale, where values of 1 and 2 indicated normobiosis, and 3–5 indicated dysbiosis.

2.7 | Adverse events and medication

The patients were asked to record their bowel habits and any adverse events in a diary. They were also asked to record their consumption of rescue medications and other new medications.

2.8 | Statistical analysis

The minimum sample sizes required in the single LI and single SI groups were calculated by assuming that the FMT responses for single LI and single SI were 60% and 90%, respectively. The total sample size was estimated to be 66 patients, with 33 in each group ($\alpha=0.05$, $1-\beta=0.80$). The minimum sample size required to compare single SI and repeated SI was calculated by assuming that the responses to single SI and repeated SI were 75% and 95%, respectively. The total sample size was estimated to be 98 patients, with 49 in each group ($\alpha=0.05$, $1-\beta=0.80$). The minimum size required for the study was therefore estimated to be 131. The differences in response, proportion of IBS patients with different IBS symptom severity or complete remission, sex, IBS subtypes, dysbiosis, and the proportion of patients who were born via caesarean section, formula-fed, smokers, ceased smoking, tried the Fermentable Oligosaccharides, Disaccharides, Monosaccharides, and Polyols (FODMAP) diet, and used medication were assessed using Fisher's exact test. Differences between single LI and single SI as well as between single and repeated SI in age, duration of IBS, IBS-SSS, BSQ, FAS, IBS-QoL, and SF-NDI scores, DI, bacterial fluorescence signals and differences

between the IBS subtypes were analyzed using the Kruskal–Wallis's test and a posttest of Dunn's multiple comparisons. Correlations were determined using nonparametric Spearman correlation.

2.9 | Outcomes

The primary endpoint was a reduction in total IBS-SSS score of ≥50 points at 12 months after FMT, and the secondary endpoint was changes in the DI and intestinal bacterial profile.

2.10 | Ethics

The study was approved by the West Regional Committee for Medical and Health Research Ethics, Bergen, Norway (approval no. 2019/6841/REK vest). This study was registered at www.clinicaltrials.gov (NCT04236843).

3 | RESULTS

3.1 | Patients and responses to FMT

At 3 months after FMT, one patient dropped out and one was excluded because of pregnancy in the repeated SI group. At 6 months, one patient dropped out in the single SI group. At 12 months, one patient dropped out and one was excluded because of pregnancy in the single LI group (Figure 1).

The proportion of patients with severe IBS symptoms decreased significantly after FMT in all groups at all intervals (Table 2). The proportion of patients with severe IBS symptoms did not differ between single LI and single SI at 3, 6, and 12 months after FMT ($p = 0.8$, 0.9, and 0.3, respectively), or between single SI and repeated SI at 3, 6 and 12 months after FMT ($p = 0.2$, 0.3, and 0.3, respectively).

The response rate did not differ between single LI and single SI at 3 and 6 months after FMT, but it was significantly higher in single SI and repeated SI than in single LI at 12 months after FMT (Figure 2A).

3.2 | Symptoms and quality of life

The total IBS-SSS and BSQ scores reduced significantly after FMT in the single LI, single SI, and repeated SI groups at all observation intervals (Supplementary Tables S2–S5). These total scores were significantly lower in repeated than in single SI (Figure 3). The total FAS scores after FMT were significantly lower than those at baseline in the three groups at all observation intervals (Supplementary Tables S6 and S7), but did differ significantly between single LI, single SI, and repeated SI (Figure 2). The total IBS-QoL scores increased significantly and those of SF-NDI significantly decreased in all groups at all observation intervals after FMT (Supplementary Tables S8–S11). Patients who received repeated SI FMT had higher and lower total IBS-QoL and SF-NDI scores, respectively, than those who received single SI FMT at all observation times after FMT (Figure 2).

3.3 | Bacterial analysis

In the single LI group, the DI at 3 months after FMT did not differ from that at baseline, but was lower at 6 and 12 months after FMT. DI decreased significantly in both the single SI and repeated SI

TABLE 2 Proportion of IBS patients in complete remission, with mild, moderate, and severe symptoms in the three groups treated with FMT at baseline and at different intervals after FMT.

	Time	Remission[a]	Mild symptoms[b]	Moderate symptoms[c]	Severe symptoms[d]
Single LI	Baseline	0%	0%	24%	76%
	3 months	14%**	23%***	32%	31%****
	6 months	16%**	14%**	41%	29%****
	12 months	11%**	17%**	33%	41%****
Single SI	Baseline	0%	0%	22%	78%
	3 months	7%***	26%****	35%	32%****
	6 months	7%***	25%****	38%	30%****
	12 months	24%***	20%**	28%	28%****
Repeated SI	Baseline	0	0	27%	73%
	3 months	26%****	18%**	39%	17%****
	6 months	27%****	29%****	24%	20%****
	12 months	28%****	28%****	20%	24%****

[a]Total IBS-SSS score < 75.

[b]Total IBS-SSS score of 75–175.

[c]Total IBS-SSS score of 175–300;

[d]Total IBS-SSS score of >300.

**$p < 0.01$.; ***$p < 0.001$.; ****$p < 0.0001$ compared with baseline.

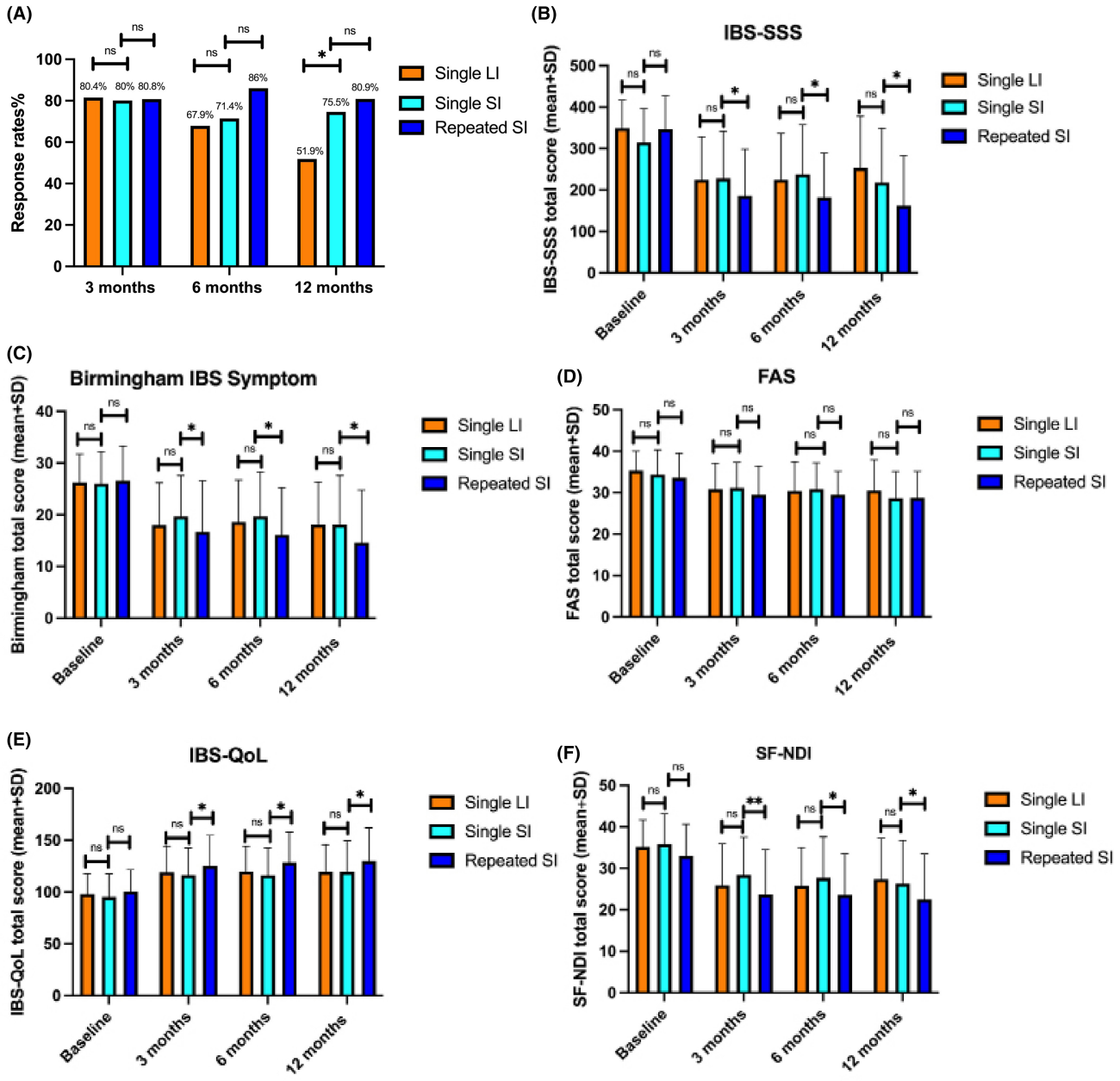

FIGURE 2 Response rates to FMT in single LI (fecal transplant administered to the colon), single SI (fecal transplant administered to the duodenum), and repeated SI (fecal transplant administered to the duodenum twice) at different intervals following FMT (A). Total scores for IBS-SSS (B), BSQ (C), FAS (D), IBS-QoL (E), and SF-NDI (F) in the single LI, single SI, and repeated SI groups at baseline and at 3, 6, and 12 months after FMT. ns, not significant; *, $p < 0.05$; **, $p < 0.01$. Statistical test: Kruskal–Wallis's test and a posttest of Dunn's multiple comparisons.

groups at all observation intervals after FMT (Figure 3), and did not differ between single LI, single SI, and repeated SI at all observation times after FMT.

The bacterial profiles for single LI, single SI, and repeated SI changed considerably after FMT at all observation times, with the changes differing between the groups (Figure 4). However, the fluorescence signals of few bacteria became more similar to the donor (Supplementary Tables S12–S14). The fluorescence signals of 20 bacterial markers changed in the single LI group after FMT (Supplementary Table S12): five at all observation intervals and eight at 12 months after FMT. In the single SI group, the bacterial fluorescence signals of 14 bacterial markers changed following FMT: four at all observation times and six at 12 months after FMT (Supplementary Table S13). Eleven fluorescence signals changed in the repeated SI group after FMT: seven at all observation times and seven at 12 months after FMT (Supplementary Table S14). *Holdemanella biformis* fluorescence signals increased significantly in all groups at 3 and 6 months after FMT, but they remained significantly higher than those at baseline in single and repeated SI at 12 months after FMT, and those of the single LI were significantly lower than baseline

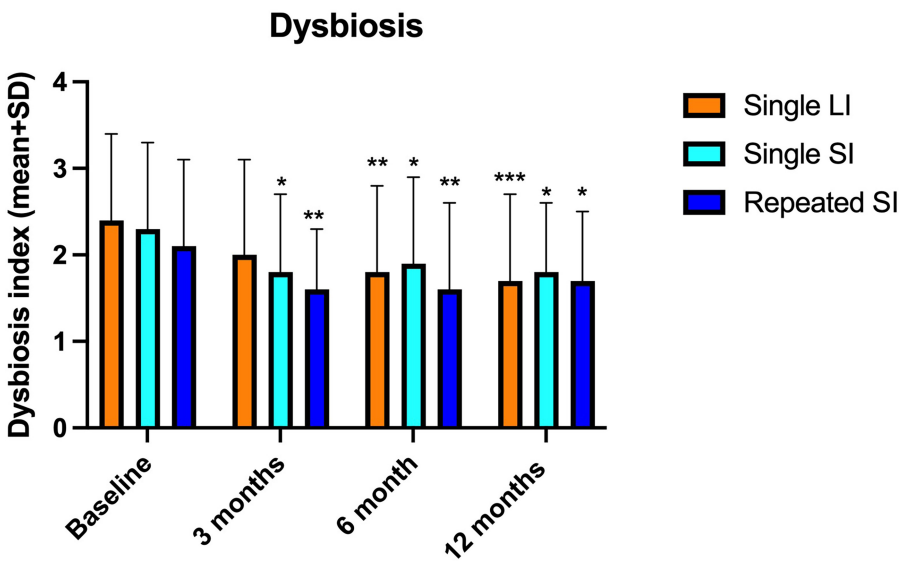

FIGURE 3 Dysbiosis indexes in the single LI, single SI, and repeated SI groups at the baseline, and at different observation intervals after FMT. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ compared with baseline values. Statistical test: Kruskal–Wallis's test and a posttest of Dunn's multiple comparisons.

values (Figure 5). Furthermore, while the *Lactobacillus* spp. fluorescence signals increased significantly in single and repeated SI at all observation intervals after FMT, those in single LI increased only at 3 months after FMT (Figure 6). Six bacterial markers whose fluorescence signals increased after FMT had inverse correlations with the total IBS-SSS score (Figure 6A). The fluorescence signals of two of these bacteria also had inverse correlations with the total FAS score (Figure 6B).

3.4 | Differences between IBS subtypes

The response rates did not differ between patients with IBS-D, IBC, and IBS-M at all observation intervals after FMT in the single LI, single SI, and in repeated Si groups (Supplementary Figure S3).

The total scores of IBS-SSS did not differ between IBS subtypes at the baseline and at different observation points in all the treated groups (Supplementary Figure S4). Birmingham IBS symptom total scores did not differ between IBS subtypes in single LI and repeated SI at the baseline and at 3, 6, and 12 months after FMT (Supplementary Figure S5). However, in the single SI group total scores of Birmingham IBS Symptom of IBS-M were higher than those of IBS-D and IBS-C at the baseline, and then those of IBS-C at 12 months after FMT (Supplementary Figure S5B). FAS, IBS-QOL, and SF-NDI scores did not differ between patients with IBS-D, IBS-C, and IBS-M in all treated groups at baseline and at 3, 6 and 12 months after FMT with only one exception (Supplementary Figures S6 and S7). This exception was seen in single SI group, where the total scores of IBS-QoL of IBS-M were lower than those of IBS-C (Supplementary Figure S7B).

DIs did not differ between patients with IBS-D, IBS-C, and IBS-M at baseline and at 3, 6 and 12 years after FMT in LI and repeated SI groups (Supplementary Figure S9). In the single SI group, DI in IBS-C was lower than that of IBS-M (Supplementary Figure S9B).

At the baseline, patients with IBS-D had higher fluorescence signals of *Bacteroides fragilis and Dorea* spp. and lower fluorescence signals of *Firmicutes* than those with IBS-C. In addition, patients with IBS-M had higher fluorescence signals of *Alistipes onderdonkii* than those with IBS-C (Supplementary Table S15).

At 3 months after FMT, patients with IBS-M had higher fluorescence signals of *Firmicutes* than those of IBS-D and IBS-C. Moreover, the fluorescence signals of *Mycoplasma hominis* in patients with IBS-C were higher than those of with IBS-D and IBS-M. In addition, fluorescence signals of *Acinetobacter junii* in patients with IBS-D, were higher than those with IBS-C and IBS-M (Supplementary Table S16).

At 6 months after FMT, the fluorescence signals of *Bacteroides* spp. and *Phascolarctobacterium* sp. in patients with IBS-D were higher than those with IBS-C. Moreover, patients with IBS-M had higher fluorescence signals of *Firmicutes* than those with IBS-D and IBS-C. Patients with IBS-M had higher fluorescence signals of *Veillonella* spp. and lower fluorescence signals of *Bacteroides* and *Prevotella* spp. than those with IBS-D (Supplementary Table S17).

At 12 months after FMT, patients with IBS-D had higher fluorescence signals of *Dorea* spp. *and Ruminococcus gnavus* and lower fluorescence signals of *Anaerobutyricum hallii* and *Mycoplasma hominis* than patients with IBS-C. Patients with IBS-M had lower fluorescence signals of *Ruminococcus gnavus than those with IBS-D* and *lower* fluorescence signals of *Eubacterium siraeum those those with IBS*-C (Supplementary Table S18).

3.5 | Adverse events and medication

Nausea, mild intermittent abdominal pain, diarrhea, and constipation were reported during the first 5 days following FMT (Supplementary Table S19). There were no significant differences between single and repeated SI regarding nausea ($p = 0.6$), abdominal pain ($p > 0.9$), diarrhea ($p > 0.9$), or constipation ($p = 0.3$).

In the single LI group, six patients (four with IBS-C and two with IBS-M) who did not response to FMT took polyethylene glycol three times daily from 1 week after FMT until the endpoint at 1 year. Five

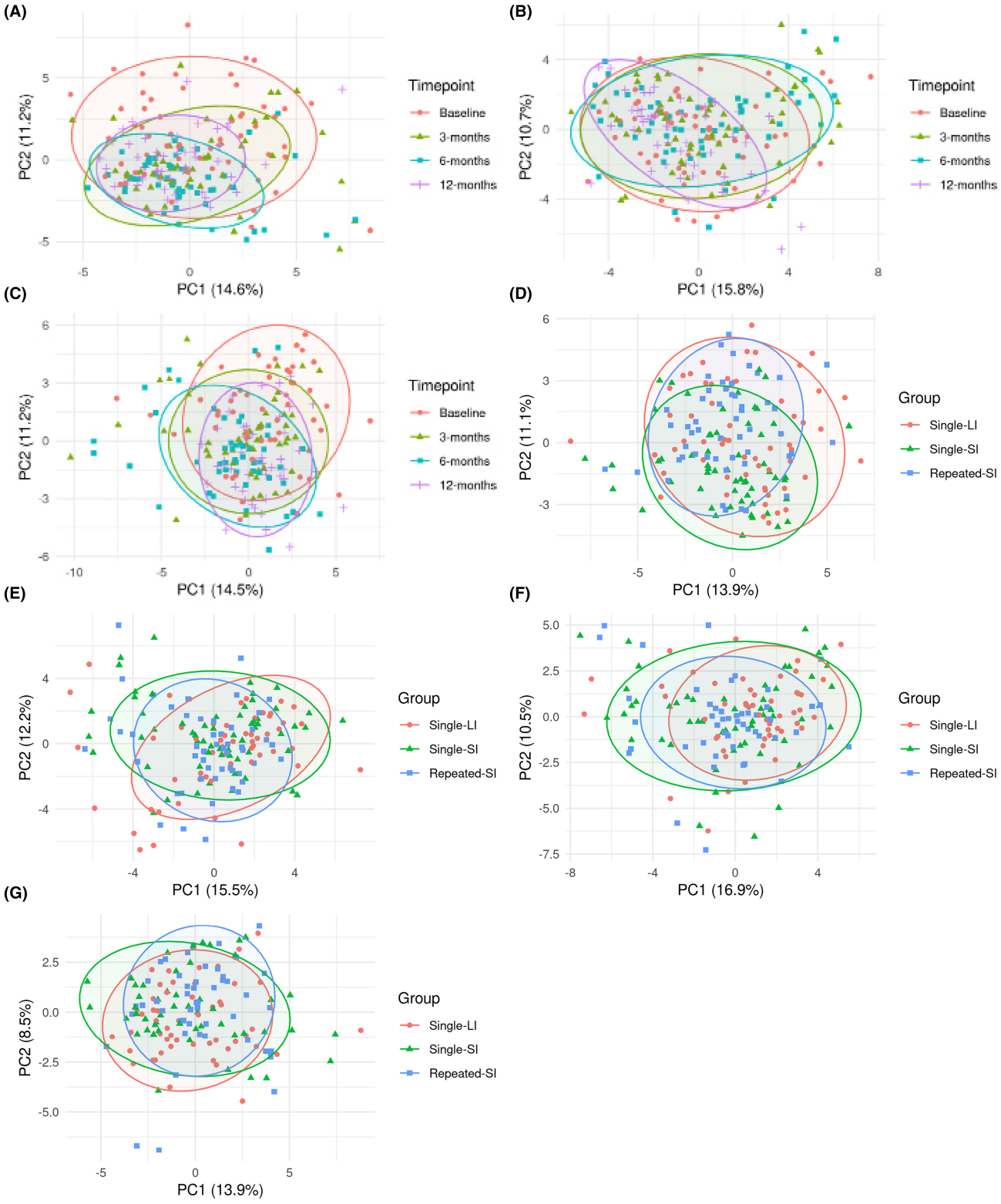

FIGURE 4 Results from scaled principal components analysis of the fecal bacterial profiles. The changes in bacterial profiles over time in single LI (A), single SI (B), and repeated SI (C). Comparisons between the bacterial profiles of the single LI, single SI, and repeated SI groups at baseline (D) and at 3 months (E), 6 months (F), and 12 months **(G)** after FMT.

patients who did not respond to FMT (three with IBS-D and two with IBS-M) regularly took loperamide. Two of the responders took loperamide: one took only one tablet at 12 days after FMT and the other took three tablets daily.

In the single SI group, eight patients (five with IBS-D and three with IBS-M) who did not respond to FMT regularly took loperamide during the trial, and three patients (two with IBS-C and one with IBS-M) took polyethylene glycol regularly. Two responders (one with

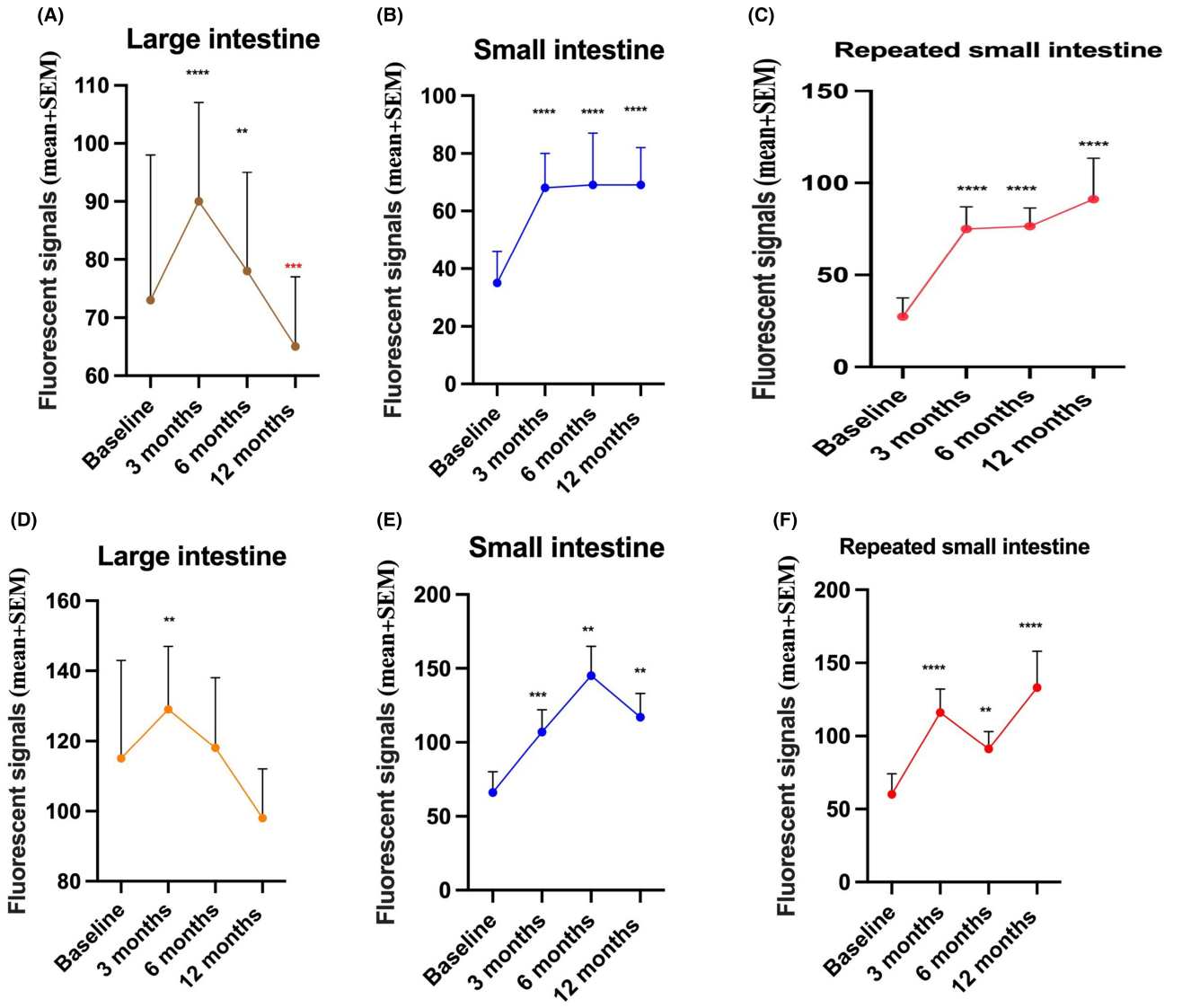

FIGURE 5 The fluorescence signals of *Holdemanella biformis* in single LI (A), in single SI (B) and in repeated SI (C). The fluorescence signals of *Lactobacillus* spp. in single LI (D), in single SI (E) and in repeated SI (F). **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$ compared with baseline values. Statistical test: Kruskal–Wallis's test and a posttest of Dunn's multiple comparisons.

IBS-D and one with IBS-C) regularly took loperamide and polyethylene glycol.

In the repeated SI group, five patients with IBS-D who did not respond to FMT regularly took Imodium, and two nonresponders (with IBS-C) regularly took polyethylene glycol. Three nonresponders (with IBS-M) regularly took either loperamide or polyethylene glycol. Two responders (with IBS-D) took one and two loperamide tablets on Days 5 and 7 after FMT, respectively.

4 | DISCUSSION

The present study showed that increasing the dose of fecal transplant from 60 g to 90 g did not improve responses. Administrating transplant to the small intestine had a long-term higher response rate than that administrated to the large intestine, and resulted in long-term colonization of beneficial bacteria, while administrating the fecal transplant to the large intestine did not. Repeating FMT had more effect on symptoms and quality of life than a single FMT.

The cohort of patients included in this study differed from that in our previous RCT in several ways.[2] The previous patients underwent a 12-hour IBS course lasting 2 days that provided information about IBS, whereas the present patients did not. While the previous patients had not previously consumed a low-FODMAP diet, 79% of the present patients had previously tried it. There were more male patients in the present study than in our previous RCT (females:-males: 2.5:1 vs. 4:1). Moreover, the present cohort included a higher proportion of patients with severe IBS than in our previous RCT(77% vs. 58%).[17] Despite these differences, the FMT response rates were comparable between the two studies.[2]

The present study showed that there was no difference between IBS-D, IBS-C and IBS-M regarding the response rates. Furthermore,

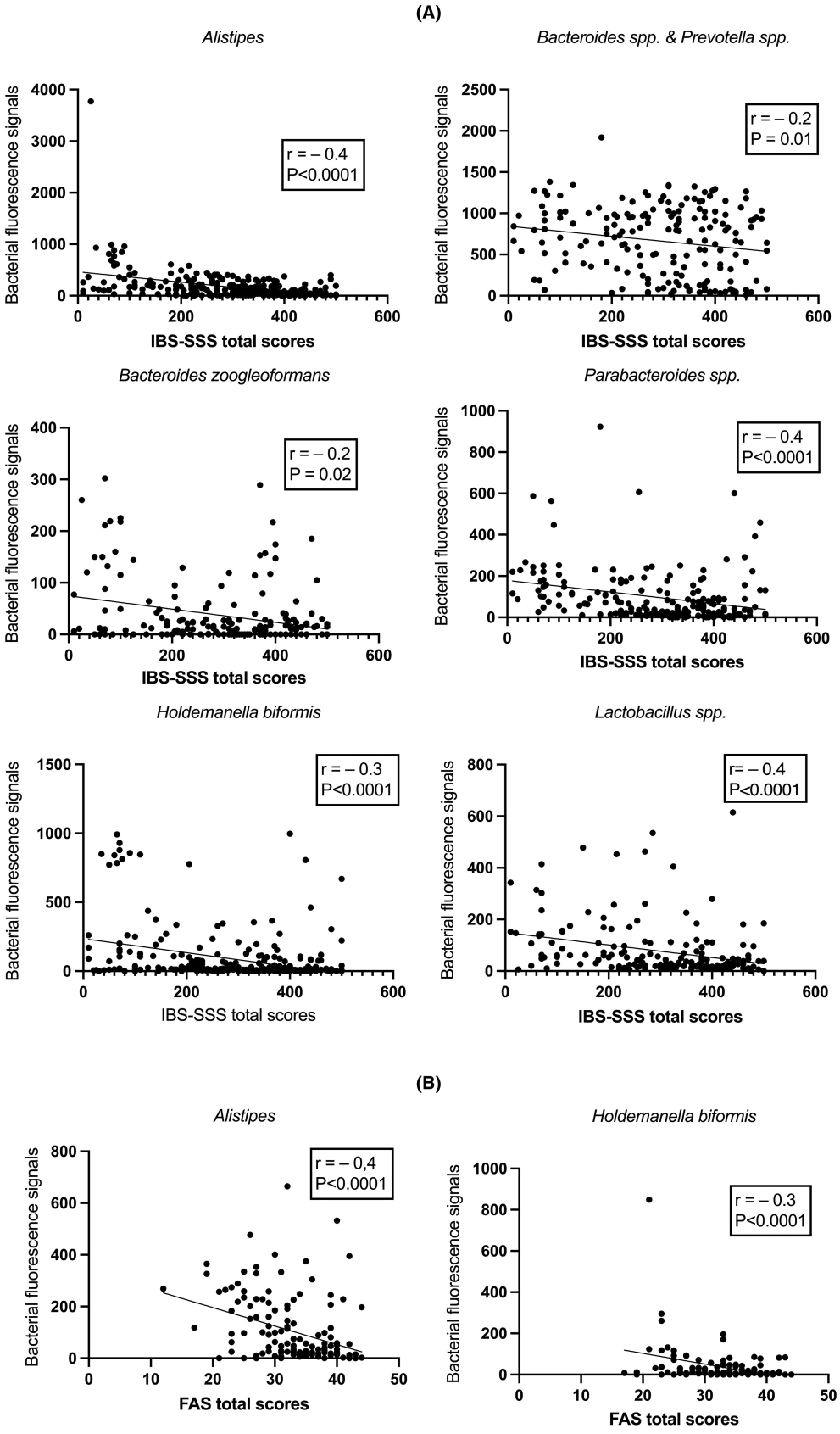

FIGURE 6 The bacteria that changed after FMT and whose fluorescence signals were correlated with total IBS-ISS scores (A), and fluorescence signals of *Alistipes* spp. and *Holdemanella biformis* fluorescence signals increased after FMT and were inversely correlated with the total FAS score (B) in IBS patients. Statistical test: nonparametric Spearman correlation.

the total scores of IBS-SSS, Birmingham IBS Symptom Questionnaire, FAS, IBS-QOL, and SF-NDI did not differ between patients with IBS-D, IBS-C, and IBS-M at baseline and at 3, 6, and 12 months after FMT. These observations are in agreement with previously published data.[2–4] The DIs did not differ between patients with IBS-D, IBS-C, and IBS-M at baseline and at 3, 6, and 12 months after FMT. Although the study included 77 patients with IBS-D, 61 patients with IBS-C and 48 patients with IBS-M, statistical error type II due to small sample size cannot be excluded. Fluorescence signals of few bacteria markers differed between the IBS subtypes at the baseline and at different intervals after FMT. The significance of these differences remains to be determined. The present findings and earlier observation suggest that FMT can be used as an intervention in the three IBS-subtypes included in the study.

In our previous RCT, the FMT response rates increased as the fecal transplant dose increased.[2] However, increasing the fecal transplant dose from 60 g in that study to 90 g in the present study did not further increase the response rates, which indicates that an optimal fecal transplant dose for IBS is 60–90 g.

The fecal levels of six bacteria that changed after FMT were correlated with total IBS-SSS scores, and two of them were correlated with total FAS scores. The roles that these bacteria play in the manifestation of IBS and fatigue remain to be determined. Among the bacteria that changed after FMT and were correlated with symptoms were *Alistipes* spp., *Holdemanella biformis*, and *Lactobacillus* spp. *Alistipes* spp. are Gram-negative, rod-shaped, anaerobic, nonspore-forming, and bile-resistant bacteria,[18] and they seem to play roles in several diseases such as depression, anxiety, chronic fatigue syndrome, autism, and cirrhosis, as well as in aging.[19] *Holdemanella biformis* is a coccus-shaped, anaerobic bacterium[20,21] that produces the long-chain fatty 3-hydroxyoctadecaenoic acid, which exerts anti-inflammatory effects on colitis and protects against intestinal tumor growth.[20,21] *Lactobacillus* spp. are Gram-positive, rod-shaped, nonsporulating, and anaerobic bacteria[22,23] whose abundance is lower in patients with IBS.[24] These bacteria are believed to contribute to the restoration of microbial homeostasis via microbe-microbe interactions, to enhance epithelial barrier function, and to modulate immune responses.[22]

The response rate did not differ between patients who received a fecal transplant into the small and large intestines at 3 and 6 months after FMT, whereas it was significantly lower for FMT into the large intestine at 12 months after transplantation. This finding agrees with previously reported observations that FMT delivered to the large intestine only provided a transient effect at 3 months after FMT.[5,6] This difference between the effects of FMT delivered to the small or large intestine can be explained by the *Holdemanella biformis* fluorescence signals being higher than at baseline in patients who received FMT into the small intestine, while those who received the transplant in the large intestine had lower *Holdemanella biformis* fluorescence signals at 12 months after FMT than those at baseline. Furthermore, the *Lactobacillus* spp. fluorescence signals significantly increased in patients who received FMT into the small intestine at all observation intervals after FMT, whereas those in patients who received it in the large intestine only increased at 3 months after FMT. These findings raise several questions: why administrating donor's fecal transplant to the small intestinal results in a long-term colonization of intestinal bacterial and consequently durable effects of FMT than administrating it to the large intestine? Do the bacteria colonize the distal small intestine (Ileum) or large intestine (colon)? Does the longer transit time when the transplant placed in the proximal small intestine play a role in the long-term colonization? Further studies are needed to address these questions.

There was no difference between patients who received FMT once or twice into the small intestine regarding FMT response rates or in bacterial profiles after FMT. However, patients who received repeated FMT experienced greater improvements in symptoms and quality of life than those who received single FMT. Could it be other intestinal microorganisms in the fecal transplant such as virus, fungi, and/or archaea that contributes to this improvement?

The efficacy of FMT in treating chronic diseases varied considerably and it has been suggested that the success FMT is donor-dependent.[25] Thus, the expression super-donor was coined for a donor with high microbial diversity.[25] Our previous clinical trial using FMT as a treatment for IBS showed a high efficacy and durable effects.[2–4] This success was attributed to the careful selection of the donor (super-donor). It has been speculated that it is difficult to obtain such efficacy in clinic as it is technically difficult to have a super-donor. However, careful analysis of our clinical trial suggested that the success of our clinical trial is due to a combination of favorable factors in our FMT protocol.[26] In addition to careful selection of the donor, high dose of the fecal transplant was among these factors. Freezing of the donor's stool immediately and keeping it frozen until transplantation was performed can be one of these favorable factors as exposing donor's stool to ambient air results in up to 12-fold reductions in the abundance of important commensal taxa.[27] Furthermore, the donor's stool was thawed at 4°C, mixed, and filtered manually. Using mixer or laboratory blender increases air flow produced and result increased oxygen-exposure and be more detrimental to oxygen sensitive species than manual.[27] The present observation showed another favorable factor in our FMT protocol, namely administrating the transplant to the small intestine.

The main strengths of this study were that it included a relatively large cohort of patients with IBS comprising three IBS subtypes, of which 26% were male, and involved a single well-defined donor. However, the limitations of this study were that it did not include placebo controls, did not include the fourth IBS subtype, IBS-U, and it only investigated a predetermined target of intestinal bacterial contents.

AUTHOR CONTRIBUTIONS

M.E.S. designed the study, obtained the funding, administered the study, recruited and followed up the patients, performed FMT, collected, analyzed, and interpreted the data, and drafted the manuscript. O.H.G. and J.G.H. contributed to designing the study and to data analysis and interpretation, and critically revised the manuscript for important intellectual content.

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CONFLICT OF INTEREST STATEMENT

M.E.S and JGH have nothing to disclose. OHG has received speaker honoraria from Bracco, GE Healthcare, Takeda AS, Ferring AS, Allergan, and Janssen-Cilag. He has served as consultant for Bracco, GE Healthcare, Takeda, and Samsung.

DATA AVAILABILITY STATEMENT

Data are available upon reasonable request from the corresponding author.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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