Clinical response to fecal microbiota transplantation in patients with diarrhea-predominant irritable bowel syndrome is associated with normalization of fecal microbiota composition and short-chain fatty acid levels

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Abstract

Objectives

Irritable bowel syndrome (IBS) may be associated with disturbances in gut microbiota composition and functions. We recently performed a study of fecal microbiota transplantation (FMT) in diarrhea-predominant IBS (IBS-D) and found that IBS symptoms improved and the gut microbiota profile changed following FMT.

We now aimed to explore the effects of FMT on the gut microenvironment in further detail by using 16S rRNA sequencing for more extended microbiota profiling and analyzing bacterial fermentation products (SCFAs: short chain fatty acids).

Materials and methods

The study included 13 patients (4 females and 9 males) with IBS-D according to Rome III criteria and 13 healthy donors. Freshly donated feces were administered into duodenum via gastroscopy. The patients completed symptom and quality of life (QoL) questionnaires and delivered feces before and 1, 3, 12 and 20/28 weeks after FMT. Microbiota analysis was performed by sequencing 16S rRNA gene with Illumina Miseq technology. Fecal concentrations of SCFAs were analyzed by vacuum distillation followed by gas chromatography.

Results

Several gut microbiota taxa and SCFAs were significantly different in the patients at baseline compared to their donors. These differences normalized by the third week following FMT in parallel with significant improvement in symptoms and QoL. Responders had different gut microbiota profile and SCFAs than non-responders. Significant correlations were found between the gut microenvironment and IBS symptoms. No adverse effects were reported.

Conclusions

FMT restores alterations of the gut microenvironment in IBS-D patients during the first 3 weeks and improves their symptoms for up to 28 weeks.

Introduction

Irritable bowel syndrome (IBS) is a common chronic gastrointestinal (GI) disorder with unclear pathogenesis. It may be associated with disturbances of gut microbiota composition and functions such as bacterial fermentation [1]. The role of gut microbiota alterations in IBS has led to increased interest in using probiotic [2] and antibiotic [3] approaches for improving IBS symptoms [2].

It has been proposed that feces from a healthy human donor may constitute "the ultimate human probiotic" [4]. The infusion of fecal preparations from a healthy donor into the GI tract of a human recipient, known as fecal microbiota transplantation (FMT), has been used to alter the gut microbiome by re-establishing the balance in the gut microbiota of the new host [4]. Therefore, FMT has been suggested as a treatment option for conditions where an altered gut microbiota has been detected, including IBS [4]. Currently, FMT is widely accepted as the recommended treatment for recurrent *Clostridium difficile* enterocolitis [5]. New data suggest that FMT is effective in improving the symptoms of IBS [6-9] and is associated with changes in the gut microbiota [9, 10]. However, the underlying mechanisms are yet to be explored.

The gut microbiota in the colon ferments unabsorbed food residues, mostly carbohydrates, to short-chain fatty acids (SCFAs) – mainly acetic acid, propionic acid, and n-butyric acid. SCFAs stimulate blood flow, fluid and electrolytes uptake in the colon, and are preferred energy substrates for the colonocytes (i.e. n-butyric acid) [11]. Previous studies of fecal SCFAs in IBS have shown conflicting results [12, 13].

We recently performed a study of FMT in diarrhea-predominant IBS (IBS-D) and

found that the symptoms improved in 75%, 85% and 65% of the total group at 1, 3 and 20/28 weeks after FMT, respectively [9]. In addition, the patients' quality of life and their stool form improved parallel to changes in their gut microbiota [9].

We now aimed to explore the effects of FMT on gut microenvironment in further detail by analyzing bacterial fermentation products (SCFAs) and more extended microbiota profiling with 16S rRNA sequencing, with a particular focus on patients responding to FMT compared with non-responders.

Material and methods

Participants

During the year of 2015, patients who were referred to the gastroenterology outpatient clinic, Haukeland University Hospital, Bergen, Norway, age between 18-70 years, with a diagnosis of IBS according to the Rome III criteria, were included in the study. Patients who scored >175 on the IBS-Symptom Severity Scoring system (IBS-SSS) questionnaire, which indicates moderate to severe symptom severity [14] were included. The exclusion criteria were previous abdominal surgery, current pregnancy or lactation, and probiotics or antibiotics treatment within 4 weeks prior to fecal installation. Patients with a history of blood in stool, inflammatory bowel disease, an immunocompromised state, GI malignancy, a history of opportunistic infections within 1 year prior to FMT, oral thrush, or disseminated lymphadenopathy were also excluded.

The donor group included healthy family members of both genders above 18 years of age. The exclusion criteria for the donors were pregnancy, history of diarrhea, blood in stool, inflammatory bowel disease, IBS, chronic abdominal pain, GI malignancy, antibiotic and probiotic use within 4 weeks prior to FMT, an immunocompromised

126 state, history of opportunistic infections within 1 year prior to FMT, oral thrush and
127 disseminated lymphadenopathy.
128
129 **Study design**
130 The participants' demographic characteristics have been described in details
131 previously [9]. In brief, the patients were scheduled for several visits; the first was
132 scheduled for screening tests one week before the FMT procedure (screening),
133 followed by the day of the FMT procedure (baseline), and then 4 control visits
134 (control 1−4) at weeks 1, 3, 12 and 28 weeks, respectively, after FMT. The screening
135 program included physical examination, and blood and stool tests (for previous
136 exposure to contagious infectious agents, inflammation and other organic diseases)
137 for both donors and patients one week before FMT as described previously [9].
138 The patients completed self-report questionnaires and delivered fresh stool samples
139 collected in containers soon after defecation at each visit before and after FMT. Stool
140 samples were temporarily stored in the refrigerator at home (4°C) before delivery to
141 our unit where they will be permanently stored in refrigerator at -80°C until analysis.
142 The patients were informed not to change their diet or life style throughout the study
143 and to immediately report health changes and/or use of new medications (if any) at
144 any point during the study.
145
146 **The FMT procedure.** Both donors and patients brought >60 g of fresh feces on the
147 FMT day [9]. Thirty grams of freshly donated feces [15] from the donors were
148 manually mixed with 60 ml of normal saline and sieved in order to avoid any hard
149 particles during the preparation of the fecal suspension just before the gastroscopy
150 procedure. After an overnight fast, gastroscopy was performed and 60 ml of the fecal

suspension was instilled, only once, in the descending part of the duodenum distal to the papilla Vateri, followed by 60 ml normal saline. The remaining feces from donors and patients were stored at –80°C until the time for analysis. T.M., G.A.L. or T.H. performed the procedures at the endoscopy unit, Haukeland University Hospital, Bergen, Norway. Control visits 1–4 were planned at weeks 1, 3, 12 and 28, respectively, after the FMT procedure when the patients delivered fecal samples for storage at –80°C until analyses.

Analysis of SCFAs. An amount of 0.5 g of fecal material was homogenized following the addition of distilled water containing 3 mmol/L of 2-ethylbutyric acid (as internal standard) and 0.5 mmol/L of H_2SO_4; 2.5 mL of the homogenate was vacuum distilled, according to the method of Zijlstra et al. [16] as modified by Hoverstad et al. [17]. The distillate was analyzed with gas chromatography (Agilent 7890 A; Agilent, CA, USA) using a capillary column (serial no. USE400345H, Agilent J&W GC columns; Agilent, CA, USA) and quantified using internal standardization. Flame ionization detection was employed. Fecal concentrations of major SCFAs (acetic, propionic and n-butyric acids) and minor SCFAs (iso-butyric, n-valeric, iso-valeric, n-caproic and iso-caproic acids) were analyzed. The results were expressed in mmol/kg wet weight.

Analysis of gut microbiota composition. Bacterial DNA were extracted from stool using MoBio PowerSoil DNA extraction kit, and submitted to sequencing of the V3-V4 regions of the 16S rRNA gene according an established protocol using the Illumina Miseq [18]. Quality control and processing of the raw reads were performed with the QIIME (qiime.org) open source software package [19]. Measures of intra-

individual (alpha) diversity as well as relative abundance of bacteria on different taxonomic levels (from phylum to genus level) were calculated. Alpha diversity was evaluated using index of diversity (Shannon) [20] and index of richness (Chao1) [21, 22].

Symptom questionnaires. Symptoms were evaluated using IBS-SSS [14]. Patients with reduced IBS-SSS scores ≥50 points at week 28 compared to baseline were defined as responders and those who achieved <50 points were defined as non-responders [14]. The IBS-SSS questionnaire was completed at all time points. In addition, IBS symptom questionnaire (IBS-SQ) [23, 24] was completed on the screening day before FMT and then daily for 20 days after FMT. Stool consistency was evaluated using Bristol stool form scale [25], ranging from 1 (constipation) to 7 (diarrhea). Quality of life (QoL) was assessed at baseline and control visits 2 and 4, using Short Form of Nepean Dyspepsia Index (SF-NDI) questionnaire where higher/lower scores represent worse/improved QoL, respectively [26].

Statistical analysis

GraphPad Prism 6 (GraphPad Software Inc., La Jolla, CA, USA) was used for statistical analyses. Repeated measures one-way ANOVA with Holm-Sidak's multiple comparisons test was used to analyse the patients' symptoms before and after FMT. Kruskal-Wallis non-parametric test with Dunn's post test was used to analyse the microbiota and SCFA data between the donors and patients before and after FMT. Multiple *t*-tests were used to compare between responders and non-responders. The data are presented as the mean ± standard error of mean (SEM). Spearman's test was used to study the correlations between the symptoms and variables of the gut

microenvironment. $P<0.05$ was considered to indicate a statistically significant difference.

Ethics Statement

The study was performed in accordance with the Declaration of Helsinki [27] and was approved by the Regional Committee for Medical and Health Research Ethics in Western Norway (reference number: 2013/1497). All of the participants were adults (>18 years old) and provided written informed consent. This study was registered at ClinicalTrials.gov (ID: NCT03333291).

Results

Participants

Sixteen patients were included in the current study. Three patients were excluded after being diagnosed with functional dyspepsia (*n*=1) and *Clostridium difficile* enterocolitis during stool screening (*n*=1) and withdrawing the consent to participate due to personal reasons (*n*=1), as previously reported [9]. Hence 13 patients (9 males and 4 females, mean age of 32 years (range 20–44 years) and 13 donors (6 males and 7 females, mean age of 33 years (range of 20–42 years) completed the whole study. Four patients and their respective donors had their last control visit scheduled eight weeks earlier than the original date (28 weeks following FMT according to the original protocol [9]) for practical reasons.

The results from the screening blood tests and stool cultures at baseline for both the donors and the patients were normal and control blood tests for the patients at the end of the study were also normal. The patients and donors did not report any changes in their diet, life style, medications or health status during the study.

226

227 **Symptom questionnaires**

228 IBS-SSS score (mean±SEM) for the asymptomatic donors was 18±8.9 and the scores
229 for the total group of IBS patients at baseline (week 0) and at the last week of the
230 study were 328.8±20.7 and 250.8±35.9, respectively. According to clinical response
231 at week 20/28, eights patients were considered responders (IBS-SSS reduction >50
232 from baseline) and five non-responders. The IBS-SSS scores for the responders and
233 non-responders' groups were similar at baseline and control visit 1, but was
234 significantly reduced compared to baseline only for the responders' group from
235 control visit 2 and onwards (Fig 1). Significant differences were noted in IBS-SSS
236 scores between the responders and non-responders' groups at control visit 3 and 4
237 (Fig 1). Clinical responses before and during the first 20 days after FMT as assessed
238 by the different domains of IBS-SQ questionnaire in responders and non-responders'
239 groups are shown in supplementary 1. The responders' group showed improved
240 quality of life scores as measured by SF-NDI at control visits 2 and 4 compared to
241 baseline (P=0.036 and 0.0095, respectively), while no improvement was shown in the
242 non-responders' group, supplementary 2. Stool consistency also improved from
243 watery to normal in the responders' group (Bristol stool scale scores changing from
244 5.4±0.6 at baseline to 3.6±0.6 at control visit 4), while it remained unchanged for the
245 non-responders' group.

246

247 **Bacterial fermentation products (SCFAs)**

248 SCFAs were analyzed in all available stool samples at all time points. Before FMT,
249 the concentrations of several SCFAs were significantly lower in IBS patients
250 compared to the donors (Table 1). After FMT, the concentrations of SCFAs in IBS

patients increased and became non-significantly different from that of the donors at control visit 2, and the increment lasted up to the end of the study (Table 1). Looking into subgroups, only the responders showed a significant difference when comparing the concentrations of the iso-butyric acid at baseline and control visit 1 to the donors (P=0.003 and 0.049, respectively), valeric acid at baseline to donors (P=0.0085), iso-valeric acid at baseline and control visit 1 to donors (P=0.002 and 0.03, respectively), Fig. 2.

Gut microbiota composition of the patients

The trajectories of the intra-individual (alpha) diversity of gut microbiota of IBS patients from baseline and after FMT are shown in comparison with the donors in Fig 3 (A and B). The diversity in the total group of IBS patients at baseline was numerically lower than that of the donors, but statistically non-significant, and increased towards the levels of the donors following FMT. The diversity of gut microbiota in the responders and non-responders' groups showed similar changing patterns, Fig 3 (A and B).

The abundance of gut microbes on phylum level in donors and patients during the course of the study is shown in Fig 4. The total group of IBS patients had significantly different gut microbial composition than their donors at baseline before FMT (Table 2). On phylum level, *Actinobacteria* was less abundant in IBS patients, which was primarily driven by the genus *Bifidobacterium*. Additional genera differing between donors and patients were *Ruminococcus gnavus*, *Clostridium sensu stricto,* and *Gardnerella*. The abundance of these four genera became more similar to the donors from baseline to control visit 2, while at control visit 3 and 4 the abundances became again significantly different compared with the donors (Table 2). Furthermore, the

abundance of *Bacteroides, Alistipes, Parabacteroides* and *Pseudomonas* became significantly different in the total group of IBS patients at the end of the study compared to the donors. An early increase of the phylum Proteobacteria was observed at control visit 1, peaking far beyond the donor abundance, primarily driven by the *Escherichia-Shigella* genus, but the abundance was reduced and no longer different from the donors at control visit 2 and later.

When investigating subgroups according to treatment response, the responders' group showed significant differences between the patients and their donors before FMT for the *Clostridium sensu stricto* and *Bifidobacterium*, which normalized by control visit 2 but some genera became significantly different again towards the end of the study as shown in Table 3.

In the non-responders' group, significant differences between the patients and their respective donors were shown only for *Bifidobacterium* before FMT and for *Bacteroides*, *Alistipes* and *Pseudomonas* towards the end of the study as shown in Table 4. No significant differences were found between the patients and their respective donors for *Akkermansia* in any of the groups and for *Ruminococcus gnavus* and Gammaproteobacteria in the responders and non-responders' groups during the whole study.

Gut microbiota composition of the donors

The donors were divided according to the patients' clinical response after receiving FMT into responders and non-responders and so the analysis of the donors' data was performed accordingly. No significant differences were found in the diversity (Shannon index) and richness (Chao1 index) of the gut microbiota between the donors of the responders' group and those of the non-responders' group.

Correlations between gut microbiota variables and IBS symptoms

In the total group of IBS patients an inverse relation is shown between microbial richness according to Chao1 index and IBS-SSS score during the study period, (Fig 5). Using Spearman test, before FMT in the total group of IBS patients, correlations were found between IBS symptoms and the gut microenvironment: nausea correlated with abundance of *Clostridium sensu stricto* (r=0.70, *P*=0.043), and diarrhea correlated with Proteobacteria (r= 0.72, *P*=0.03) and *Escherichia-Shigella* (r= 0.72, *P*=0.03). Concentrtion of butyric acid in feces correlated with abundance of Firmicutes *(r*=0.79, *P*=0.016) and Actinobacteria (r= 0.85, *P*=0.005). The scores for IBS-SSS correlated with SF-NDI in responders' group before FMT (r=0.73, *P*=0.046) and in non-responders' group before FMT (r= 1, *P*=0.02) and after FMT at control visit 2 (r= 0.97, *P*=0.03). In addition, scores for Bristol stool form scale correlated with IBS-SQ-abdominal pain (r=0.79, *P*=0.02) and diarrhea (r=0.72, *P*=0.046) in the responders' group before FMT and after FMT at control visit 1 (r=0.82, *P*=0.03 and r=0.78, *P*=0.04, respectively).

Complications

No complications or adverse events were reported or recorded during or following the FMT procedure or at any point of the study.

Discussion

The current study investigated the effects of transplanting feces from healthy donors to IBS patients and focused on exploring the effects on the gut microenvironment by measuring fecal SCFAs, which are end products of bacterial fermentation, and

326 microbiota profiling with 16S rRNA sequencing. The results suggest that both gut
327 microbiota and SCFAs in IBS patients are different from that of the donors at baseline
328 and seem to normalize after 3 weeks following FMT in parallel to improved IBS
329 symptoms and quality of life for the patients during the same period.
330
331 Previous studies have shown that IBS is associated with altered gut microbiota profile
332 and fecal SCFAs concentrations [1, 12]. In our study, the major SCFA– n-butyric acid
333 – and several minor SCFAs were significantly different between patients and donors
334 at baseline.
335 The major SCFAs are acetic, propionic and n-butyric acids [11] and their levels
336 depend on factors such as diet, microbiota composition, and host factors such as
337 orocecal transit time [28]. IBS is associated with altered (either lower [12, 29] or
338 higher [12, 13]) fecal levels of SCFAs. More abdominal rumbling was noticed for IBS
339 patients with lower concentrations of SCFAs [12]. These studies suggested using
340 fecal SCFAs as diagnostic markers for IBS [13, 29].
341 In a previous publication, IBS patients were classified according to their acetic acid
342 levels, high vs. low [1]. In the current study, IBS patents had lower levels of acetic
343 acids than healthy donors, but it did not reach the level of significance. The branched-
344 chain fatty acids (iso-valeric and iso-butyric acids) are mainly products of protein
345 degradation, fermented increasingly through progression to the distal colon. They
346 were found to be significantly different in patients compared to donors at baseline,
347 which maybe is due to differences in microbiota associated with protein fermentation
348 [30].
349

In general, IBS patients seem to have low microbial diversity and richness [10, 31], decreased levels of Actinobacteria and *Bifidobacterium* [32], and increased levels of Bacteroidetes and Proteobacteria in the feces [32, 33], which is consistent with our findings. The microbial diversity, richness and several bacterial genera seem to normalize following FMT in the total and responders' groups, which is in line with previous publications [10, 31], and indicates that FMT alters the gut microbiota [10].

Actinobacteria and *Bifidobacterium* are important for mucosal barrier of the gut to keep pathogens from crossing over [32]. They were present in low levels in IBS patients at baseline in the current study, which is consistent with a previous publication [32]. Actinobacteria *and Bifidobacterium* produce acetic acid during colonic fermentation to be used as substrates by other butyrate-producing bacteria [34], which may explain the correlations between these bacteria and SCFAs in this study. Higher levels of n-butyric acid promote the virulence in *E. coli* and its ability to colonize the colon, which may explain the increase in *Escherichia* levels one week after FMT [35]. The correlations of diarrhea with *Escherichia-Shigella* and Proteobacteria are consistent with previous publications [36, 37].
Akkermansia (belongs to Verrucomicrobia phylum) are butyrate-producing bacteria and have been shown to restore gut barrier function and appropriate tight junction expression [38]. On the other hand, *Ruminococcus g*navus cause degradation of the mucus layer [32, 39]. *Akkermansia* correlated positively with *Ruminococcus gnavus* [40], both of which were found in higher abundance in our patients at baseline compared to their donors, which may allow us to speculate whether *Akkermansia* levels reflect their gut permeability-protective actions against *Ruminococcus gnavus*.

About 62% of the patients (8/13) were considered as clinical responders by the end of the study [9]. The inverse relation between the severity of IBS symptoms (IBS-SSS) and low microbial richness (Fig 5) is consistent with previous publications [41, 42]. The same observation applies on low microbial diversity and richness, and worsening of diarrhea [31, 42]. The correlations between IBS-SSS and quality of life and between IBS-SQ-abdominal pain and diarrhea with Bristol stool form scale are consistent with previous publications [10, 14, 25, 42, 43]. The improvement in the stool form as evaluated by Bristol stool form scale following FMT has also been observed in a previous study [31].

Several techniques have been described for the laboratory preparation of fecal suspension and can be classified into rough filtration, filtration plus centrifugation, and microfiltration plus centrifugation [44, 45]. The method used in the current study i.e. 30 g of feces that have been manually suspended in saline and sieved in order to avoid the clogging of infusion syringes and tubes) is consistent with the European consensus on FMT in clinical practice [15]. In the current study, the patients received FMT only once. The time intervals for repeated FMT is crucial and might affect the clinical outcome but at the time being it is still unknown what is the best time interval for repeated FMT procedures and future research should focus on this point.

A meta-analysis of four randomized control trials comparing FMT to placebo (either autologous FMT or other) show conflicting results regarding the effect of FMT on IBS symptoms and concludes that current evidence does not suggest a benefit of FMT for global IBS symptoms [46]. However, despite the limitations of the current study, it shows that FMT has positive effects on IBS symptoms that lasts at least 6 months in

most of the patients who responded to the treatment. The limitations of this study are the small sample size and the lack of placebo group. In addition, we, unfortunately, unintentionally missed the fecal samples from some of the patients at baseline. In order to circumvent the issue of missing data, comparisons between the donors and the patient groups at different time points were performed. Still we cannot exclude the possibility that missing data may have had an impact on the results. The strengths of the present study include the simultaneous assessment of microbiota, SCFAs and IBS symptoms along with their correlations. It is important to specify that this was an exploratory study and multiple statistical tests were performed, increasing the likelihood that some of these are significant by chance. However, we considered the importance with an exploratory study to generate hypotheses for subsequent research and to use as basis for improvements in design for future trials.

In conclusion FMT helps in restoring the alterations in the gut microbiota and their functions in IBS patients and improves their symptoms for up to 28 weeks after FMT. Thus, normalizing both the levels of SCFAs and gut microbiota may be beneficial in IBS [47]. This study confirms the associations between gut microbiota, SCFAs and IBS symptoms.

Conflict of interests: The authors declare that they have no conflict of interests.

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Authors contribution list

TM was involved in study planning and design, recruited patients, performed gastroscopies with FMT, data analysis and interpretation and drafted the original manuscript.

TH was involved in study planning and design, recruited patients, performed gastroscopies with FMT, data interpretation and commented on the manuscript.

JRH performed 16s sequencing and microbiota analysis, data interpretation and commented on the manuscript.

JV performed SCFAs analysis, data interpretation, and commented on the manuscript.

DAS was in involved in study planning and design, recruited patients and commented on the manuscript.

MES was involved in study planning and design and commented on the manuscript

OHG was in involved in study planning and design and commented on the manuscript

JGH was involved in study planning and design, data interpretation and commented on the manuscript.

GAL was involved in study planning and design, recruited patients, performed gastroscopies with FMT, data interpretation and commented on the manuscript.

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655 ## Figure legends

656 **Fig 1. The scores of IBS-SSS in the responders vs. non-responders' groups**

657 **before (at screening and baseline) and after fecal microbiota transplantation**

658 **(control visits 1–4).**

659

660 **Fig 2. Concentrations of short-chain fatty acids (mmol/kg) for donors and**

661 **patients in the responders vs. non-responders' groups before (baseline) and**

662 **after fecal microbiota transplantation (control visits 1–4).**

663

664 **Fig 3. Alpha diversity in the responders and non-responders and total groups**

665 **of donors and patients with irritable bowel syndrome before (baseline) and**

666 **after (control visits 1–4) fecal microbiota transplantation as presented by A)**

667 **Shannon index, B) Chao1 index.**

668

669 **Fig 4. Taxonomy levels (%) in total, donors, patients at FMT (baseline) and**

670 **control visits 1–4.**

671

672 **Fig 5. Chao1 index vs. IBS-SSS scores for the total group of IBS patients before**

673 **(baseline) and after fecal microbiota transplantation (control visits 1–4).**

674

675

676

677 **Table 1:** Short-chain fatty acids (SCFAs) concentrations (mmol kg^{-1}) in fecal samples

678 collected from total groups of donors and patients with irritable bowel syndrome

679 (IBS) before and after fecal microbiota transplantation.

SCFAs	Donors (n=13)	Patients									
		Baseline (n=9)	Control visit 1 (n=12)	Control visit 2 (n=10)	Control visit 3 (n=13)	Control visit 4 (n=12)	P baseline	P 1	P 2	P 3	P 4
Acetic acid	33.9±2.8	23.6±6	31.1±4.9	35.5±3.9	25.8±4.4	28.5±2.4	0.77	>0.9	>0.9	0.3	>0.9
Propionic acid	9.5±1	6.2±1.6	7.9±1.5	8.2±1.5	7.3±1.9	8.1±1.2	0.18	>0.9	>0.9	0.2	>0.9
n-butyric acid	10.4±1.6	4.7±1.2	7.7±1.8	8.4±1.5	5.8±1.4	5.96±1.11	**0.049**	0.78	>0.9	0.095	0.25
Iso-butyric acid	1.27±0.17	0.67±0.11	0.77±0.12	0.92±0.13	0.7±0.12	0.98±0.2	**0.03**	0.089	>0.9	**0.025**	0.96
n-valeric acid	1.4±0.18	0.68±0.008	1.05±0.2	1.06±0.15	0.77±0.13	0.93±0.093	**0.013**	0.67	>0.9	**0.042**	0.47
Iso-valeric acid	1.6±0.2	0.8±0.2	0.9±0.15	1.16±0.2	0.8±0.14	1.27±0.2	**0.014**	**0.046**	>0.9	**0.011**	>0.9
n-caproic	0.8±0.02	0.3±0.1	0.5±0.2	0.5±0.1	0.2±0.08	0.3±0.09	0.2	>0.9	>0.9	0.059	0.17
Iso-caproic	0.01±0.005	0.02±0.02	0.008±0.006	0.013±0.01	0.01±0.005	0±0	>0.9	>0.9	>0.9	>0.9	0.6
Total SCFAs	58.8±5.4	37±8	49.9±8	55.7±6.2	41.4±7.1	46±4.7	0.17	>0.9	>0.9	0.15	0.6

Data are presented as mean±SEM. Comparison: Kruskal-Wallis multiple comparisons test with Dunn's post test. ^{Pbaseline} Donors at the beginning of the study vs. patients on FMT day before fecal installation, ^{P1} Donors at the beginning of the study vs. patients at 1st control following FMT, ^{P2} Donors at the beginning of the study vs. patients at 2nd control following FMT, ^{P3} Donors at the beginning of the study vs. patients at 3rd control following FMT, ^{P4} Donors at the beginning of the study vs. patients at 4th control following FMT. FMT: fecal microbiota transplantation. SCFAs: short-chain fatty acids.

680

681

682 **Table 2: Gut microbiota differences in the total group between donors at**
683 **baseline and IBS patients before and at each control visit after fecal microbiota**
684 **transplantation.** The left part of the table shows the microbial taxa for donors and
685 patients before fecal microbiota transplantation **(**FMT) and at each control visit after
686 FMT and the directionality towards or away from that of the donors at baseline. The
687 right part of the table shows the *P*-values when comparing the different microbial
688 abundances for the patients at different time points to that for the donors at baseline.

Bacteria	**Donors (*n*=13)**	**Patients**									
		Baseline (n=9)	**Control visit 1 (n=12)**	**Control visit 2 (n=9)**	**Control visit 3 (n=13)**	**Control visit 4 (n=12)**	**P baseline**	**P 1**	**P 2**	**P 3**	**P 4**
Ruminococcus gnavus	0	0.0005±0.0003	6.7e-005±5.15e-005	0.0001±0.0001	0.0002±0.0002	1.675e-005±1.675e-005	**0.003**	>0.9	>0.9	>0.9	>0.9
Bacteroides	0.11±0.03	0.16±0.05	0.18±0.04	0.17±0.05	0.31±0.05	0.28±0.03	>0.9	>0.9	>0.9	**0.001**	**0.007**
Alistipes	0.02±0.006	0.025±0.01	0.026±0.009	0.036±0.01	0.045±0.006916	0.058±0.008	>0.9	>0.9	>0.9	0.06	**0.004**
Parabacteroides	0.006±0.002	0.0058±0.002	0.009±0.003	0.016±0.008	0.014±0.002	0.018±0.004	>0.9	>0.9	>0.9	**0.04**	**0.03**
Clostridium sensu stricto	0.008±0.006	0.0008±0.0005	0.0004±0.0002	0.001±0.0007	0.0003±0.001	0.0002±0.002	**0.02**	**0.03**	0.2	**0.005**	**0.006**
Pseudomonas	0.0002±3.84e-005	0.0002±0.0002	8.375e-005±5.224e-005	0.0001±8.863e-005	0.0003±0.0002	0	>0.9	0.2	0.6	0.3	**0.002**

Actinobacteria	0.1±0.03	0.047±0.02	0.057±0.01	0.09±0.02	0.03±0.006	0.03±0.01	**0.03**	0.3	>0.9	**0.01**	**0.002**
Bifidobacterium	0.081±0.02	0.0079±0.002	0.014±0.005	0.039±0.02	0.018±0.004	0.01±0.003	**0.0007**	**0.004**	0.5	**0.03**	**0.0005**
Gardnerella	0.002±0.0006	2.233e-005±2.233e-005	0.0003±0.001	0.0004±0.002	0.0002±0.001	0.0002±0.001	**0.0002**	0.05	0.3	**0.004**	**0.003**
Proteobacteria	0.02±0.009	0.08±0.06	0.2±0.05	0.01±0.005	0.03±0.01	0.02±0.008	>0.9	**0.001**	>0.9	0.5	>0.9
Gammaproteobacteria	0.01±0.009	0.07±0.06	0.2±0.05	0.01±0.004	0.02±0.01	0.009±0.008	>0.9	**0.01**	>0.9	>0.9	>0.9
Escherichia-Shigella	0.004±0.002	0.07±0.06	0.2±0.05	0.008±0.004	0.02±0.01	0.008±0.008	>0.9	**0.01**	>0.9	>0.9	>0.9

Data are presented as the mean±SEM. Comparison: Kruskal-Wallis multiple comparisons test with Dunn's post test: ^{Pbaseline} Donors at the beginning of the study vs. patients on FMT day before fecal installation, ^{P1} Donors at the beginning of the study vs. patients at 1st control following FMT, ^{P2} Donors at the beginning of the study vs. patients at 2nd control following FMT, ^{P3} Donors at the beginning of the study vs. patients at 3rd control following FMT, ^{P4} Donors at the beginning of the study vs. patients at 4th control following FMT. FMT: fecal microbiota transplantation.

689

690 **Table 3:** Gut microbiota differences in the responders' group between donors at
691 baseline and IBS patients before (baseline) and at each control visit after fecal
692 microbiota transplantation.

Bacteria	**Donors (*n*=8)**	**Patients**									
		Baseline (n=6)	**Control visit 1 (n=7)**	**Control visit 2 (n=5)**	**Control visit 3 (n=8)**	**Control visit 4 (n=8)**	**P baseline**	**P 1**	**P 2**	**P 3**	**P 4**
Clostridium sensu stricto	0.010±0.009	0.0009±0.0008	0.00022±0.0002	0.00020±0.0001	0.00018±8e-005	0.00027±0.0002	**0.04**	**0.03**	0.2	**0.04**	**0.02**
Actinobacteria	0.1±0.04	0.06±0.03	0.06±0.02	0.08±0.02	0.03±0.007	0.03±0.009	**0.01**	**0.02**	0.7	0.08	**0.003**
Bifidobacterium	0.08±0.03	0.008±0.002	0.01±0.006	0.03±0.01	0.02±0.006	0.008±0.003	**0.01**	**0.01**	0.7	0.1	**0.003**
Proteobacteria	0.01±0.004	0.03±0.02	0.16±0.08	0.01±0.006	0.04±0.02	0.2±0.01	>0.9	**0.03**	>0.9	>0.9	>0.9

Data are presented as the mean±SEM. Comparison: Kruskal-Wallis multiple comparisons test with Dunn's post test: ^{Pbaseline} Donors at the beginning of the

study vs. patients on FMT day before fecal installation, ^{P1} Donors at the beginning of the study vs. patients at 1st control following FMT, ^{P2} Donors at the beginning of the study vs. patients at 2nd control following FMT, ^{P3} Donors at the beginning of the study vs. patients at 3rd control following FMT, ^{P4} Donors at the beginning of the study vs. patients at 4th control following FMT. FMT: fecal microbiota transplantation.

693

694 **Table 4:** Gut microbiota differences in the non-responders' group between donors at
695 baseline and IBS patients before (baseline) and at each control visit after fecal
696 microbiota transplantation.

Bacteria	**Donors (*n*=5)**	**Patients**									
		Baseline (n=3)	**Control visit 1 (n=5)**	**Control visit 2 (n=4)**	**Control visit 3 (n=5)**	**Control visit 4 (n=4)**	**P baseline**	**P 1**	**P 2**	**P 3**	**P 4**
Bacteroides	0.07±0.04	0.26±0.1	0.18±0.06	0.15±0.08	0.35±0.08	0.23±0.04	0.4	>0.9	>0.9	**0.02**	0.2
Alistipes	0.01±0.003	0.008±0.003	0.02±0.004	0.03±0.02	0.04±0.01	0.06±0.02	>0.9	>0.9	>0.9	0.3	**0.008**
Pseudomonas	0.0002±4.02 e-005	0	4.02 e-005±4.02 e-005	0.0003±0.0002	0	0	0.086	0.2	>0.9	**0.03**	**0.046**

Data are presented as the mean±SEM. Comparison: Kruskal-Wallis multiple comparisons test with Dunn's post test: ^{Pbaseline} Donors at the beginning of the study vs. patients on FMT day before fecal installation, ^{P1} Donors at the beginning of the study vs. patients at 1st control following FMT, ^{P2} Donors at the beginning of the study vs. patients at 2nd control following FMT, ^{P3} Donors at the beginning of the study vs. patients at 3rd control following FMT, ^{P4} Donors at the beginning of the study vs. patients at 4th control following FMT. FMT: fecal microbiota transplantation.

697

698

699 **Supplementary figures**

700 **Suppl. 1. The scores of IBS-SQ in the responders vs. non-responders' groups**
701 **one week before and daily for 20 days after fecal microbiota transplantation in**
702 **the different domains: a) nausea, b) bloating, c) abdominal pain, d)**
703 **constipation, e) diarrhea and f) anorexia/loss of appetite.**

704

Suppl. 2. Short form- Nepean dyspepsia index (SF-NDI) scores in responders and non-responders patients with irritable bowel syndrome before and after fecal microbiota transplantation.