

The efficacy of *Bifidobacterium bifidum* G9-1 in improving quality of life in patients with chronic constipation

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Research article

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Abstract

Background: Chronic constipation is a functional disorder that decreases patient's quality of life (QOL). Because dysbiosis has been associated with constipation, we aimed to investigate the efficacy of *Bifidobacterium bifidum* G9-1 (BBG9-1) in improving QOL in constipated patients.

Methods: This was a prospective, single-center, non-blinded, single-arm, feasibility trial. A total of 31 patients received BBG9-1 treatment for 8 weeks, followed by a 2-week washout period. The primary endpoint was the change in the overall Patient Assessment of Constipation of QOL (PAC-QOL) score relative to that at the baseline after probiotic administration. Secondary endpoints included changes in intestinal flora, stool consistency, frequency of bowel movement, degree of straining, sensation of incomplete evacuation, and frequency of rescue drug use.

Results: The overall PAC-QOL scores and frequency of bowel movement were significantly improved after BBG9-1 administration compared to those at baseline ($p < 0.05$, $p < 0.05$, respectively). There were no statistically significant changes in other clinical symptoms. Subset analysis revealed that patients with the initial Bristol Stool Form Scale of < 4 had improvements in stool consistency, a significant increase in the frequency of their bowel movements, and a significant alleviation in their degree of straining following BBG9-1 administration. *Sarcina* at the genus level and *Sarcina maxima* at the species level were significantly increased. Functional analysis showed that butanoate metabolism increased significantly, whereas methane metabolism decreased significantly.

Conclusions: BBG9-1 is safe and improves the QOL of constipated patients. The underlying improvements may be due to changes in stool consistency. Trial registration: UMIN 000029969. Registered 15 November 2017, <https://www.umin.ac.jp/>

Introduction

Chronic constipation is a common gastrointestinal disorder with a high prevalence (14%) in the general population, and its prevalence increases modestly with increasing age [1]. It is therefore expected that as society ages, the number of patients suffering from constipation will increase. Constipated patients often suffer from an impaired quality of life (QOL) [2]. Such an impaired QOL creates a large economic burden on society, reducing work productivity and increasing the frequency of visits to the hospital or emergency room [3]. Improvements in lifestyle, fiber supplements, and pharmacological therapies (osmotic and stimulant laxatives) are the currently recommended treatments to manage chronic constipation [4]. Despite this, nearly half of the constipated patients are not fully satisfied with their current treatment, mainly because of the lack of efficacy [5]. Since treatment of constipation may improve a patient's QOL [2], there is an ongoing need to improve strategies for treating constipation.

Numerous studies have reported that gastrointestinal microbiota play an important role in gastrointestinal function [6–8], and an association between dysbiosis and constipation has been noted.

Loading [MathJax]/jax/output/CommonHTML/fonts/TeX/fontdata.js } significantly decreased levels of

Bifidobacterium or Lactobacillus compared to those in healthy individuals [9]. Therefore, treatment with probiotics could be an effective therapy for chronic constipation.

Probiotics are living microorganisms that confer a health benefit on the host when administered in adequate dosage [10, 11]. Several previous randomized control trials have reported that probiotics improve stool frequency and stool consistency in chronically constipated patients [12–14]; however the results from these previous studies are equivocal and there is a lack of strong evidence for the effectiveness of probiotics. In this regard, the effects of probiotics have been reported to be species- and strain- specific [11]. With respect to Bifidobacterium, one of the major bacteria that occupy the intestinal flora, the efficacy of Bifidobacterium lactis [12, 15, 16] and Bifidobacterium breve [17] has been demonstrated in constipated patients. However, Bifidobacterium bifidum G9-1 (BBG9-1), one of the probiotics that has been used as an intestinal medicine for decades, has been shown to be effective for treating constipation, but only in animal studies [18, 19]. Furthermore, few studies are available to assess the QOL in patients with constipation, and the effect of probiotics on QOL is not fully understood.

Therefore, the aim of this feasibility study was to investigate the efficacy of BBG9-1 on QOL in patients with constipation.

Materials And Methods

Study design

This trial was a prospective, single-center, non-blinded, single-arm, feasibility trial using constipated patients who suffered from a decreased QOL. Patients were recruited from among gastroenterology outpatients at Yokohama City University Hospital from June 2017 to February 2019.

Participants

Eligibility criteria

Consecutive 20-79-year-old patients who were diagnosed with functional constipation according to the Rome IV criteria or who were already under treatment for chronic constipation were recruited for this study.

The Rome IV criteria for functional constipation are as follows [20]:

1. Must include two or more of the following:
 - a. Straining during more than one-fourth (25%) of defecations
 - b. Lumpy or hard stools (Bristol Stool Form Scale [BSFS] 1-2) more than one-fourth (25%) of defecations

- c. Sensation of incomplete evacuation more than one-fourth (25%) of defecations
 - d. Sensation of anorectal obstruction/blockage more than one-fourth (25%) of defecations
 - e. Manual maneuvers to facilitate more than one-fourth (25%) of defecations (eg, digital evacuation, support of the pelvic floor)
 - f. Fewer than three spontaneous bowel movements per week
2. Loose stools are rarely present without the use of laxatives
 3. Insufficient criteria for irritable bowel syndrome

These criteria should be fulfilled for the last 3 months, with symptom onset at least 6 months prior to diagnosis, and for research studies, patient meeting the criteria for opioid-induced constipation that is exacerbated or developed after opioid initiation, or after increasing opioid dosage or changing opioid therapy, should not be given a diagnosis of functional constipation.

The other proposed inclusion criteria for the study were as follows:

- 1) Aged 20 to 79 years as of the date of informed consent.
- 2) Willingness to provide written informed consent.

The proposed exclusion criteria were as follows:

- 1) Type 1 or 7 stool consistency scored by the BSFS.
- 2) Bowel movements less than once a week.
- 3) Presence of rectal anal dysfunction.
- 4) The overall Patient Assessment of Constipation of QOL (PAC-QOL) score of less than 1.
- 5) The presence of mechanical disorders confirmed by colonoscopy within 5 years before trial entry.
- 6) Concurrent serious cardiovascular, respiratory, renal, hepatic, gastrointestinal (excluding constipation), blood, or neurological diseases.
- 7) History or current evidence of celiac disease or inflammatory bowel disease.
- 8) Current treatment with steroids or biological products.
- 9) Current evidence of severe psychiatric diseases that could affect the evaluation of study drug efficacy.
- 10) History or current evidence of abuse of drugs or alcohol.
- 11) History of *Bifidobacterium* allergies.

- 13) Adjustment of medication within 4 weeks before entry.
- 14) Administration of drugs currently in development.
- 15) Participating or have participated in other clinical trials within 12 weeks before entry.
- 16) Administration of other probiotics.
- 17) Patients judged as being inappropriate candidates for the trial by the investigators.

Study Protocol

Patients who met the Rome IV criteria for functional constipation and/or who had already underwent constipation therapy were recruited. Patients were monitored for a 2-week baseline period during which data on their backgrounds, blood tests, and bowel habits were collected. Patients were also assessed for disease-specific QOL using the Japanese version of PAC-QOL [21, 22]. Patients satisfying all eligibility and exclusion criteria and who provided written informed consent were then enrolled in the trial. The enrolled patients received two tablets of BBG9-1 three times per day for 8 weeks, followed by a 2-week washout period (Fig. 1).

If the participants were already receiving any medication for constipation, these medications were allowed to be continued during the study period without any adjustment in dosing. No new medications for the treatment of constipation other than the study product were allowed during the study period. In the absence of defecation for several days, participants were allowed to take 48 mg of sennoside. During the 10-week study period, participants recorded their daily bowel movements as during the screening period. Records containing more than 5 days per week were required for the data to be considered valid. Fecal samples were collected before and 8 weeks after BBG9-1 administration for microbiota analysis. All subjects were instructed to visit the study site at 4, 8, and 10 weeks after therapy initiation. At each visit, patients completed the Japanese version of PAC-QOL and were interviewed about any side effects, and their defecation diaries were checked.

Outcome Measurements

Primary endpoint

The primary endpoint of this study was the change in the overall PAC-QOL score comparing before and 8 weeks after the administration of BBG9-1. Furthermore, the changes in the overall PAC-QOL scores comparing before and 4 weeks after the administration, and 8 weeks after administration and after a 2-week washout period were analyzed. The PAC-QOL is a reliable and specific self-administered questionnaire that has been developed and validated to assess QOL impairment in patients with chronic

Loading [MathJax]/jax/output/CommonHTML/fonts/TeX/fontdata.js 5-point Likert scale response (1: not at all, 2:

slightly, 3: moderately, 4: quite a bit, 5: extremely or a great deal). It is important to note that we used the Japanese version of PAC-QOL in this study [22].

The PAC-QOL measure also contains four subscales: physical discomfort, psychosocial discomfort, worries/concerns, satisfaction. The overall score and each subscale score are expressed as the average score of each item [21].

Secondary Endpoints

The secondary endpoints were changes in stool consistency, frequency of bowel movement, degree of straining, incomplete sensation of evacuation, frequency of rescue drug use, and alternation of intestinal flora following BBG9-1 intervention.

During the baseline and study period, patients were instructed to record their bowel habits every day. Stool consistency was scored using the BSFS. The incomplete sensation of evacuation was assessed on a binary scale (0, absent; 1, present), and the degree of straining was assessed on a 5-point ordinate scale (1, none; 2, mild; 3, moderate; 4, strong; 5, extremely strong). The mean scores of the baseline period, and those of last two weeks of 4 and 8 weeks of BBG9-1 administration periods, and those of the washout period were analyzed. Patients who had incomplete sense of evacuation in more than half of their defecation events were defined as having an incomplete sense of defecation. The frequency of bowel movements was defined as the number of days with at least one bowel movement.

Analysis Of Fecal Microbiota

To analyze intestinal flora, fecal samples were collected before and 8 weeks after BBG9-1 administration. DNA extraction was performed as previously described [23], and the resulting DNA was stored at -80°C until use. Analysis of the V3-V4 region of bacterial 16S rRNA was performed as previously described with minor modifications [24]. Briefly, the amplicons representing the V3-V4 region of 16S rRNA with unique indices incorporated by The Illumina Nextera XT Index kit (Illumina.K.,Japan) were purified using AMPure XP beads. The purified barcoded library was diluted to 4 nM using 10 mM Tris-HCl (pH 8.0), and then, the same volume was pooled for multiplex sequencing. The multiplexed library pool (10 pM) was spiked with 40% PhiX control DNA (10 pM) and was sequenced using a 2 × 250-bp paired-end run on a MiSeq platform using MiSeq Reagent Kit v2 chemistry (Illumina).

Sequence analysis was conducted using the 16S Metagenomics cloud application provided by Illumina, which calculates the number of reads and annotates sequences with GreenGenes. QIIME Preprocessing and QIIME Visualizations were used for linear discriminant analysis and to construct the dendrogram, respectively. Representative reads for each Operational Taxonomic Unit (OTU) were then assigned to the 16S rRNA gene database with $\geq 97\%$ identity. Beta diversity was estimated by computing the weighted UniFrac distance between samples, a phylogenetic tree-based metric [25]. Additionally, the predicted

functional composition of the gut microbiome was inferred for each stool sample using PICRUSt. Based on the fact that phylogeny and function are closely linked, this method accurately predicts the abundance of gene families from the 16S rRNA information [26]. A previous study has shown that the PICRUSt imputed and shotgun sequenced metagenomes have a very good correlation with an average Spearman's coefficient of around 0.8 [26]. Briefly, metagenome inference was performed with 16S rRNA gene sequences clustered at a 97% identity threshold using a closed reference of the GreenGenes (version 13.5) database. The resulting OTU table was then normalized using the 16S rRNA gene copy number, and the predicted gene family abundance was inferred for each sample.

Study Product

The study products were tablets of BBG9-1 (Biofermin® tablets). One tablet contained 12 mg (viable cell count: $1 \times 10^6 - 1 \times 10^9$ CFU/g) of *Bifidobacterium bifidum*. Participants were instructed to take two tablets of BBG9-1 after each meal every day. Compliance was monitored by counting the remaining drug tablets at the end of the 8-week drug administration. Participants with less than 80% compliance were excluded from the analysis.

Safety And Adverse Events Monitoring

Adverse events were monitored by a doctor at every follow-up visit to the study site. Adverse events were graded according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI-CTCAE), version 4.0. The protocol stated that if a Grade 3 or more severe adverse event occurred, the patient was to be immediately withdrawn from the study.

Statistical analysis

Efficacy analyses were performed by per protocol set. The per protocol set was defined as among the full analysis set, population who had serious deviations from the protocol. Safety analyses were performed for all patients who were administered at least one dose of the study product. Changes in the PAC-QOL overall scores, stool consistency, frequency of bowel movement, degree of straining, and frequency of rescue drug use were analyzed using a Student's paired t-test. Incomplete sensation of defecation and safety were compared using a chi-square test. A p value of < 0.05 was regarded as statistically significant.

Regarding the gut microbiota analysis, the Shannon Index, which accounts for both richness and evenness, was calculated using the basic count data to assess for alpha diversity in each group of patients, and a Student's t-test was used to assess intergroup differences. A weighted UniFrac distance was measured using data normalized by regularized logarithm transformation [27] and used for the principal coordinate analysis (PCoA). Samples were classified as pre- and post-treatment; compositional analysis of variance on the distance matrix. The

two groups of patients were then compared by the linear discriminant analysis (LDA) effect size (LEfSe) method, which emphasizes both statistical significance and biological relevance. The algorithm performs a nonparametric factorial Kruskal-Wallis sum-rank test and LDA to determine statistically significant different features among taxa and estimates the size effect of these differences [28]. Differences were considered significant at adjusted p values of < 0.05 and a logarithmic LDA score cutoff of ≥ 2 . Key bacterial taxa that emerged from the LEfSe analysis (adjusted P values < 0.05 and logarithmic LDA score cutoff ≥ 2) were displayed using the package qgraph. The analyses were performed using the R statistics program (version 3.4.0).

Trial steering committee and data monitoring committee

The Trial Steering Committee and Data Monitoring Committee were located at the Department of Clinical Research, Yokohama City University School of Medicine. The management team monitored the trial progress and data every month.

Results

A total of 34 patients were enrolled; 31 patients were analyzed in the study. An overview of the study design is shown in Fig. 1. A flow chart describing the patient selection and exclusion is shown in Fig. 2. Demographic data for patients who were analyzed are shown in Table 1.

Table 1
Demographic data of analyzing patients, Mean \pm SD

Variables (Mean \pm SD)	0w	4w	8w	10w
	N=31			
Age (years)	63.7 \pm 11.8			
Gender (M/F)	11/20			
History of gastrointestinal operation, n (%)	4(14.3%)			
Combination use of laxative				
none, n	4			
Osmotic laxative, n	18			
Stimulant laxative, n	11			
lubiprostone/Linaclotide, n	7			
Kampo medicine, n	8			
Over-the-counter laxative, n	3			
Over 2 kinds of laxative, n	17			
Mean frequency of bowel movements (2 weeks)	10.2 \pm 3.42	10.9 \pm 3.07	11.3 \pm 2.77**	11.1 \pm 2.58
Mean stool consistency (Bristol stool form scale) (2 weeks)	3.82 \pm 1.25	3.55 \pm 1.22	3.98 \pm 1.25	3.8 \pm 1.16
Mean extent of straining (2 weeks)	2.96 \pm 0.78	2.98 \pm 0.97	2.79 \pm 0.97	2.85 \pm 0.92
Mean overall PAC-QOL score	1.73 \pm 0.54	1.07 \pm 0.63**	0.97 \pm 0.65**	1.1 \pm 0.68
Mean frequency of using rescue laxative (2 weeks)	1.0 \pm 2.1	0.81 \pm 2.2	0.88 \pm 2.6	0.84 \pm 2.7
The mean proportion of patients who have sense of incomplete evacuation	71(54.0-87.9)	58.1(39.7-76.5)	67.7(50.3-85.2)	61.3(43.1-79.5)
*p < 0.05, **p < 0.01, versus baseline (0w)				

Qol Assessment

The mean overall PAC-QOL score at baseline was 1.73 ± 0.54 , whereas the PAC-QOL scores after 4 and 8 weeks of BBG9-1 administration, and after a 2-week washout period, were 1.07 ± 0.63 , 0.97 ± 0.65 and 1.1 ± 0.68 , respectively. The PAC-QOL score for all patients significantly improved after 4 and 8 weeks of BBG9-1 administration (Fig. 3a. $p < 0.05$, $p < 0.05$, respectively). Furthermore, there was no significant difference between the score after 8 weeks of administration and that after the 2-week washout period. Participants in this study showed an equivalent QOL after discontinuance of the probiotic for 2 weeks.

All the subscales score in the PAC-QOL (physical discomfort, worries/concerns, psychosocial discomfort, and satisfaction) were significantly decreased after 4 and 8 weeks of BBG9-1 administration ($p < 0.05$, $p < 0.05$, $p < 0.05$, $p < 0.05$ respectively) (Fig. 3b-3e).

Stool Consistency Assessment

The mean BSFS score at baseline was 3.82 ± 1.25 , whereas the BSFS scores after 4 and 8 weeks of BBG9-1 administration and after a 2-week washout period were 3.55 ± 1.22 , 3.98 ± 1.25 , and 3.8 ± 1.16 , respectively (Fig. 4a). These scores were not significantly different from baselines.

We conducted a post hoc analysis of the BSFS scores based on the scores pre-intervention. For patients who showed had the BSFS score of 4 or more at the start of the study, the mean BSFS score at baseline was 4.71 ± 0.54 , whereas the BSFS scores after 4 and 8 weeks of BBG9-1 administration and after a 2-week washout period were 4.35 ± 0.74 , 4.5 ± 0.7 , and 4.33 ± 0.9 , respectively. The BSFS score at 4 weeks of BBG9-1 administration was significantly different from baseline (Fig. 4b, $p = 0.03$). For patients who had the BSFS score of less than 4 at the start of the study, the mean score at baseline was 2.59 ± 0.83 , and the BSFS scores after 4 and 8 weeks of BBG9-1 administration and after a 2-week washout period were 2.43 ± 0.78 , 3.27 ± 0.82 , and 3.07 ± 1.1 , respectively. The BSFS score significantly increased after 8 weeks of BBG9-1 administration (Fig. 4c, $p = 0.03$).

Frequency Of Bowel Movement

The mean overall frequency of bowel movements at baseline was 10.2 ± 3.42 , whereas the frequency of bowel movements after 4 and 8 weeks of BBG9-1 administration and after a 2-week washout period were 10.9 ± 3.07 , 11.3 ± 2.77 , and 11.1 ± 2.58 , respectively. The frequency of bowel movements was significantly increased after 8 weeks of BBG9-1 administration compared to baseline (Fig. 4d, $p < 0.01$).

For patients who had the BSFS score of 4 or more at the start of the study, the mean overall frequency of bowel movements at baseline was 10.5 ± 3.75 , whereas the frequency of bowel movements after 4 and 8 weeks of BBG9-1 administration and after a 2-week washout period were 10.9 ± 3.24 , 11.1 ± 3.75 , and 11.4 ± 3.02 , respectively. None of these were significantly different compared to baseline (Fig. 4h). For patients that had the BSFS score less than 4 at the start of the study, the mean frequency of bowel movements at baseline was 9.9 ± 3.02 whereas the frequency of bowel movements after 4 and 8 weeks

of BBG9-1 administration and after a 2-week washout period were 10.8 ± 2.95 , 11.5 ± 2.47 , and 10.6 ± 2.18 , respectively. The frequency of bowel movements significantly increased after 8 weeks of BBG9-1 administration compared to baseline (Fig. 4f, $p = 0.04$).

Degree Of Straining Assessment

The mean degree of straining at baseline was 2.96 ± 0.78 , whereas the degree of straining after 4 and 8 weeks of BBG9-1 administration and after a 2-week washout period were 2.98 ± 0.97 , 2.79 ± 0.97 , and 2.85 ± 0.92 , respectively (Fig. 4g). These values were not significantly different from the baseline. For patients that had the BSFS score of more than 4 at the start of the study, the mean degree of straining at baseline was 2.75 ± 0.73 , whereas the mean degrees of straining after 4 and 8 weeks of BBG9-1 administration and after a 2-week washout period were 2.79 ± 0.78 , 2.75 ± 1.03 , and 2.84 ± 0.89 , respectively. These values were not significantly different than baseline (Fig. 4h). However, for patients who had the BSFS score of less than 4 at the start of the study, the mean degree of straining at baseline was 3.25 ± 0.79 , whereas the mean degrees of straining after 4 and 8 weeks of BBG9-1 administration and after a 2-week washout period were 3.25 ± 1.16 , 2.8 ± 0.9 , and 2.87 ± 0.99 , respectively. The degree of straining was significantly alleviated after 8 weeks of administration (Fig. 4i, $p = 0.03$).

Sense Of Incomplete Evacuation Assessment

The mean proportion of patients who had sense of incomplete evacuation was 71% (95% CI, 54.0–87.9), whereas the mean proportion of patients who had a sense of incomplete evacuation after 4 and 8 weeks of BBG9-1 administration and after a 2-week washout period were 58.1% (95% CI, 39.7–76.5), 67.7% (95% CI, 50.3–85.2), and 61.3% (95% CI, 43.1–79.5), respectively (Table 1). These were not significantly different than at baseline.

Frequency Of Rescue Drug Use Assessment

The mean frequency of rescue drug use at baseline was 1.0 ± 2.1 , whereas the frequencies of rescue drug use after 4 and 8 weeks of BBG9-1 administration and after a 2-week washout period were 0.81 ± 2.2 , 0.88 ± 2.6 , and 0.84 ± 2.7 , respectively (Table 1). These were not significantly different than at baseline.

In summary, the overall and all the subscales scores in the PAC-QOL significantly improved after BBG9-1 administration. For all patients, there were no significant differences observed comparing before and after BBG9-1 intervention in terms of the BSFS score, degree of straining, sense of incomplete evacuation, and frequency of rescue drug use (Table 1), whereas the frequency of bowel movements was significantly increased after BBG9-1 intervention.

Subset analysis revealed that patients with the initial BSFS score of < 4 had tended to improve to have

Loading [MathJax]/jax/output/CommonHTML/fonts/TeX/fontdata.js bowel movements, and a significant alleviation

in their degree of straining following BBG9-1 administration. Furthermore, patients with the initial BSFS score ≥ 4 tended to improve to have harder stool following BBG9-1 administration.

Analysis of fecal microbiota

For fecal microbiota, there were no significant changes in the Shannon index (evenness) and OTU observed following BBG9-1 treatment (Fig. 5a and 5b). However, chao1 (richness) was significantly increased (Fig. 5c). At the phylum level, Nitrospora were significantly increased after Bifidobacterium treatment. At the genus level, Sarcina, Johnsonella, Thermodesulfovibrio, Lentibacillus, Yaniella, Marinitoga, Arcanobacterium, Phyllobacterium, Kineosporia, Pseudidiomarina, Ectothiorhodospira, Sphaerisporangium, Rhodobacter, Anaeromusa, and Halanaerobacter were significantly increased, and Neisseria, Leptotrichia, Pasteurella, Abiotrophia, and Achromobacter were significantly decreased after BBG9-1 treatment (Table 2). Additionally, at the species level, Sarcina maxima was significantly increased (Table 3). In the LefSe analysis, the absolute value of the LDA score was 2 or more for Phyllobacteriaceae, Aerococcaceae, Neisseria, Aggregatibacter, Alphaproteobacteria, Haemophilus, and Pasteurellales (Fig. 5e). The cladogram is shown in Fig. 5f. The functional potential of the bacterial assemblies associated with each stool sample was predicted with PICRUSt using level 3 of the Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologs. As assessed with LefSe at a P value < 0.05 , the gut microbiome post-treatment was significantly enriched in twenty-six functional categories, compared to pre-treatment. These enriched functional categories (Table 4) were related to carbohydrate metabolism (e.g., propanoate metabolism, butanoate metabolism, starch and sucrose metabolism, and galactose metabolism), amino acid metabolism (e.g., phenylalanine, tyrosine, and tryptophan biosynthesis, tyrosine metabolism, valine, leucine, and isoleucine biosynthesis) and metabolism of cofactors, energy metabolism (methane metabolism) and vitamins (e.g., pantothenate and CoA biosynthesis, retinol metabolism, and lipoic acid metabolism). Surprisingly, propanoate and butanoate metabolism were significantly increased and methane metabolism significantly decreased after BBG9-1 administration.

Table 2
Change of gut-microbiota abundance between pre- and post-Bifidobacterium administration in Genus levels

Genus	Pre (%)	Post (%)	p Value	
Sarcina	0.042	0.14	0.046	*
Neisseria	0.028	0.015	0.002	**
Johnsonella	0.058	0.071	0.036	*
Thermodesulfovibrio	0.013	0.019	0.013	*
Leptotrichia	0.0029	0.0017	0.035	*
Lentibacillus	0.0067	0.0089	0.047	*
Yaniella	0.0045	0.0066	0.014	*
Marinitoga	0.0041	0.0058	0.033	*
Arcanobacterium	0.0020	0.0033	0.027	*
Phyllobacterium	0.00064	0.0022	0.009	**
Kineosporia	0.0020	0.0030	0.037	*
Pasteurella	0.0016	0.00033	0.003	**
Pseudidiomarina	0.00042	0.0010	0.032	*
Abiotrophia	0.0014	0.000044	0.029	*
Ectothiorhodospira	0.00021	0.00070	0.043	*
Sphaerisporangium	0.00013	0.00057	0.012	*
Rhodobacter	0.00017	0.00059	0.035	*
Achromobacter	0.00033	0	0.012	*
Anaeromusa	0	0.00020	0.044	*
Halanaerobacter	0	0.00020	0.046	*
*p < 0.05, **p < 0.01, versus baseline (0w)				

Table 3
Change of gut-microbiota abundance between pre- and post- Bifidobacterium
administration in Species levels

Species	Pre (%)	Post (%)	p Value
Anaeromusa acidaminophila	0	0.00042448	0.043 *
Bacteroides rodentium	0.57	0.31	0.020 *
Bacteroides uniformis	1.5	0.92	0.044 *
Bifidobacterium ruminantium	0.008	0.013	0.020 *
Blautia hydrogenotrophica	0.12	0.24	0.024 *
Campylobacter faecalis	0	0.00042	0.043 *
Cohnella laeviribosi	0.0025	0.0049	0.027 *
Ectothiorhodospira imhoffii	0.00042	0.00127	0.030 *
Euzebya tangerina	0.0072	0.0099	0.031 *
Ferrimicrobium acidiphilum	0.00032	0.00117	0.043 *
Fructobacillus pseudoficulneus	0.0031	0.0060	0.044 *
Fusobacterium nucleatum	0.0023	0.00032	0.012 *
Fusobacterium periodonticum	0.0129	0.0041	0.045 *
Halanaerobacter chitinivorans	0	0.00042	0.043 *
Johnsonella ignava	0.11	0.13	0.044 *
Kineosporia mikuniensis	0.0035	0.0056	0.028 *
Lactobacillus pobuzihii	0.00042	0.00000	0.043 *
Leuconostoc carnosum	0	0.0011	0.048 *
Marinitoga okinawensis	0.00021	0.0011	0.018 *
Mycobacterium lepromatosis	0.00053	0.00011	0.043 *
Neisseria lactamica	0.0011	0.00011	0.048 *

Species	Pre (%)	Post (%)	p Value
<i>Neisseria mucosa</i>	0.041	0.027	0.001 **
<i>Nocardia devorans</i>	0.00042	0	0.043 *
<i>Paenibacillus filicis</i>	0.00011	0.00053	0.043 *
<i>Pasteurella pneumotropica</i>	0.0024	0.00064	0.007
<i>Porphyromonas canis</i>	0.0087	0.014	0.038 *
<i>Prevotella enoeca</i>	0.00042	0	0.043 *
<i>Prevotella veroralis</i>	0.00084	0.0030	0.001 **
<i>Sarcina maxima</i>	0.080	0.26	0.046 *
<i>Sphaerisporangium rubeum</i>	0.00021	0.0011	0.009 **
<i>Streptococcus salivarius</i>	0.00011	0.0014	0.021 *
<i>Streptomyces roseogilvus</i>	0.00021	0.0011	0.043 *
<i>Syntrophomonas bryantii</i>	0	0.00042	0.043 *
<i>Tepidanaerobacter syntrophicus</i>	0.011	0.016	0.049 *
<i>Thermodesulfovibrio thiophilus</i>	0.026	0.035	0.040 *
<i>Veillonella denticariosi</i>	0.014	0.006	0.009 **
<i>Vibrio porteresiae</i>	0.0016	0.0040	0.037 *
*p < 0.05, **p < 0.01, versus baseline (0w)			

Table 4

Functional profile of the gut-microbiota from pre- or post- treatment by Bifidobacterium

Level 1	Level 2	Level 3	Pre (N = 30)	Post (N = 30)	P value	
Metabolism	Metabolism of Terpenoids and Polyketides	Limonene and pinene degradation	0.080	0.088	0.005	**
Metabolism	Carbohydrate Metabolism	Propanoate metabolism	0.509	0.523	0.007	**
Metabolism	Amino Acid Metabolism	Phenylalanine, tyrosine and tryptophan biosynthesis	0.889	0.866	0.013	**
Metabolism	Carbohydrate Metabolism	Butanoate metabolism	0.583	0.607	0.013	*
Metabolism	Carbohydrate Metabolism	Starch and sucrose metabolism	1.139	1.114	0.014	*
Metabolism	Metabolism of Cofactors and Vitamins	Pantothenate and CoA biosynthesis	0.644	0.632	0.018	*
Metabolism	Xenobiotics Biodegradation and Metabolism	Drug metabolism - cytochrome P450	0.036	0.041	0.024	*
Metabolism	Energy Metabolism	Methane metabolism	1.413	1.375	0.027	*
Metabolism	Xenobiotics Biodegradation and Metabolism	Benzoate degradation	0.209	0.226	0.028	*
Metabolism	Xenobiotics Biodegradation and Metabolism	Metabolism of xenobiotics by cytochrome P450	0.034	0.040	0.028	*
Metabolism	Amino Acid Metabolism	Tyrosine metabolism	0.352	0.363	0.028	*
Metabolism	Metabolism of Cofactors and Vitamins	Retinol metabolism	0.032	0.035	0.032	*
Metabolism	Metabolism of Terpenoids and Polyketides	Biosynthesis of vancomycin group antibiotics	0.061	0.058	0.033	*

Level 1	Level 2	Level 3	Pre (N = 30)	Post (N = 30)	P value	
Human Diseases	Infectious Diseases	Epithelial cell signaling in Helicobacter pylori infection	0.098	0.095	0.037	*
Organismal Systems	Nervous System	Glutamatergic synapse	0.110	0.107	0.037	*
Metabolism	Xenobiotics Biodegradation and Metabolism	Aminobenzoate degradation	0.103	0.112	0.040	*
Metabolism	Carbohydrate Metabolism	Galactose metabolism	0.860	0.842	0.042	*
Unclassified	Metabolism	Nucleotide metabolism	0.037	0.046	0.042	*
Human Diseases	Neurodegenerative Diseases	Amyotrophic lateral sclerosis (ALS)	0.008	0.011	0.045	*
Metabolism	Metabolism of Terpenoids and Polyketides	Polyketide sugar unit biosynthesis	0.214	0.206	0.045	*
Metabolism	Lipid Metabolism	Synthesis and degradation of ketone bodies	0.021	0.024	0.045	*
Metabolism	Metabolism of Cofactors and Vitamins	Lipoic acid metabolism	0.021	0.024	0.047	*
Metabolism	Xenobiotics Biodegradation and Metabolism	Dioxin degradation	0.079	0.084	0.047	*
Human Diseases	Neurodegenerative Diseases	Huntington's disease	0.016	0.019	0.049	*
Metabolism	Xenobiotics Biodegradation and Metabolism	Caprolactam degradation	0.021	0.029	0.050	*
Metabolism	Amino Acid Metabolism	Valine, leucine and isoleucine biosynthesis	0.831	0.816	0.0498	*

*p < 0.05, **p < 0.01, versus baseline (0w)

All participants showed over 80% medication compliance. No adverse events were observed during the study period.

Discussion

This is the first study to assess the efficacy of BBG9-1 for QOL in constipated patients. The overall PAC-QOL score and all subscale scores of the PAC-QOL were improved, and an equivalent QOL score was observed 2 weeks after discontinuance of BBG9-1. With respect to constipation-related clinical symptoms, the frequency of bowel movements was significantly increased after 8 weeks and 2 weeks after discontinuance. A previous meta-analysis has reported that probiotics significantly reduce whole gut transit time, increase stool frequency, and improve stool consistency in patients with functional constipation [29]. Therefore, our results on the frequency of bowel movements are consistent with previous reports.

On the other hand, for all patients, stool consistency, degree of straining, sense of incomplete evacuation, frequency of rescue drug use, were not significantly different following BBG9-1 treatment. However, when the degree of stool consistency at the start of the study is taken into account, there is a hint that BBG9-1 is effective.

The mean BSFS score of all patients at baseline was 3.82 ± 1.25 . This was probably because most of the patients were already under some form of treatment at the registration of this study. This is clear reflection of the fact that this study targeted constipated patients with a low QOL. We consider this to be the reason that no statistical differences in BSFS score were found following BBG9-1 treatment. However, when we divided the enrolled patients into two groups according to their initial stool consistency, the stool consistency of patients with soft stools having the BSFS score of 4 or more improved to have harder stools, whereas patients with hard stools having the BSFS of 4 or less tended to improve to have softer stools following BBG9-1 administration. Previously, a stool consistency with the BSFS score of 4 has been showed to contribute to an improved QOL in constipated patients [30]. BBG9-1 may there have the potential to change stool consistency to around the BSFS score of 4, and this may contribute to improving QOL.

With respect to degree of straining, patients with hard stools with the BSFS score under 4 tended find alleviation of symptoms following BBG9-1 administration.

In summary, BBG9-1 may help normalize stool consistency, and in particular may contribute to improving stool frequency and straining in patients with hard stools (i.e. those with the BSFS score less than 4).

With respect to fecal microbiota, there were no significant changes in Bifidobacterium following BBG9-1 administration. However, at the genus levels, Sarcina was significantly increased, and at the species level, Sarcina maxima, which is known to be a butyric acid-producing bacterium, was significantly increased (Table 3) and a functional analysis showed supporting data on butyric acid production (Table 4).

Furthermore, *Bacteroides uniformis* and *Bacteroides rodentium* were both significantly decreased following BBG9-1 administration.

Butyric and propionic acid are two types of short-chain fatty acids (SCFAs), and are known to be reduced in patients with constipation [31]. Previous studies have reported that SCFAs can affect gut motility and reduce gut transit time [31]. One of the most popular theories on the mechanism of action of probiotics in constipation is that probiotics increase the production of lactate as well as SCFA levels, and thereby enhance colonic peristalsis [31]. SCFA production by *Sarcina maxima* may contribute to some of the positive effects on QOL in constipated patients. Furthermore, *Bacteroides* has been reported to be increased in constipated patients. The decrease in *Bacteroides* may also be related to the improvement of QOL in constipated patients.

To further explore these hypotheses linking SCFA to *Bifidobacterium* treatment, we used PICRUST to assess the metagenomic profile of the gut microbiota in our patients [26]. Interestingly, this functional approach showed that *Bifidobacterium* treatment was associated with significant shifts in metabolic function in the gut microbiota, mainly impacting the KEGG pathways that relate to metabolism of carbohydrates, especially propanoate and butanoate metabolism. Surprisingly, a decrease in methane metabolism was observed after BBG9-1 administration. Previous studies have been reported that increases in methane /methane-producing bacteria in the colon inhibits the colonic transit time [32–35]. These results provide exciting, new insights about the potential roles of gut microbiota in *Bifidobacterium* treatment. However, they must be confirmed by further “classical” metagenomics studies to precisely identify which metabolic pathways of the gut microbiota are associated with *Bifidobacterium* treatment.

Although intriguing, this study has several limitations. First, a placebo effect was not evaluated because this was a non-blinded, single-arm trial. Second, this was a single-center study at a university hospital, which make it difficult to generalize our conclusions beyond the population we studied. Third, the sample size was also too small to generalize our conclusions. Fourth, most of the patients enrolled in this study had already taken some medication for their constipation. Therefore, stool frequency or other clinical symptoms caused by constipation were likely already moderately controlled. However, the discontinuation of current medications is not ethical, meaning that had to add the probiotic in addition to current medications.

In conclusion, in this study, probiotics containing BBG9-1 were found to be safe and improved the QOL of constipated patients. BBG9-1 may be an effective treatment option for chronic constipation. The mechanism of the improvement in QOL remains to be explored. To confirm these data, a placebo-controlled, double-blinded randomized controlled trial should be conducted.

Abbreviations

BBG9-1

Bifidobacterium bifidum G9-1

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Bristol Stool Form Scale
KEGG
Kyoto Encyclopedia of Genes and Genomes
LDA
linear discriminant analysis
LEfSe
linear discriminant analysis effect size
NCI-CTCAE
National Cancer Institute Common Toxicity Criteria for Adverse Events
OTU
Operational Taxonomic Unit
PAC-QOL
Patient Assessment of Constipation of quality of life
PCoA
principal coordinate analysis
QOL
Quality of life
SCFAs
short-chain fatty acids
UMIN
University Hospital Medical information Network

Declarations

Ethics approval and consent to participate

The study protocol was in compliance with the Declaration of Helsinki [36] and the Ethics Guidelines for Clinical Research published by the Ministry of Health, Labor, and Welfare, Japan. We obtained approval for this study from the Ethics committee of Yokohama City University Hospital in June 2017 (B170601003). This trial has been registered in the University Hospital Medial Information Network (UMIN) Clinical Trials Registry as UMIN 000029969. Written informed consent for participation in the study was obtained from all the participating patients.

Consent for publication

Not applicable

Availability of data and materials

The data generated and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

AN received research funding from Biofermin Pharmaceutical Co., Ltd.

Funding

This trial was sponsored by Biofermin Pharmaceutical Co., Ltd.

Authors` contributions

AF, TH, and AN conceived the study. AF and TH conducted the study. TK, HO, AK, YT, and NM recruited the patients. KW and HU analyzed the fecal microbiome. AF, TK, and MI analyzed the data and AF drafted the initial manuscript. TH was responsible for the revision of the manuscript. AN supervised the study. All authors have read and approved the final manuscript.

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Figures

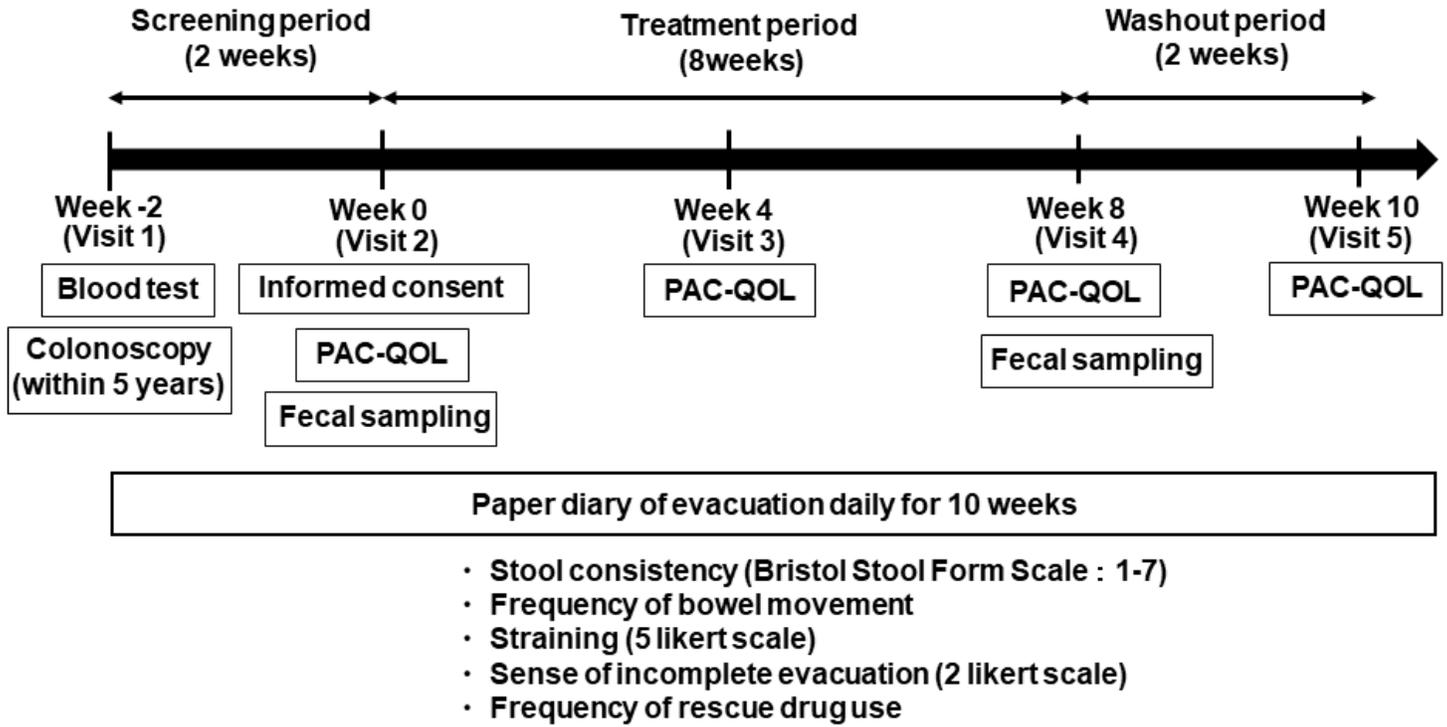


Figure 1.

Figure 2

Study design

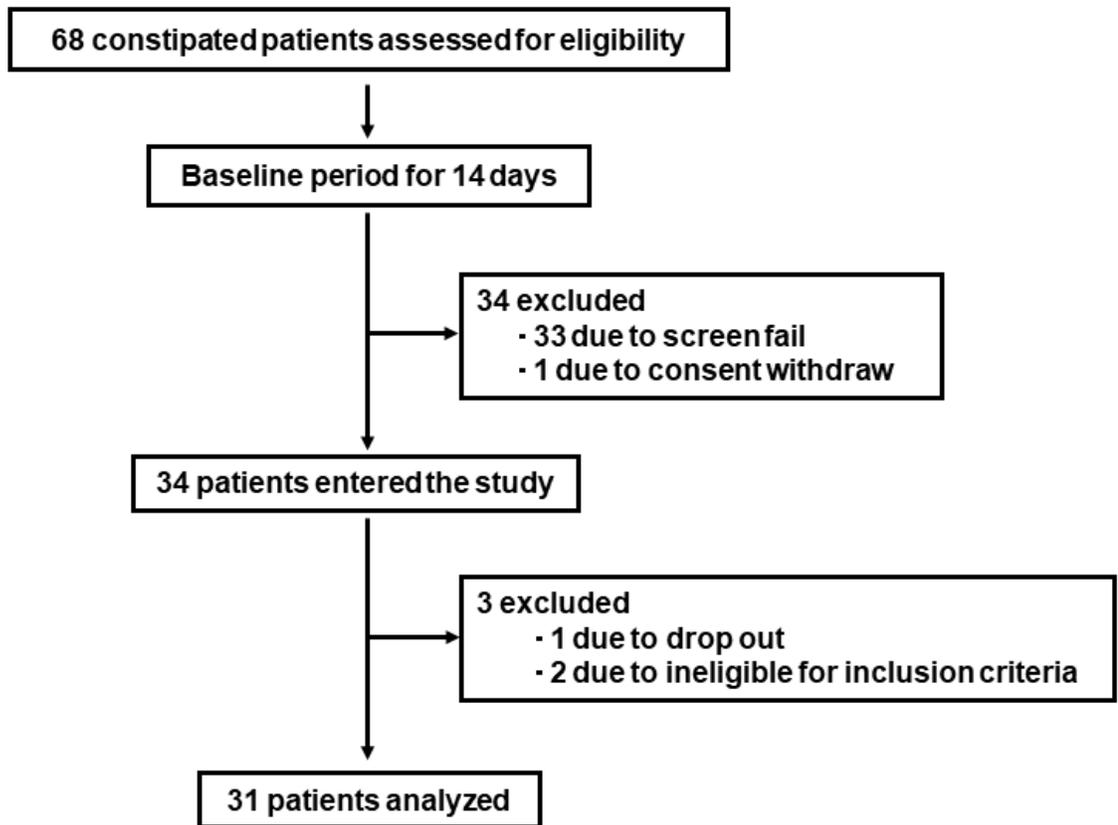


Figure 2.

Figure 4

Patient exclusion/inclusion flow chart

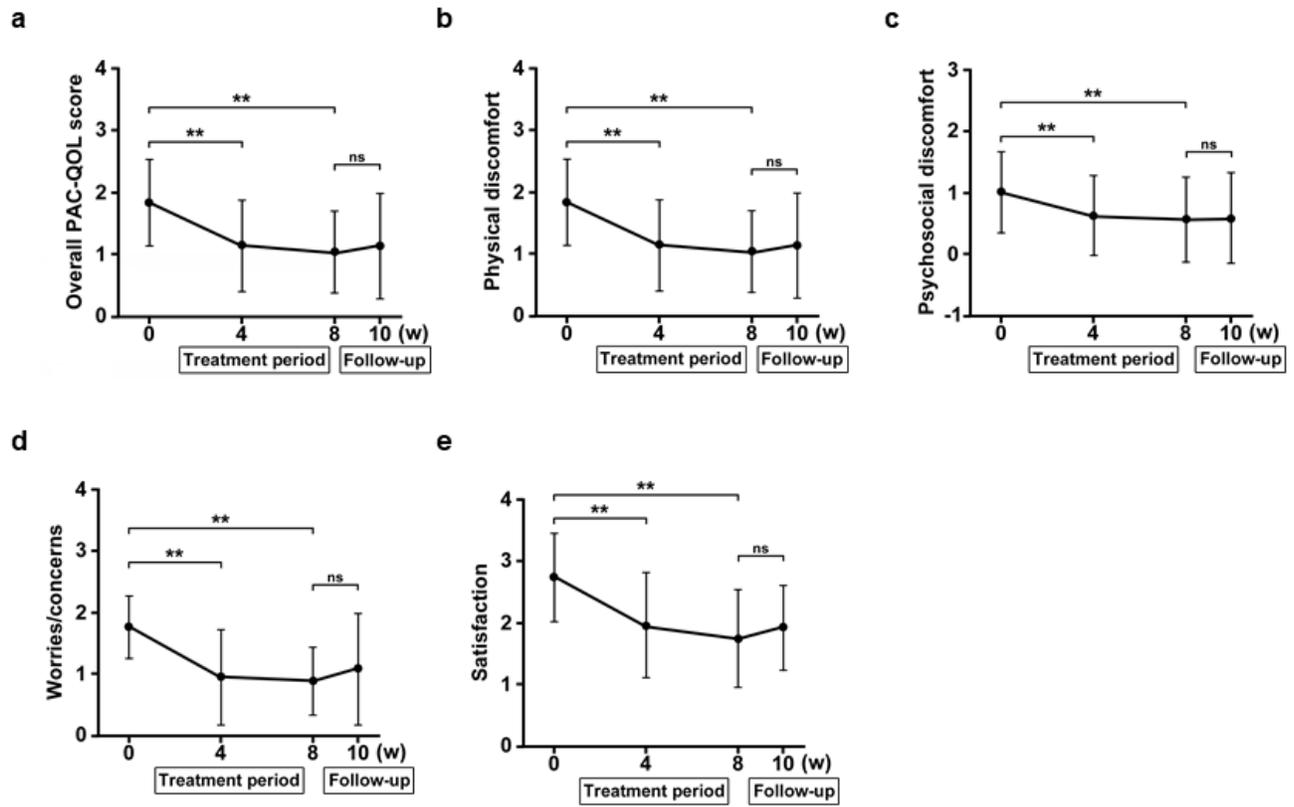


Figure 3

Figure 6

(a) The overall PAC-QOL scores. (b-e) subscales of the PAC-QOL. (b) physical discomfort, (c) psychosocial discomfort, (d) worries/concerns, and (e) satisfaction. Statistical differences were evaluated using the Student's paired t-test. * $p < 0.05$, ** $p < 0.01$, versus baseline (0 w).

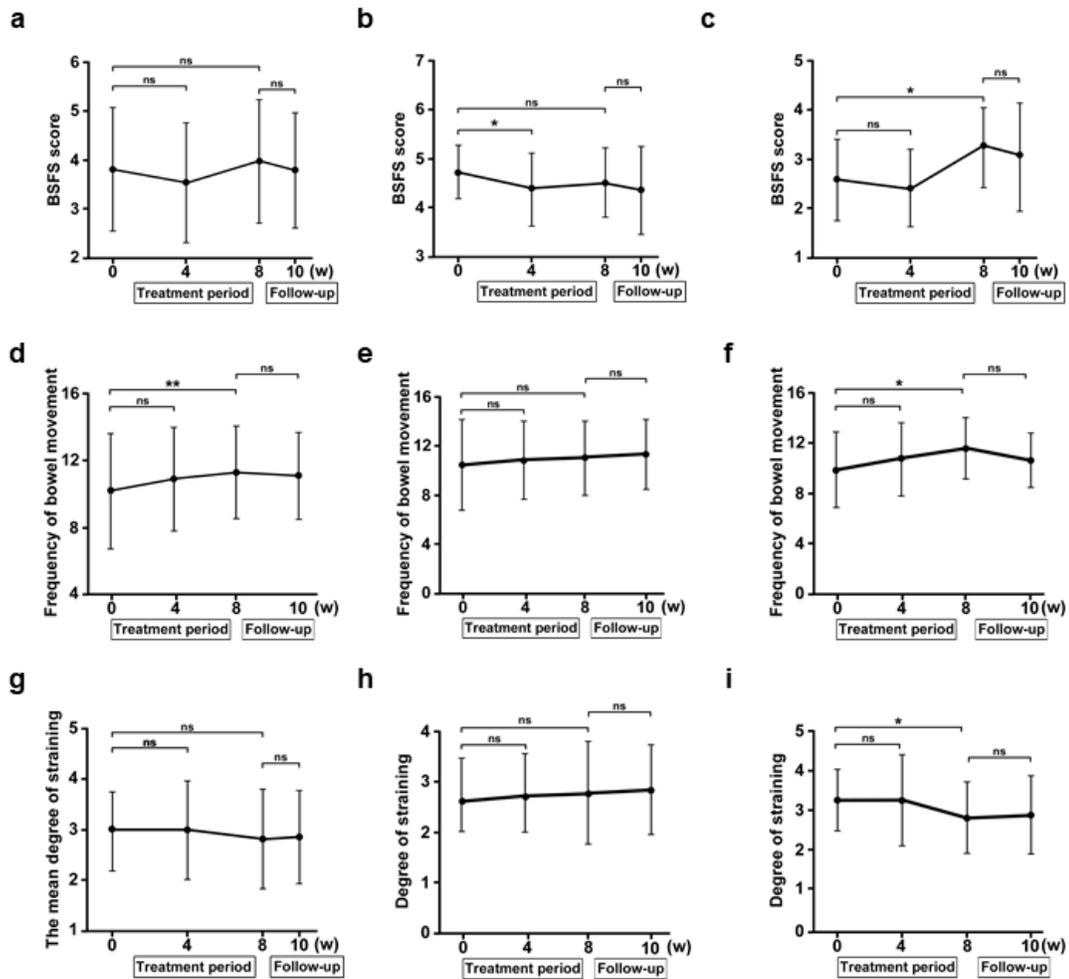


Figure 4.

Figure 8

a-c, Mean BSFS scores (a; all patients, b; subset of patients with the BSFS score of 4 or more, c; subset of patients with the BSFS score of less than 4). d-f, Mean frequency of bowel movements (d; all patients, e; subset of patients with the BSFS of 4 or more, f; subset of patients with the BSFS of less than 4). g-i, Mean degree of straining (g; all patients, h; subset of patients with the BSFS of 4 or more, i; subset of patients with the BSFS of less than 4). Statistical differences were evaluated using the Student's paired t-test. *p < 0.05, **p < 0.01, versus baseline (0 w).

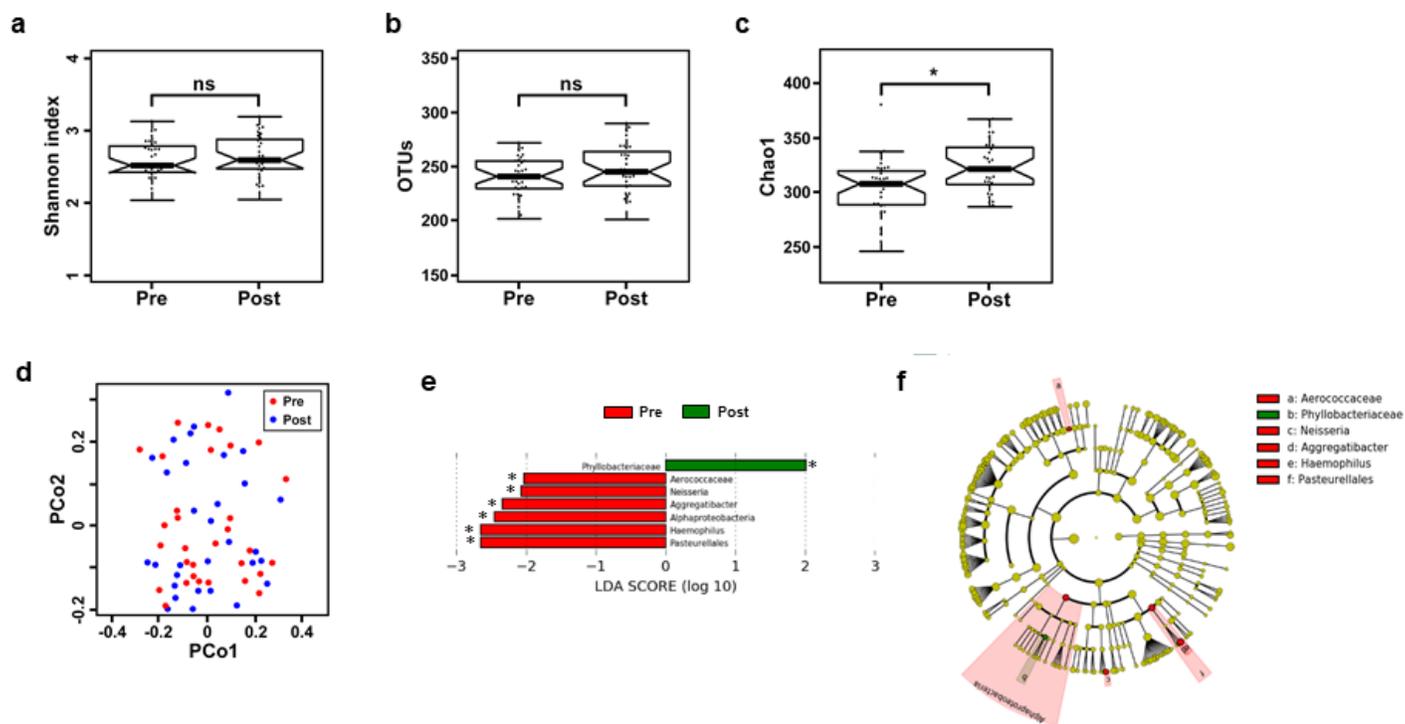


Figure 5

Figure 10

Changes in microbial diversity following *Bifidobacterium* treatment measured by (a) the Shannon index, (b) OTU, (c) chao1. The whiskers denote the lowest and highest values within 1.5 IQR from the first and third quartiles. The circles represent outliers beyond the whiskers. The notches show the 95% confidence interval for the medians. The analysis was performed using the paired Student's t-test. ns; not significant. (d) PCoA ordination plot based on weighted UniFrac distance matrix. Each subject is represented by a dot. Pre-treatment values are represented in red and post-treatment values in blue. (e) LefSe analysis of whole patients population both pre-and post-treatment with *Bifidobacterium*. *Features with an LDA score > 2. (f) Cladogram displaying the taxonomic tree of differentially abundant taxa. The histogram represents the LDA scores of bacteria with significant differential abundance between the compared groups, identified by different colors.