ARTIFICIAL INTELLIGENCE-BASED PERSONALIZED DIET: A PILOT CLINICAL STUDY FOR IBS

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ABSTRACT

Background and aims: Certain diets are often used to manage functional gastrointestinal symptoms in irritable bowel syndrome (IBS) patients. Personalized diet-induced microbiome modulation is being preferred method for symptom improvement in IBS. Although personalized nutritional therapies targeting gut microbiota using artificial intelligence (AI) promise great potential, this approach has not been studied in patients with IBS. Therefore, in this study, we investigated the efficacy of an AI-based personalized microbiome diet in patients with IBS-Mix (M).

Methods: This study was designed as a pilot, open-labeled study. We enrolled consecutive IBS-M patients (n=25, 19 females, 46.06 ± 13.11 years) according to Rome IV criteria. Fecal samples were obtained from all patients twice (pre- and post-intervention), and high; throughput, 16S rRNA sequencing was performed. Patients were divided into two groups based on age, gender, and microbiome matched. Six weeks of AI-based microbiome diet (n=14) for Group 1 and standard IBS diet (Control Group, n=11) for Group 2 were followed. AI-based diet was designed based on optimizing a personalized nutritional strategy by an algorithm regarding individual gut microbiome features. An algorithm assessing an IBS index score using microbiome composition attempted to design the optimized diets based on modulating the microbiome towards the healthy scores. Baseline and post-intervention IBS-SSS (symptom severity scale) scores and fecal microbiome analyses were compared.

Results: The IBS-SSS evaluation for pre- and post-intervention exhibited significant improvement ($p<0.02$ and $p<0.001$ for the control and intervention groups, respectively). While the IBS-SSS evaluation changed to moderate from severe in 82% (14 out of 17) of the intervention group, no such change was observed in the control group. After six weeks of intervention, a significant shift in microbiota profiles in terms of alfa- or beta-diversity was not observed in both groups. A trend of decrease in the Ruminococcaceae family for the intervention group was observed ($p=0.17$). A statistically significant increase in the Faecalibacterium genus was observed in the intervention group ($p = 0.04$). Bacteroides and putatively probiotic genus Propionibacterium were increased in the intervention group; however, Prevotella was increased in the control group. The change (delta) values in IBS-SSS scores (before-after) intervention and control groups were significantly higher in the intervention group.

Conclusion: AI-based personalized microbiome modulation through a diet significantly improves IBS-related symptoms in patients with IBS-M. Further large-scale, randomized placebo-controlled trials with long-term follow-up (durability) are needed.

***K*eywords:** Functional bowel disorder · Bacteria · Microbiome · Diet · Artificial intelligence

1 Introduction

Irritable bowel syndrome (IBS) is a chronic functional gastrointestinal disorder that negatively impacts the quality of life and healthcare sources [1]. The exact causes of IBS remain largely unknown. These factors are multifactorial and varied among patients. The pathophysiology of IBS is complex, but recent evidence suggests that the gut microbiome may play an essential role in the development, progression, and severity of these symptoms [2]. The advent of next-generation sequencing has increased investigations to identify changes in the gut microbiome related to IBS. Some investigators reported increased fecal Streptococcus [3] and Proteobacteria levels in the gut mucosa [4]. IBS severity was also associated with lower alpha diversity [5]. A recent systematic review of 24 studies performed before 2018 has found that while there was some overlap, none of the studies reported the same differences in gut microbiota [6, 7]. This inconsistency can be the result of a unique microbiome composition for each patient and each disease state. In other words, discovering disease biomarkers of IBS might be challenging due to diverse and heterogeneous microbiome compositions across populations. The second reason for this inconsistency might be that the dynamic alterations of the microbiome complicate the interpretation of data in gut microbiome studies over time. For this reason, a snapshot of observations from cross-sectional studies lacks temporal resolution and does not reflect clinical features of IBS. Diet is increasingly gaining popularity as an interventional approach in IBS treatment. There are specific evidence-based diets used for IBS-symptom relief. The most popular and studied diet is the FODMAP diet [8]. Although the FODMAP diet induces rapid symptom-relief (especially for bloating/distension), it has detrimental effects on gut microbiota (lowering microbiome diversity). The temporary symptom relief by the FODMAP diet is a consequence of the decreased gut abundance of the bacterial population, and it is not a healthy state for the host.

To overcome these microbiome-related inconsistencies in clinical studies, we need to personalize microbiota-modifying therapies. This can be done through specific personalized diets created by machine-learning algorithms, which can handle complex gut microbiome data harboring intrinsic correlations.

In this pilot study, we aimed to modulate the gut microbiota of IBS patients with an individualized diet. The secondary outcome is to measure the therapeutic effect of this diet on disease-specific parameters.

2 Materials and methods

Study cohorts

This study was designed as a pilot, open-labeled study. We enrolled consecutive IBS-M patients (n=25, 19 females, 46.06 ± 13.11 years) according to Rome IV criteria and a healthy control group (n=34) used to model IBS classification models. The healthy group consisted of subjects without chronic diseases affecting microbiome and antibiotic/probiotic consumption in the previous six week-period. IBS-M patients were excluded if they had severe cardiac, liver, neurological, psychiatric diseases or a gastrointestinal disease other than IBS (e.g., celiac disease or inflammatory bowel disease). The patients were not enrolled in the study if they were following a restricted diet for any purpose. Certain medications involving spasmolytics, antidepressants, etc., were allowed if administered at stable doses for the previous four weeks. Probiotics and antibiotics (including rifaximin) were not allowed for the previous six weeks. Paired fecal samples were obtained (pre- and post-intervention), and high; throughput, 16S rRNA sequencing was performed to reveal the microbiota compositions at the baseline and post-intervention. Patients were divided into two groups based on age and gender. Moreover, baseline microbiota compositions were clustered to form subpopulations, and each treatment group was populated to represent similar subpopulation diversity. Six weeks of personalized microbiome diet (n=14) for Group 1 and standard IBS diet (Control Group, n=11) for Group 2 were followed.

Fecal sampling and 16S ribosomal RNA gene sequencing

Fecal samples were collected using BBL culture swabs (Becton, Dickinson and Company, Sparks, MD) and transported to the laboratory in a DNA/RNA shield buffer medium. DNA was extracted directly from the stool samples using a Qiagen Power Soil DNA Extraction Kit (Qiagen, Hilden, Germany). The final concentrations of extracted DNA were measured using a NanoDrop (Shimazu). dsDNA quantification was done using the Qubit dsDNA HS Assay Kit and a Qubit 2.0 Fluorimeter (Thermo Fisher Scientific, Waltham, MaA USA), and then they were stored at 20°C for further analysis.

The sequencing of 16S rRNA was performed according to the protocol of the manufacturer (16S Metagenomic Sequencing Library Preparation Preparing 16S Ribosomal RNA Gene Amplicons for the Illumina MiSeq System) using Illumina MiSeq (Illumina, San Diego, CA, USA) system. In brief, 2-step PCR amplification was used to construct the sequencing library. The 1st step of PCR is to amplify the V4 hypervariable region. The entire length of the primers was: 515F, forward 5' GTGCCAGCMGCCGCGGTAA3' and 806R, reverse 'GGACTACHVGGGTWTCTAAT3' [9].

PCR amplification was performed using a 25L reaction volume that contained 12.5L of 2X KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Wilmington, MA USA), 0.2M each of forward and reverse primer, and 100ng of the DNA template. The reaction process was executed by raising the solution temperature to 95°C for 3min, then performing 25 cycles of 98°C for 20sec, 55°C for 30sec, and 72°C for 30sec, ending with the temperature held at 72°C for 5min. Amplicons were purified using the AMPure XP PCR Purification Kit (Beckman Coulter Life Sciences, Indianapolis, IN, USA). The second step of PCR is to add the index adaptors using a 10-cycle PCR program. The PCR step adds the index 1 (i7), index 2 (i5), sequencing, and common adapters (P5 and P7). PCR amplification was performed on a 25L reaction volume containing 12.5L of 2X KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Wilmington, MA USA), 0.2M of each index adaptor (i5 and i7), and 2.5L of the first-PCR final product. The reaction process was executed by raising the solution temperature to 95°C for 3min, then performing 10 cycles of 98°C for 20sec, 55°C for 30sec, and 72°C for 30sec, ending with a 72°C hold for 5min. Amplicons were purified using the AMPure XP PCR Purification Kit (Beckman Coulter Life Sciences, Indianapolis, IN, USA).

All amplified products were then checked with 2% agarose gel electrophoresis. Amplicons were purified using the AMPure XP PCR Purification Kit (Beckman Coulter Genomics, Danvers, MA, USA) and quantified using the Qubit dsDNA HS Assay Kit and a Qubit 2.0 Fluorimeter (Thermo Fisher Scientific, Waltham, MA, USA). Approximately 15% PhiX Control library (v3) (Illumina, San Diego, CA, USA) was combined with the final sequencing library. The libraries were processed for cluster generation. Sequencing with 250PE MiSeq runs was performed, generating at least 50.000 reads per sample.

Sequencing data were analyzed using the QIIME pipeline [10] after filtering and trimming the reads for PHRED quality score 30 via the Trimmomatic tool [11]. Operational taxonomic units were determined using the Uclust method, and the units were assigned to taxonomic clades via PyNAST using the Green Genes database [12] with an open reference procedure. Alpha- and beta-diversity statistics were assessed accordingly by QIIME pipeline scripts. The graph-based visualization of the microbiota profiles was performed using tmap topological data analysis framework with Bray-Curtis distance metric.

IBS-index Scoring

The baseline group of IBS-M patients (n=25) and the healthy controls (n=34) were compared in terms of their microbiota compositions. The detected microbiota profiles were used to characterize the disease in a classification setting. Based on Gradient Boosted Trees (GBT) [13] classification algorithm, a stochastic gradient boosting classification model (XGBoost, version 0.90 [14]) was used in Dropouts meet multiple Additive Regression Trees (DART) booster with binary logistic regressor. Five-fold cross-validation, with 10 random seeding trials, was used to observe the disease classification performance. The logistic regression scores of XGBoost models were used as IBS-index scores. The dataset was utilized for training the final IBS-index model. The hyperparameters of the XGBoost model were optimized using the Bayesian optimization tool Optuna [15] in a 5-fold-cross validation setting.

The AI-based personalized nutrition model

The Enbiosis personalized nutrition model estimates the optimal micronutrient compositions for a required microbiome modulation. The present study computed the microbiome modulation needed for an IBS case based on the IBS indices generated by the machine learning models. The baseline microbiome compositions are perturbed randomly with a small probability p. Perturbed profiles are accepted with a probability proportional to the decrease in the IBS-index as suggested by Metropolis sampling [16]. This Monte-Carlo random walk in the microbiome composition space is expected to meet a low IBS-index microbiome composition nearby the baseline microbiome composition of the patient with a minimal modulation. Then, the personalized nutrition model estimates the optimized nutritional composition needed for this individual, expecting to drive the IBS-index to lower values.

Therefore, an algorithm assessing an IBS index score using microbiome composition attempted to design the optimized diets based on modulating the microbiome towards the healthy scores.

3 Results

Gut microbiota communities between IBS patients and Healthy Controls

The gut microbiome genus-level abundance profile is shown in Figure 1. The gut microbiome profile of the recruited patients and the healthy controls showed significant differences in beta diversity. Based on unweighted UniFrac dissimilarity measurement of microbiota sample pairs, the patient and the healthy control groups showed different community profiles ($p < 10^{-6}$, PERMANOVA test with 1,000,000 random permutations). The stratified profiles can

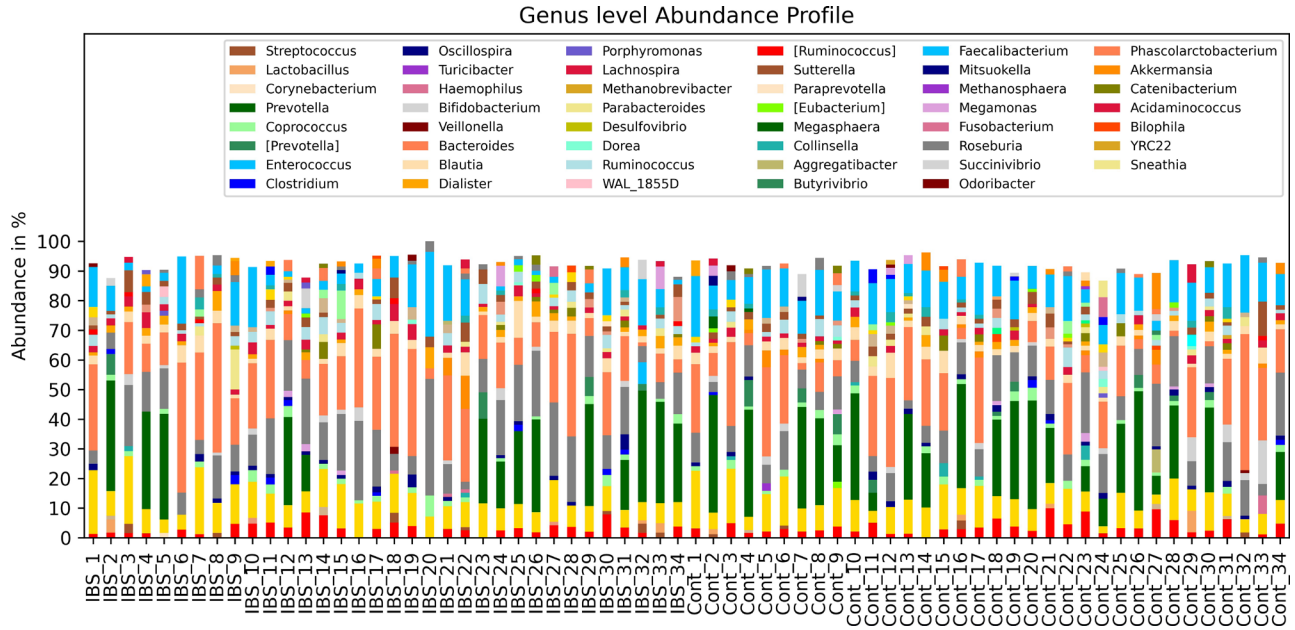

Figure 1: Genus level abundance profiles.

Table 1: IBS-SSS scores (mean ± standard deviation) before and after the interventions.

	Pre-intervention	Post-intervention	P-value (paired t-test)
Personalized nutrition	357.1 ± 18.2	232.5 ± 61.5	**< 0.001**
Control	363.1 ± 16.7	331.8 ± 42.9	**< 0.02**

be observed in the tmap visualization in Figure 2. Clear subgroupings between the IBS cases and the healthy controls can be observed from these topological maps. When bacterial taxa are considered individually, the most significant differences between the IBS and healthy control groups are observed in Ruminococcaceae ($p = 0.014$, Mann-Whitney U-test) and Clostridiaceae ($p = 0.022$, Mann-Whitney U-test) families and Ruminococcus ($p = 0.023$, Mann-Whitney U-test) and Faecalibacterium ($p = 0.0005$, Mann-Whitney U-test) genera (Figures 3,4).

Disease classification and microbiome-derived IBS index scores

A machine learning (ML) based classifier trained and tested on pre-interventional microbiota profiles exhibited a strong classification performance. Using 5-fold cross-validation on the held-out XGBoost classifier models, an averageROC-AUC of 0.964 and average classification accuracy of 0.91 were determined. The microbiome-derived IBS index scores, which are the inferred disease probability measurements obtained from XGBoost classification models, were significantly different ($p < 10-5$, Mann-Whitney U-test), as shown in Figure 5.

Evaluating the IBS-index scores on the held-out validation cohorts, we observed that the score distributions of the IBS-patients and the healthy controls differ significantly ($p = 0.001$, Mann-Whitney U-test), implying that the machine-learned IBS-index is a strong indicator of the disease.

Clinical Evaluation of Personalized nutrition vs. control groups

The IBS-SSS evaluation for both pre-intervention and post-intervention conducted for both groups exhibited significant improvement ($p<0.02$ and $p<0.001$ for the control and the personalized nutrition interventions, respectively). It was observed that the score improvement for the personalized nutrition group was significantly greater than the control group (Table 1, Figure 6).

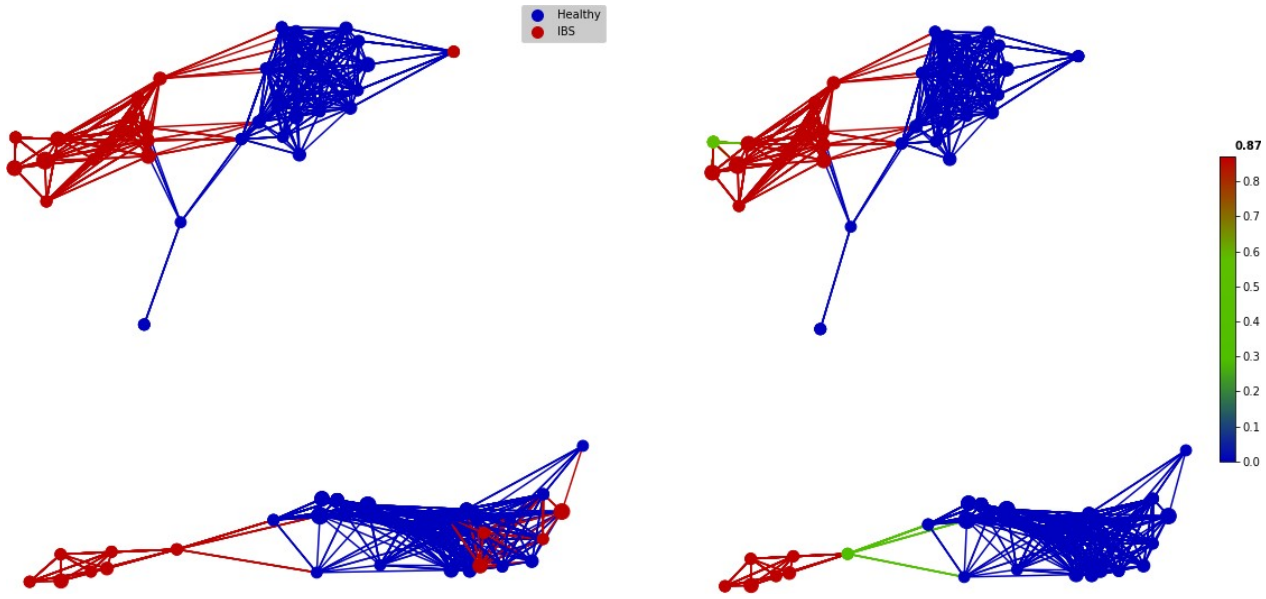

Figure 2: Two-dimensional network visualization of the microbiota profiles using tmap network analysis (constructed by Bray-Curtis metric). Two major enterotypes (up: Bacteroides dominant, down: Preveotella Dominant) nearly form different disease subgroups. Left: network nodes labeled by disease phenotype. Right: SAFE enrichment analysis of the disease scores. Blue-to-red indicates lower to higher IBS scoring.

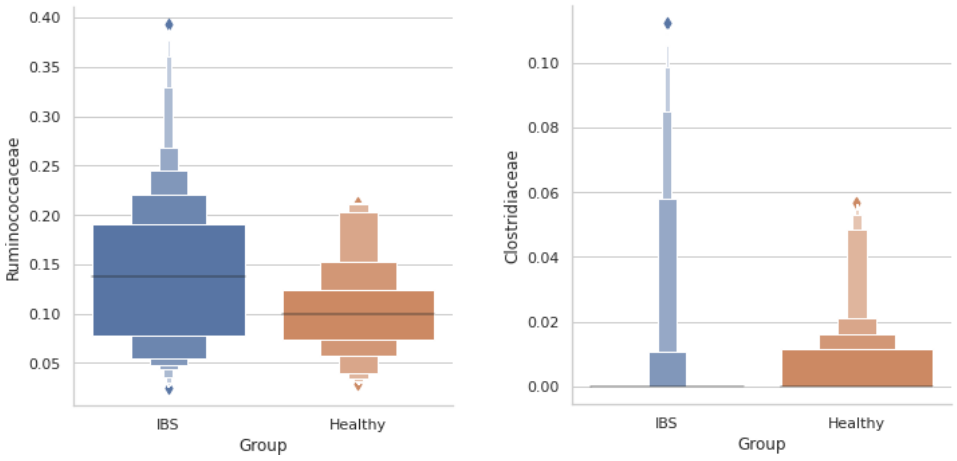

Figure 3: The Ruminococcaceae family are observed in higher abundance in the IBS Group (p-value 0.014, Mann-WhitneyU-test), where the Clostridiaceae family is decreased in IBS patients (p-value 0.022, Mann-Whitney U-test)

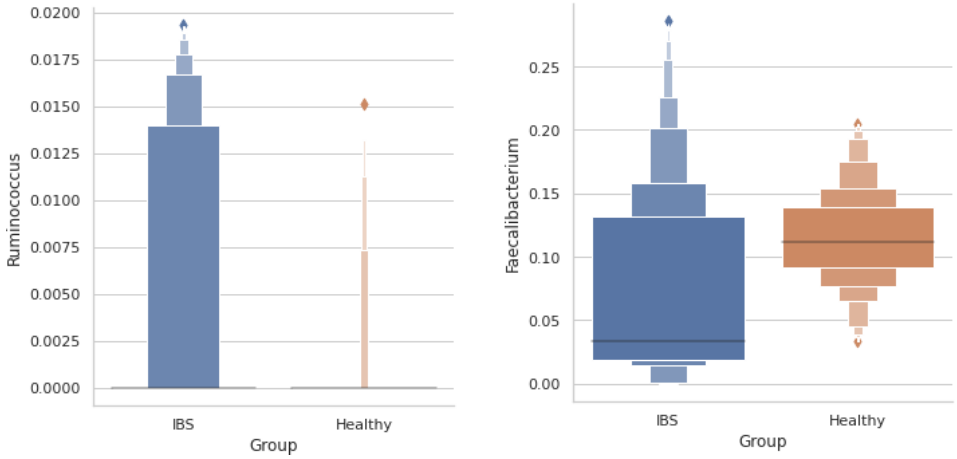

Figure 4: Ruminococcus genus is observed more abundantly in the IBS Group (p-value 0.023, Mann-Whitney U-test), where Faecalibacterium is observed in significantly lower abundances in IBS patients (p-value 0.0005, Mann-Whitney U-test).

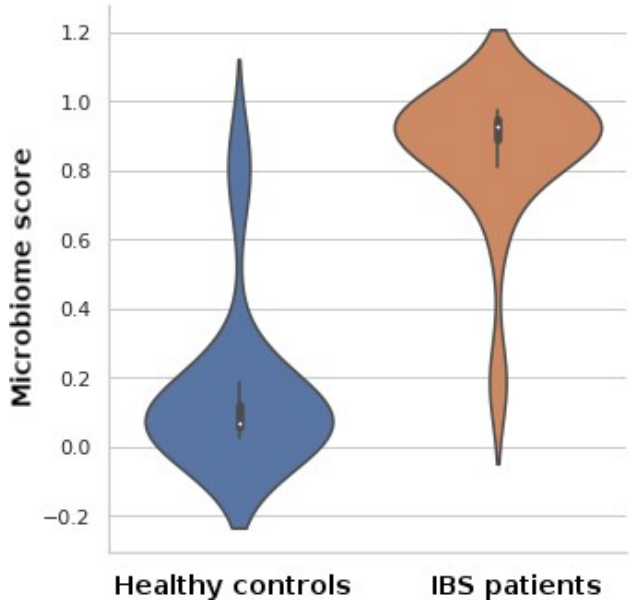

Figure 5: The microbiome scores evaluated for the healthy controls and the IBS patients.

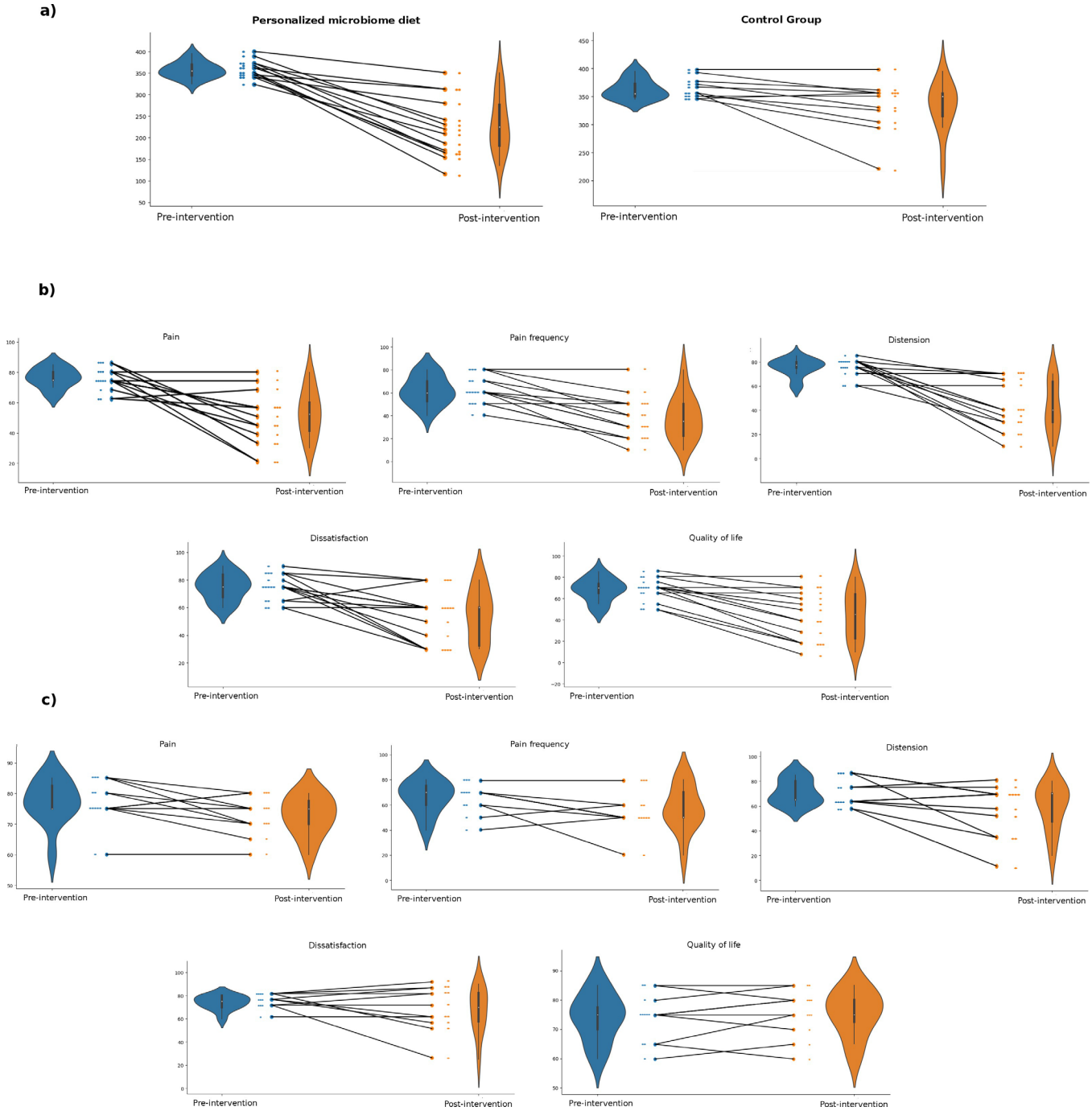

Figure 6: a) IBS-SSS scores for personalized nutrition intervention and IBS-SSS scores for the control intervention, b) IBS-SSS score categories for personalized nutrition pre- and post-intervention, c) IBS-SSS score categories for the control treatment pre- and post-intervention.

Table 2: IBS-SSS score categories (mean ± standard deviation) before and after the interventions.

Personalized nutrition			
	Pre-intervention	**Post-intervention**	**P-value (paired t-test)**
Abdominal pain	76.4 ± 6.4	53.2 ± 15.0	**< 0.001**
Abdominal pain frequency	62.1 ± 12.0	37.9 ± 18.2	**< 0.001**
Distension	75.4 ± 7.2	42.9 ± 19.9	**< 0.001**
Dissatisfaction with bowel habits	75.0 ± 9.3	53.6 ± 18.3	**< 0.01**
IBS-related quality of life	68.2 ± 10.3	45.0 ± 21.7	**< 0.001**
Control			
	Pre-intervention	**Post-intervention**	**P-value (paired t-test)**
Abdominal pain	77.3 ± 6.7	72.7 ± 6.2	**0.043**
Abdominal pain frequency	66.4 ± 12.3	57.3 ± 17.1	0.074
Distension	71.4 ± 9.6	59.1 ± 17.7	**0.041**
Dissatisfaction with bowel habits	74.1 ± 6.0	67.3 ± 18.6	0.246
IBS-related quality of life	74.1 ± 7.6	75.5 ± 7.5	0.391

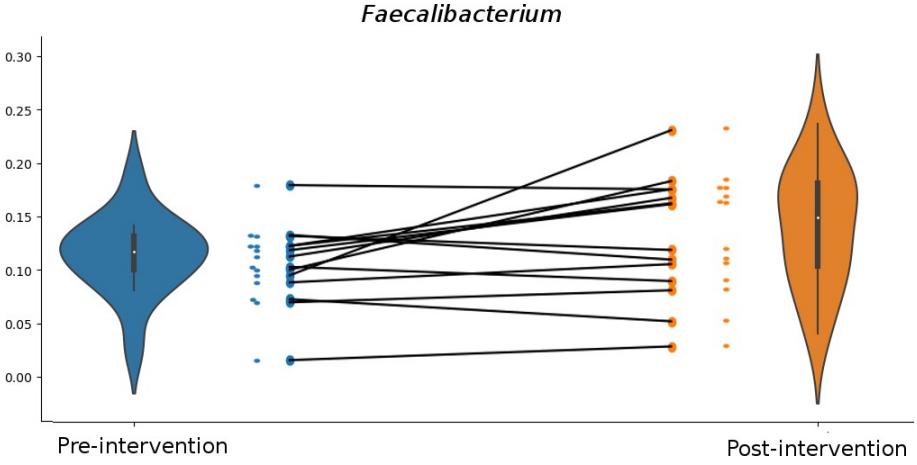

Figure 7: Faecalibacterium relative abundances in the personalized nutrition group pre- and post-intervention.

The personalized nutrition was effective on all, considering each of the 5 IBS-SSS items. In contrast, abdominal pain frequency, dissatisfaction with bowel habits, and IBS-related quality of life were not changed significantly in the control group (Table 2).

Post-interventional changes in microbiota profiles

After six weeks of intervention, a significant shift in microbiota profiles in terms of alfa- or beta-diversity was not observed in both groups. A trend of decrease in the Ruminococcaceae family for the personalized nutrition intervention group was observed; however, this change was not observed to be statistically significant ($p = 0.17$, paired t-test). A statistically significant increase in the Faecalibacterium genus was observed in the personalized nutrition group ($p = 0.04$), whereas no meaningful change was reported for the control group ($p = 0.63$) (Figure 7).

Both Bacteroides rich and Preveotella rich enterotypes were represented in personalized nutrition and control intervention groups without significantly different Bacteroides and Prevotella abundances ($p = 0.34$ for Bacteroides and $p = 0.36$ for Prevotella, Mann-Whitney U-test). However, we have observed an increase in Bacteroides for the personalized nutrition group ($p > 0.05$), while an increasing trend in Prevotella ($p = 0.057$) was noticeable in the control group. Along with that, a significant increase in the putatively probiotic genus Propionibacterium ($p = 0.027$) was apparent in the personalized nutrition group, whereas no such increase was observed in the control group.

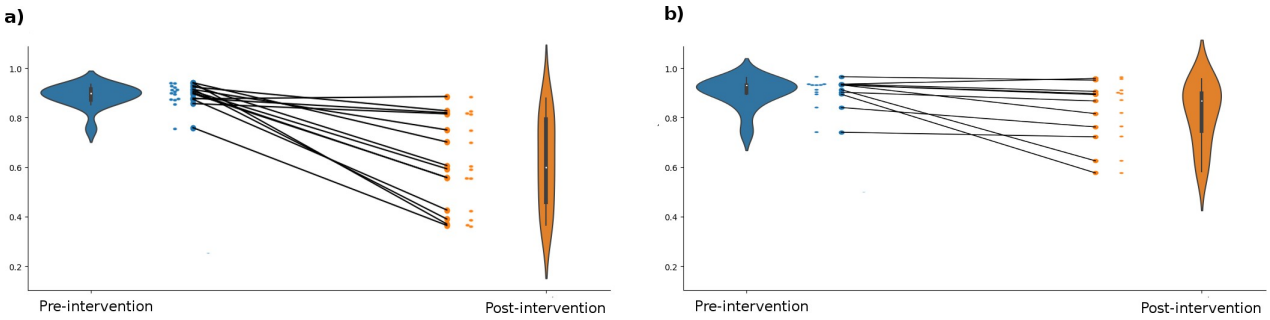

Figure 8: a. Microbiome scores for personalized nutrition intervention, b. Microbiome scores for the control intervention.

Table 3: Microbiome scores (mean ± standard deviation) before and after the interventions.

	Pre-intervention	Post-intervention	P-value (paired t-test)
Personalized nutrition	0.89 ± 0.04	0.62 ± 0.18	**< 0.001**
Control	0.87 ± 0.05	0.79 ± 0.11	**0.03**

The evaluation of microbiome-derived IBS index scores

The microbiome-derived IBS index scores improved towards lower scores in both intervention groups. The improvement in the personalized nutrition group was observed to be greater (Table 3, Figure 8). To observe the correlation between the microbiome-derived IBS scores and the clinical evaluations (i.e., IBS-SSS), we have measured the explained variance of IBS-SSS with respect to microbiome scores (Figure 9). Including the corresponding scores of both intervention groups, an R^2 score of 0.652 was found, indicating that the microbiome scores contribute significantly to the explanation of the clinical scores.

4 Discussion

Dietary habits constitute a strong driver of interpersonal variance in the gut microbiome composition, and their influence prevails over genetics by most estimates [17]. Our study investigated the therapeutic effect of the personalized diet on the individual gut microbiome and the disease-specific symptoms. Most IBS patients regard diet as an essential trigger for their gastrointestinal symptoms. Based on the subjective correlation between diet and IBS symptoms, there have been many attempts to design specific diets to relieve IBS-related complaints. Recent studies indicate that a low FODMAP diet relieves some IBS symptoms, such as abdominal gas, bloating, distension, and even abdominal pain [18]. Elimination of fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs) is also recommended by the guidelines [19]. FODMAPs are sugars that ferment in the gut due to inadequate digestion; common ones are lactose, fructose, fructans, and sorbitol. Foods containing FODMAPs include wheat, some fruits and vegetables, corn syrup, and onions. Initial positive study of a low FODMAP diet was performedin IBS patients with a positive fructose breath test and without a control group [20]. Another randomized controlled study compared a low FODMAP diet with a typical Australian diet, and it has found a 30% decrease in IBS-symptom severity [21]. However, subsequent randomized trials failed to detect significant clinical differences between classicalIBS diets and low FODMAP diets. All the diets were nearly 50% effective in relieving IBS symptoms, and the low FODMAP diet is not an exception [22].

Another critical but neglected issue in IBS treatment is the diet-related gut microbial changes. In the last decade, there have been many studies on the gut microbiome in IBS patients [23, 24, 25, 26, 27]. A recent systematic review analyzed 24 studies, mainly from Europe and North America. They have found that Bifidobacterium, Faecalibacterium genus are decreased, and Lactobacillaceae, Bacteroides, Enterobactericeae families are increased in IBS [28]. To overcome the reduced levels of Bifidobacteria, prebiotic or sometimes probiotic supplements might be advised to the IBS patients on a low FODMAP diet. While this increases the abundance of Bifidobacteria, it has some detrimental effects on gut health in animal studies caused by disruption of the mucosal barrier, increased mucosal inflammation, and visceral hypersensitivity [29]. Rapid colonic fermentation is central to the identified mechanisms that include injury from high luminal concentrations of short-chain fatty acids and low pH and the inflammatory effects of increased endotoxin load and glycation of macromolecules [29].

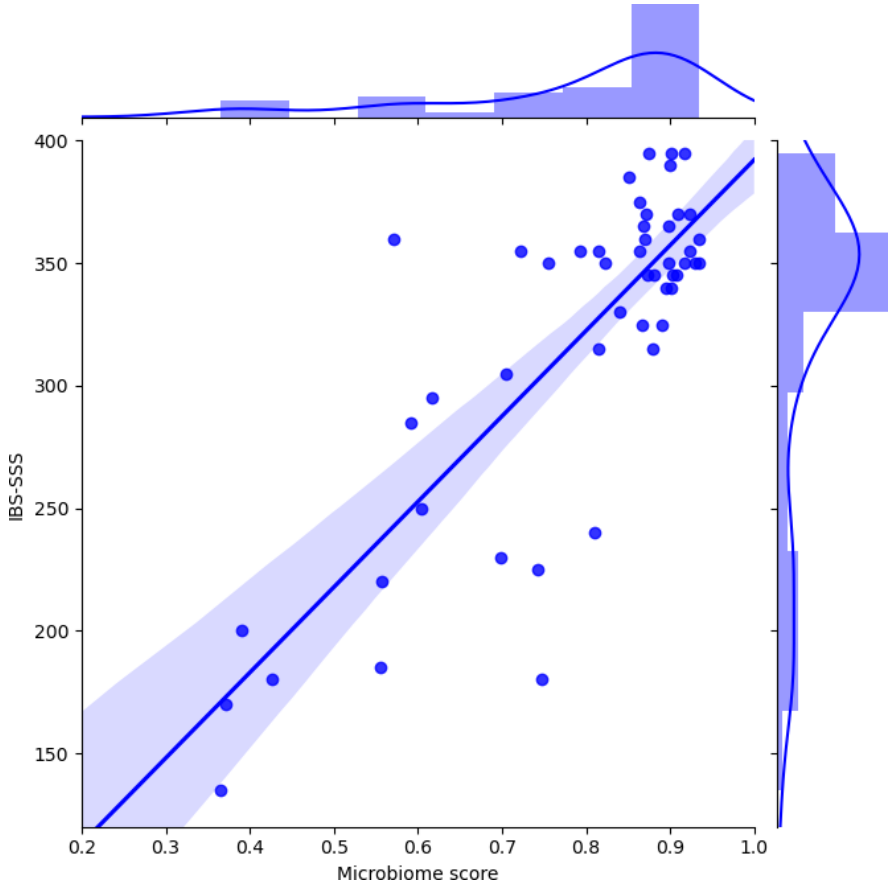

Figure 9: The plot shows the scatter and the marginal histograms of IBS-SSS and microbiome-derived IBS scores. The linear regression line represents the positive correlation.

Currently, the optimal diet for the treatment of IBS patients is lacking. The ideal diet should be effective on (at least) most of the symptoms in IBS and maintain a healthy state of the gut microbiome. It should be sustainable and personalized. Our study is the first attempt to reach these therapeutic goals in IBS. We used machine-learning for determining personalized diet to modulate the IBS microbiota to an individually similar 'healthy' state. In other words, we tried to formulate a personalized microbiome-modulating diet for patients with IBS-M. The gut microbiome genus level IBS and healthy controls showed significant differences in beta diversity. When we look at the bacterial taxa, the most significant differences between the IBS and healthy control groups were observed in the Ruminococcaceae and Clostridiaceae families. Ruminococcaceae was increased, and Clostridiaceae was decreased in the IBS group. In the genera level, Ruminococcus was increased, and Faecalibacterium was decreased in the IBS group. In a recent systematic review, the Ruminococcaceae family and Faecalibacterium genus were not different in the IBS vs. healthy groups [28]. Although there are inconsistencies between the literature and our results, these differences might stem from patients' geographic, cultural, and dietary habits.

The IBS-index scores on the held-out validation cohorts were different between IBS-patients and the healthy controls. This implies that the machine-learned IBS-index is a strong indicator of the presence of disease. We detected a significant improvement in IBS-SSS values for both pre- and post-intervention periods. The score improvement for the personalized diet group of IBS patients was greater than the control group (Table 1, Figure 6). For each of the five items of IBS-SSS evaluated, the personalized diet group showed significant improvement on all parameters. However, the control group showed no improvement in abdominal pain frequency, dissatisfaction with bowel habits, and IBS-related quality of life parameters. Böhn et al. reported that low FODMAP and standard IBS diet were similar for relieving IBS symptoms. In their study, abdominal pain frequency and IBS-related quality of life parameters were improved with low FODMAP diet, but the dissatisfaction with bowel habits did not improve [22]. They have noticed a nearly 50% response rate to both diets. This study concluded that a low FODMAP diet shows similar clinical benefits with standard IBS diets.

The post-intervention gut microbiome changes were also different between groups. After six weeks of intervention, a major shift in microbiota profiles in terms of alfa- or beta-diversity was not observed in both groups. A statistically significant increase in the Faecalibacterium genus was observed in the personalized nutrition group ($p = 0.04$), whereas no meaningful change was reported for the control group ($p = 0.63$). Peter J et al. investigated the role of the microbiome in IBS-related psychological distress and found that depression was negatively associated with Lachnospiraceae abundance; the distress, anxiety, depression, and stress perception were associated with higher abundances of Proteobacteria. The feeling of anxiety was characterized by elevated Bacteroidaceae [30]. In our study, we have observed an increase in Bacteroides for the personalized nutrition group ($p > 0.05$), while an increasing trend in Prevotella ($p = 0.057$) was noticeable in the control group. The increase in the Bacteroides group might have affected our IBS patients' anxiety statusin the intervention group and improved the quality-of-life scores in IBS-SSS evaluation.

The microbiome-derived IBS index scores improved towards lower scores in both intervention groups. The improvement in the personalized nutrition group was observed to be more significant. IBS severity is also correlated with gut microbiome features. Tap J et al. investigated the correlation between gut microbiota signatures and IBS severity. They found that IBS symptom severity to be associated negatively with microbial richness, exhaled CH4, presence of methanogens, and enterotypes enriched with Clostridiales or Prevotella species. This microbiota signature could not be explained by differences in diet or the use of medications [31]. In our study, the post-interventional analysis showed an increasing trend of Prevotella species (although statistically insignificant) in the control group.

As a result, our study is the first trial in the literature comparing the therapeutic effect of AI-based personalized diet for patients with IBS-M. We had limited clinical and gut microbiome-related benefits after six weeks of intervention. Further, more extensive randomized controlled trials are needed to determine this treatment's safety, effectiveness, and durability.

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