Fecal microbiota Profiling in Irritable Bowel Syndrome and Inflammatory Bowel Disease Patients with Irritable Bowel Syndrome-Type Symptoms

Xiufang Cui
First Affiliated Hospital of Nanjing Medical University https://orcid.org/0000-0003-2299-802X
Haiyang Wang
The Affiliated Sir Run Run Hospital of Nanjing Medical University
Ziping Ye
First Affiliated Hospital of Nanjing Medical University
Yi Li
The Affiliated Sir Run Run Hospital of Nanjing Medical University
Xinyun Qiu
First Affiliated Hospital of Nanjing Medical University
Hongjie Zhang (hjzhang06@163.com)
First Affiliated Hospital of Nanjing Medical University https://orcid.org/0000-0003-4497-0503

Research

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Abstract

BACKGROUND

The intestinal microbiota is thought to be involved in the occurrence of Inflammatory Bowel Disease in remission (IBDR) with Irritable Bowel Syndrome (IBS)-type symptoms, but the specific distinct profile of these bacteria remains unclear. Therefore, the purpose of this research is to investigate this issue by conducting a cross-sectional study.

METHODS

IBS patients were diagnosed according to Rome criteria, IBD diagnosed according to the criteria of European Crohn & Colitis Organization (ECCO), IBDR patients with IBS-type symptoms were defined according to related IBS-type symptoms meeting the Rome IV criteria in IBDR patients, and were included Crohn's disease in remission (CDR) and ulcerative colitis in remission (UCR) based on Crohn's Disease Activity Index (DAI) and Mayo Scoring System respectively. Healthy controls come from the physical examination center and exclude people with underlying diseases. All enrolled subjects were divided into six groups, as followed: Health Control, IBS, CDR with IBS-type symptoms (CDR-IBS$^+$), CDR without IBS-type symptoms (CDR-IBS$^-$), UCR-IBS$^+$ and UCR-IBS$^-$. We collected fresh fecal samples from all subjects and applied 16S rRNA sequencing analysis to detect the structure and diversity of the microbiota among different groups.

RESULTS

A total of 97 subjects were included in this study, of which 18 were health controls, 34 IBS patients, 25 CDR and 20 UCR. The richness of intestinal microbiota in CDR-IBS$^-$was significantly lower than that in the control and IBS groups based on the analysis of observed species and Chao index ($P < 0.05$). The observed species index in CDR-IBS$^+$ was significantly higher than CDR-IBS$^-$ group (median index: 254.8 vs 203, $P = 0.036$). No difference was found in Alpha diversity between UCR-IBS$^+$ and UCR-IBS$^-$. At phylum level, there was no significant difference between UC or CD with IBS-type symptoms and those without related symptoms. At genus level, the number of *Faecalibacterium* in CDR-IBS$^+$ increased significantly while *Fusobacterium* decreased compared with CDR-IBS$^-$(mean relative abundance of *Faecalibacterium*: 20.35% *vs* 5.18%, $P < 0.05$; *Fusobacterium*: 1.51% *vs* 5.2%, $P < 0.05$). However, compared with UCR-IBS - group, the number of Faecalibacterium in UCR-IBS + group decreased, while the number of Streptococcus increased, but there was no statistical difference in the genus structure. Regardless of the phylum or genus level, the abundance and composition of the microbiota of IBS patients were not distinct from those of healthy people.

CONCLUSIONS

CD patients in remission with IBS-type symptoms may be related to the increase of *Faecalibacterium* and decrease of *Fusobacterium*. UC patients in remission with IBS-type symptoms cannot be explained by changes in the abundance and structure of intestinal microbiota from our across-sectional study.

Introduction

Changes in intestinal microbiota can result in the loss of intestinal homeostasis, which have been found in a variety of intestinal disorders including Inflammatory Bowel Diseases (IBD) and Irritable Bowel Syndrome (IBS)[1]. IBD is a chronic relapsing inflammatory disease of the gastrointestinal tract with unknown etiology, including Crohn's disease (CD) and ulcerative colitis (UC). The pathogenesis of IBD remains incompletely understood, and it is currently recognized that it is closely related to genetic susceptibility, environment, disruption of intestinal microbiota, and immune disorders, especially intestinal microbiota. A large number of studies have confirmed that the interaction between intestinal flora and genetic susceptibility can be considered a contributor to the pathogenesis of IBD by triggering exacerbated immune response[2]. Previous studies indicate that alterative gut microbiota composition was found in IBD patients including a reduction of microbial diversity and richness[3]. Specifically, the beneficial bacteria decrease, while the harmful bacteria increase[4, 5].

Irritable bowel syndrome (IBS) is a functional bowel disease characterized by recurrent abdominal pain, bloating, and altered bowel habits. IBD patients at active stage often have abdominal pain, diarrhea, bloody stools and other uncomfortable symptoms. Some IBD patients in remission (IBDR) have persistent gastrointestinal symptoms including abdominal pain, diarrhea and abdominal discomfort. For IBDR, these symptoms meet criteria for IBS and can be defined as IBS-type symptoms[6–8]. According to the previous studies, the prevalence of IBS-type symptoms in patients with IBD with clinically quiescent disease ranged from 25% and 60% due to the different definition of remission and population sizes[9, 10]. There is a lack of evidence-based therapeutic options available for the management of such patients, who experience a reduced quality of life equivalent to patients with overt inflammatory disease activity[10].

Although IBS and IBD belong to functional and organic diseases respectively, they have some common points in the etiology, especially intestinal microbiome[11, 12]. Emerging evidences suggest an important role of the intestinal microbiota in the pathophysiology of IBS[13, 14]. Previous observational studies have shown that intestinal infections may cause IBS and probiotics can be used to treat IBS[15, 16]. The etiology of IBS-type symptoms in IBDR patients is still unclear and remains controversial. In addition, at present, there is still a lack of knowledge and effective treatment of the disease in patients with IBDR accompanied by IBS symptoms.

Therefore, we hypothesized that the IBS-type symptoms of IBD patients in remission would be closely related to alterations in intestinal microbiota. To clarify this hypothesis, we performed a cross-sectional study to initially explore the alterations in the intestinal microbiota of IBD patients in remission with IBS-type symptoms.

Materials And Methods

Ethics approval

The current study protocol and procedure were approved by Ethics Committee of the First Affiliated Hospital of Nanjing Medical University and informed consents were obtained from all enrolled participants before the data and sample collection (Ethics number: 2018-SR-061).

Participants and Setting

IBD patients: All participants with IBD had an established radiological, histological, or endoscopic diagnosis of CD or UC according to the criteria of European Crohn & Colitis Organization (ECCO)[17]. In this study, IBD patients in clinical remission were included and the inclusive criteria as follows[18]: 1) Mayo scores ≦ 2, and single item score is < 1; 2) Crohn disease activity index(CDAI) ≦ 150; 3) Participants have not taken antibiotics, probiotics, colon cleansing liquid, etc. at least 2 months prior to the study. Exclusive criteria including an inability to understand the Chinese version of the informed consent form, uncategorized IBD, a history of gastroenterology surgery, female subjects who were lactating or pregnant.

IBS patients: IBS was evaluated by excluding organic diseases on the basis of Rome III diagnostic items as follows[19]: 1) Symptoms occur at least six months before diagnosis; 2) Onset of abdominal analgesia or discomfort more than three days of each month within the previous three months; 3) At least two of the following characteristics: improvement after defecation, relationship with changes in stool frequency or stool form.

IBD-IBS

Patients with confirmed IBD had symptoms of abdominal pain and changes in bowel habits, and these symptoms met the Rome IV criteria[19] and were defined as IBD with IBS-type symptoms (IBD-IBS) according to the previous studies[20, 21].

Healthy control

Normal healthy control selected from healthy people in the physical examination center. The data was collected from the First Affiliated Hospital of Nanjing Medical University, from August 2018 to September 2019.

Sample collection and genomic DNA extraction

We collected fresh fecal samples from all enrolled subjects and immediately stored them in the − 80℃ refrigerator within 2 hours to avoid bacteria overgrowth in oxygen environment. According to the manufacturer's instructions, genomic DNA for microbiome analysis was extracted using special kits QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). Briefly, fecal sample (200 mg) was added in a 2 ml microcentrifuge tube and tube wea placed on ice. 1 ml InhibitEX Buffer was added to each fecal sample and the tube was vortexed continuously for 1 min until the sample thoroughly homogenized. The

subsequent extraction protocol is carried out in strict compliance with recommendation from QIAamp DNA Stool Mini Kit' instructions.

Sequencing

All DNA samples were quality controlled and before being amplified by polymerase chain reaction (PCR) of the V4 hypervariable region of the 16S rRNA gene, a reliable indicator of bacterial taxonomy[22]. Diluted genomic DNA as a template, amplified PCR were performed by using specific primers with Barcode, Phusion®High-Fidelity PCR Master Mix with GC Buffer from New England Biolabs, and high-efficiency and high-fidelity enzymes according to the selection of the sequencing region. PCR products were mixed and purified by Qiagen Gel Extraction Kit. Magnetic beads were used to screen the target Amplicon fragments, and finally, the qualified library was used for cluster preparation and paired-end sequencing through the Illumina platform (Hiseq or Miseq) following manufacturer's instructions.

Data collection and analysis

Samples were merged to build a library through barcode, and after getting clean data, the barcode sequence was used to split the sample through an internally written program. The allowed number of mismatches between barcode sequence and sequencing reads is 0 bp. Using this method, Paired-end sequencing is performed on the Illumina platform (Hiseq or Miseq), and the off-machine data is removed from low-quality reads. Paired End Reads are spliced into Tags through the overlap relationship between reads by using FLASH (V1.2.11, http://ccb.jhu.edu/software/FLASH/)[23]. The low-quality raw tags were removed and the high-quality tags were remained for subsequently analysis according to the QIIME (V1.8.0, http://qiime.org/index.html)[24]. Finally, the obtained effective clean tags were analyzed using UCHIME algorithm (UCHIME Algorithm, v7.0.1090, http://www.drive5). Use the software USEARCH (v7.0.1090) to cluster the spliced Tags into operational taxonomic units (OTUs), the procedure as follows: 1) Use UPARSE to perform clustering at 97% similarity to obtain the representative sequence of OTU;2) Use UCHIME (v4.2.40) [4] to remove the chimera generated by PCR amplification from the OTU representative sequence by comparing the sequence with reference database (Gold database, v20110519, http://drive5.com/uchime/uchime_download.html; UNITE, v20140703); 3) Use the usearch_global method to align all tags back to the OTUs representative sequences and obtain the abundance statistics table of each sample in each OTUs. After obtaining the representative sequence of OTUs, the representative sequence of OTUs were compared with the database Greengene_2013_5_99 through the RDP classifer (v2.2) software, and the species were annotated, and the confidence threshold was set to 0.6. After the spliced tags were optimized, all samples were selected with the smallest number of tags, and clustered into OTUs (Operational Taxonomic Units) for species classification at 97% similarity. In the abundance information, the abundance of OTU preliminarily indicates the species richness of the sample. In this study, the alpha diversity value of the sample was calculated by mothur (v1.31.2) software. The differences of sample diversity index in groups were analyzed and displayed with R (v3.1.1) software bases on the standardized output data.

Statistical Analysis

All the data were analyzed using Statistical Package for Social Sciences version 25.0 (IBM Company, Armonk, NY). Age parameter data were expressed as mean ± standard deviation. Unless specifically explained, majority of microbiota data were non-normal distributions and the data were expressed as the median (maximum, minimum). Kruskal-Wallis one-way analysis of variance were used to compare the microbiota data. Partial graphs were drawn using Graphpad software 8.0 (Graphpad Inc, San Diego, CA). *P* value lower than 0.05 was considered statistically significant.

Results

1. The demographics and Clinical Characteristics of Study subjects

A total of 97 subjects were enrolled in the study including 34 IBS patients, 45 IBD patients in remission, 18 healthy controls. All the subjects met the enrolling criteria from the First Affiliated Hospital of Nanjing Medical University were recruited from August 2018 to September 2019. The mean age was 42.9 years in IBS group, 30.9 years in CDR-IBS+ group, 29.8 years in CDR-IBS- group, 37.1 years in UCR-IBS+ group, 42.4 years in UCR-IBS- group and 37.9 years in control group. The proportion of male subjects is 54.6% (53/97). However, there were more female subjects in IBS group (61.7%, 21/34), which might be closely related to the obvious gender difference in the incidence of this kind of disease. Detailed demographic data and clinical characteristics of all included subjects are listed in Table 1.

Overall sequencing results

The paired end reads were optimized to remove low-quality reads, and clustered into operational taxonomic units (OTUs) for species classification at 97% similarity, and the abundance information of each sample in each OTUs was counted. The abundance preliminarily explains the species richness of the sample. A total of 4869075 high-quality tags were obtained, and the average number of tags for each sample were 50197. According to the 97% similar clustering principle, a total of 1118 OTUs were generated from 97 samples. Compared with the control group, the number of OTUs in IBS, CD patients in remission with IBS-type symptoms (CDR-IBS$^+$), CD patients in remission without IBS-type symptoms (CDR-IBS$^-$), UC patients in remission with IBS-type symptoms (UCR-IBS$^+$) and UC patients in remission without IBS-type symptoms (UCR-IBS$^-$) reduced, but only the OTUs of patients in CDR-IBS$^-$ had a statistical difference (163.7±65.98 *vs* 240.8±66.75, $P<0.05$), suggesting that this group of patients may have the lowest species abundance. The detailed results were shown in Table 2.

The Characteristics of Microbial diversity in Different Groups

The rarefaction curve was used to reflect the rationality of the amount of sequencing data. Each sample was obtained a rarefaction curve according the number of the bacterial OTUs on sequence counts at different sequencing depths. As shown in Supplementary Figure 1, as the number of sequencing continues to increase, the rarefaction curve of each sample tended to be saturated, indicating that the final sequencing data in the study was reliable. Alpha diversity was used to evaluate the differences in the microbiota of samples in Control, IBS, CDR-IBS$^+$, CDR-IBS$^-$, UCR-IBS$^+$, and UCR-IBS$^-$ groups. The observed

species index, Chao index and Ace index were calculated to reflect the species richness of the microbial community in the sample, while the shannon index and simpson index reflected species diversity, which is affected by the species richness and species evenness of the sample community. The results of comparison among the Alpha Diversity Index groups indicated that observed species index, chao and Ace of CDR-IBS$^-$ were statistically significant decreased compared with Control groups, respectively, while no difference was found among other groups (Figure 1A-C). Meanwhile, the observed species of microbiota in CDR-IBS$^-$ was lower than CDR-IBS$^+$ group, Chao index was lower than IBS group. Therefore, the richness of microbiota in fecal samples from CDR-IBS$^-$ groups were significantly decreased base on the analysis of alpha diversity. There were no differences in shannon index and simpson index among the six groups (Figure 1D-E). Although there is no statistical difference in a diversity analysis of patients between IBS, CDR-IBS$^+$ and UCR-IBS$^+$ groups, including observed species, chao and Ace index. Furthermore, the overall microbiota structures were analyzed according the number of shared or unique OTUs. The results were displayed in partial least-squares discriminant analysis (PLS-DA) plots and Venn plots (Figure 1F-G) and heat maps in phylum level (Supplementary Figure 2). PLS-DA and heat maps exhibited that the microbiota structure had slight differences among the groups, but majority of bacteria community overlapped.

Overall Taxonomic Composition of IBDR, IBS and Control Groups in Phylum

From the Figure 2, the proportions of different species in phylum were summarized detail for each sample. The phylum level of taxonomic composition in fecal samples of all groups with major microbiota were as following: *Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Fusobacteria,* and *Verrucomicrobia.* (Figure 2A-B). There was no statistical difference of microbiota composition in phylum among the six groups. Compared with Control, CDR patients with or without IBS-type symptoms had a trend decrease in abundance of *Bacteroidetes*, but they did not obtain a statistically differences. A relatively decrease trend in abundance of *Bacteroidetes* was found in CDR groups compared with IBS (median proportional abundance CDR-IBS$^+$ *vs* IBS: 30.0% *vs* 47.6%, CDR-IBS$^-$ *vs* IBS: 34.7% *vs* 47.6%, Figure 2C), but no statistical difference was obtained (P>0.05). The proportion of *Firmicutes* displayed a relative trend to increase in CDR-IBS$^+$ and UCR-IBS$^+$ groups. No difference of *Fusobacteria* in phylum abundance was determined in current populations, although there was an increase trend for IBDR in different type no matter with or without IBS-type symptoms. Overall, there was no statistical difference of microbiota among IBS, CDR-IBS$^+$ and UCR-IBS$^+$ groups. Further analysis showed that there was no statistical difference in the alteration of microbiota community in the phylum level between UCR with or without IBS-type symptoms, as wells as CDR groups.

Comparison of Microbiota Composition among the Different Groups in Genus Level

The overall genera from each sample were displayed in bar-plot of taxonomic analysis. The main microbiota composition in genus level were as follows: *Bacteroides, Faecalibacterium, Prevotella, Escherichia, Roseburia, Blautia, Streptococcus, Fusobacterium, Haemophilus, Lachnospira,* (Figure 3A). There was no statistical difference in the changes of the genus level between Control and IBS subjects (Figure 3B). The relative abundance of *Bacteroides* were slightly increased in IBS, UCR-IBS$^+$ and UCR-IBS$^-$

patients compared to that in Controls, but the differences was not statistically significant. The mean abundance of Bacteroides was a trend to decrease in CDR-IBS$^+$ while to increase in UCR-IBS$^+$ compared with IBS groups, although the difference was no statistically significant. Compared with CDR-IBS$^-$, the abundance of *Faecalibacterium* and *Roseburia, Streptococcus* were a trend to increase while *Prevotella*, *Escherichia, and Fusobacterium* decrease in CDR-IBS$^+$ group, of which the number of *Faecalibacterium* was significantly higher while *Fusobacterium* lower in CDR-IBS$^+$ compared with CDR-IBS$^-$ (mean relative abundance of *Faecalibacterium*: 20.35% *vs* 5.18%, $P<0.05$; *Fusobacterium*: 1.51% *vs* 5.2%, $P<0.05$). In addition, the changes of microbiota community in UCR subjects were not the same as CDR. The results indicated that the abundance of the *Fusobacterium, Streptococcus* were increased, but *Faecalibacterium, Escherichia, Lachnospira* and were a slightly decrease in the group of UCR-IBS$^+$ compared to that in UCR-IBS$^-$, despite of all of the difference without statistically significant. Differences were also found between CDR and UCR at the genus level. There was a significantly greater abundance of *Fecalibacterium* in UCR-IBS$^-$ relative to CDR-IBS$^-$(mean proportional abundance 5.4% *vs* 16.6%, $P=0.012$) and a greater abundance of *Fusobacterium* in CDR-IBS$^-$ relative to UCR-IBS$^-$ (mean proportional abundance 5.6% *vs* 0.04%, $P=0.001$). The genera differences between CDR-IBS$^+$ and UCR-IBS$^+$ were not statistically significant. The difference of microbiota community among IBS, CDR-IBS$^+$ and UCR-IBS$^+$ groups were also been analyzed. The results showed that the mean abundance of *Faecalibacterium* and Streptococcus were a trend to increase while the level of *Prevotella* and *Lachnospira* were a trend to decrease in CDR-IBS$^+$ and UCR-IBS$^+$ groups compared with IBS groups. But further analysis of the genera among IBS, CDR-IBS$^+$, UCR-IBS$^+$ did not reveal a statistically significant difference.(Figure 3B). The difference of genera was displayed in detail in Table 3. As shown, other genera including: *Butyricimonas, Odoribacter, Enterococcus, Clostridium, Megasphaera, Megasphaera, Ruminofilibacter, Gemmiger, Desulfovibrio, Actinomyces, Akkermansia.* The increase and decrease trend of relative abundance in genera were listed in Table 3.

Discussion

The gut microbiome contains more than 100 trillion different microorganisms, including bacteria, fungi, viruses and protozoa[25]. Majority of the intestinal bacteria belong to the four phyla including *Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria*, and in healthy adults *Firmicutes* and *Bacteroidetes* are the main ones[26]. A number of studies have confirmed that changes in the structure and abundance of intestinal microbiota play an important biological role, including: immune regulation, nutrition, metabolism and defense against pathogens[27]. The more richness and diversity of microbiota seen as an indicator of good health, while decrease diversity and imbalance in microbiota may be closely related to a large range of diseases, especially in intestinal diseases. Studies have confirmed that disorders of intestinal bacteria are involved in the occurrence and development of intestinal diseases including IBS and IBD[1]. Partial IBD patients in remission may also suffer IBS-like symptoms, which may be related to the intestinal microbiota[28]. However, the results of our cross-sectional study indicate that the onset of IBS might not be related to the obvious alteration in intestinal bacteria. Further analysis revealed that CD patients in remission with IBS-type symptoms might be related to the increase of *Faecalibacterium* and decrease of *Fusobacterium*. There was no statistically significant difference in the abundance of intestinal bacteria

between the UCR-IBS$^+$ and UCR-IBS$^-$ groups at any taxonomic levels. UC patients in remission with IBS-type symptoms cannot be explained by changes in the abundance and structure of intestinal microbiota from our across-sectional study.

Our study found that the proportion of *Bacteroidetes* in IBS patients at the phylum level was a trend to increase, while the Firmicutes decreases compared with Control subjects, but the difference was not statistically significant. At genus taxonomic, the alterations of bacteria composition in IBS patients were also not apparently differed from control, while this result was not inconsistent with partial previous research. Indeed, the role of fecal microbiota in IBS is still controversial due to different sample source, study population, dietary habitual and environment factors. A recent prospective study by comparing 110 IBS patients and 39 health controls demonstrated that the diversity of fecal microbiota and the number of *Prevotella* were reduced in IBS patients[29]. A systematic review involving microbiota in IBS revealed that genus *Bacteroide* were increased in IBS patients compared with controls[30]. As shown in Fig. 3B, our results also shown an increase trend of *Bacteroide* compared with Controls, but failed to achieve statistically significant, this might be explained by different population, sample size. Consistent with our results, one previous study also found that no difference among major phyla or genera between IBS patients and controls[31].Our results do not support a role for fecal microbiota in the pathogenesis of IBS and correlate with some other studies that reported significantly differences between IBS patients and healthy controls in the composition of fecal microbiota[11, 32]. The discrepancy may be explained by different population, inter-individual variation, and no further classification of IBS. Further large-sample cohort study is needed to confirm the characteristics of the fecal microbiota of IBS patients.

The interaction between the intestinal microbiota and enteric intestinal immune system is the general mechanism of the pathogenesis of IBD, especially in active stage. Previous studies have confirmed that the disease activity of IBD patients is closely related to a decrease of anti-inflammatory bacteria species and an increase of pro-inflammatory bacteria species, as well as the decrease of overall alpha diversity[33]. But some patients in remission of IBD suffer from varying degrees of IBS-like symptoms[34, 35]. Is there any relationship between the intestinal flora of IBD patients with IBS like symptoms and that of IBS patients? Therefore, we focus to explore the alteration of microbiota community and diversity in IBDR patients with IBS-type symptoms. The results indicated that the decreased richness (Chao1 and ACE index) were observed in CDR-IBS$^-$ group, while not in CDR-IBS+, UCR-IBS$^+$, UCR-IBS$^-$ group compared with controls. Furthermore, there was trend to decrease number of *Bacteroidetes* and an increase number of *Fusobacteria* in CDR-IBS$^+$ and CDR-IBS$^-$ based on the analysis of phylum taxonomic levels compared with control and IBS group, this might be due to the different disease. For genus taxonomic analysis, the bacteria community of fecal sample from CDR-IBS$^-$ exhibited an apparently difference from other groups by a markedly higher numbers of *Fusobacterium* and increased trend of *Escherichia*, while lower numbers of *Lachnospira* and *Faecalibaterium* compared to that in CDR-IBS$^+$. However, there was no difference in richness and diversity across the CDR-IBS$^+$, CDR-IBS$^-$ group, as well as between UCR-IBS$^+$ and UCR-IBS$^-$. A recent study conducted in IBS subjects have shown that *Fusobacterium* might exacerbate visceral hypersensitivity[36], which is not consistent with our study. It may be that the study focused on diarrhea

predominant-IBS (IBS-D) and we focused on the relationship between IBS symptoms and microbiota during IBD remission. In addition, *Faecalibaterium* belong to butyrate-producing genera[37], is elevated in fecal sample of patients with functional bowel disease. In consistent to previous studies, the relative abundance of *Faecalibaterium* was significantly higher than that in CDR-IBS^{+} group, indicating the kind of genera might play an important role in formation of IBS-type symptoms. To date, our current results could not draw an insight that there is a possible association between the presence of IBS-type symptoms in CD or UC patients in remission. Therefore, it is impossible to comment on any causal relationship between specific microbiome characteristics and the development of IBS-type symptoms which is consistent with previous study[21].

Of course, this study has certain limitations. Firstly, it is a cross-sectional study and do not compare the dynamic changes of intestinal bacteria in the development of IBD patients with IBS-type symptoms. Meanwhile, the associated microbiota in mucosa may more accurately reflect the relationship between microbiota and disease, but in our study only fecal microbiota was detected and analyzed. Secondly, IBS is clinically divided into several types, including constipation (IBS-C),diarrhea (IBS-D), or a combination of both (IBS-mixed) according the Rome IV Diagnostic Criteria[38]. The pathogenesis of different types of IBS is somewhat different. However, due to the small sample size in this single center study, no subgroup analysis was performed on the type of confirmed IBS patients, and IBS-type symptoms. In addition, this study used CDAI and Mayo scores to define the active and remission stage of CD and UC respectively, which are not the gold standard for intestinal inflammation. This is a possible reason that our inability to account the significantly alteration of microbiota in CDR-IBS^{-}.

Despite of observation study by our study and previous study[21] which failed to achieve any difference in CDR-IBS^{-}composition and diversity in IBDR patients reporting IBS-type symptoms. However, clinical trials including probiotics and a low fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAP) diets have obtained promising results in IBS, indicating the role involving intestinal microbiota[39, 40]. Also, the effect of probiotics and fecal bacteria transplantation (FMT) in the treatment of IBD are multiple beneficial, and no attention is focus to the impact of these treatments on IBD-like symptoms[41, 42].

In conclusion, the obtained results from our study did not find any difference in intestinal microbiota between IBD patients in remission with IBS-type symptoms and those without IBS-type symptoms. This result provides a certain basis for recommendations for future trials on the management of IBS-type symptoms. In the future, we still need to have a better understanding of the mechanism of IBD with IBS-type symptoms in the absence of persistent disease activity and strive to find effective treatments to relieve clinical symptoms and improve life treatment.

Declarations

Data Availability Statement

The original data supporting this research conclusion will be availability from the corresponding author.

Ethics Statement

The study protocol and procedure were approved by Ethics Committee of the First Affiliated Hospital of Nanjing Medical University and informed consents were obtained from all enrolled participants before the data and sample collection (Ethics number: 2018-SR-061).

Conflicts of Interest

The authors declare that they have no conflict of interest.

Author Contributions

HJ Zhang, XF Cui and HY Wang designed the study and developed the concept. XF Cui and HY Wang performed experiments, performed statistical analysis. XF Cui wrote the manuscript. ZP Ye, XY Qiu collected fecal samples. Yi Li helped to modify the manuscript. HJ Zhang revised and finalized the manuscript. HJ Zhang supervised the report. All authors read and approved the final version of the manuscript.

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Tables

Table 1. Clinical characteristics of all included patients

	Control	IBS	CDR-IBS+	CDR-IBS-	UCR-IBS+	UCR-IBS-
n	18	34	10	15	10	10
Age, mean±SD, yr	37.9±8.3	42.9±14.0	30.9±13.9	29.8±10.5	37.1±13.1	42.4±12.7
Sex, male/female	10/5	13/21	5/5	11/4	8/2	8/2
Disease duration, median(range)		12(6-240)	24(12-60)	24(10-120)	27(12-120)	27(9-120)
Montreal A (Age of onset, yr) , n (%)						
A1(<16)			0	1(6.7)		
A2(17~40)			8(80)	12(80)		
A3(>40)			2 (20)	2(13.3)		
Montreal A (Location) , n (%)						
L1(ileal)			3(30)	5(33.3)		
L2(colonic)			4(40)	3(20)		
L3(ileocolonic)			3(30)	7(46.7)		
L4(upper gastrointestinal tract)			0	0		
Montreal B (Behavior) , n (%)						
B1(nonstricturing, nonpenetrating)			9(90)	13(86.7)		
B2(stricturing)			1(10)	1(6.7)		
B3(penetrating)			0	1(6.7)		
Montreal E, n (%)						
E1(ulceration proctitis)					3(30)	3(30)
E2(left sided ulceration colitis)					3(30)	4(40)
E2(extensive ulceration colitis)					4(40)	3(30)
Therapy, n (%)						
5-ASA			2(20)	1(6.7)	8(80)	7(70)
Azathioprine			3(30)	2(13.3)	0(0)	0(0)
Steroids			0(0)	0(0)	2(20)	3(30)
infliximab			5(50)	11(73.3)	0(0)	0(0)
nutritional treatment			1(10)	2(13.3)	0(0)	0(0)

CD: Crohn’s disease, CDR: Crohn’s disease in remission, CDR-IBS+: CDR with IBS-type symptoms, CDR-IBS-: CDR without IBS-type symptoms. UC: ulcerative colitis, UCR: ulcerative colitis in remission, UCR-IBS+: UCR with IBS-type symptoms, UCR-IBS-: UCR without IBS-type symptoms; SD: standard deviations; 5-ASA: 5-aminosalicylic acid

Table 2: The differences of the number of Tags and OUTs

	Control	IBS	CDR-IBS$^+$	CDR-IBS$^-$	UCR-IBS$^+$	UCR-IBS$^-$
Tag number	48231±3293	50825±2820	48491±3337	51135±2373	51259±4455	50836±1891
OUT numbers	240.8±66.75	221.4±70.84	225.5±70.48	163.7±65.98*	204.3±79.91	213.2±78.62

CD: Crohn’s disease, CDR: Crohn’s disease in remission, CDR-IBS+: CDR with IBS-type symptoms, CDR-IBS-: CDR without IBS-type symptoms. UC: ulcerative colitis, UCR: ulcerative colitis in remission, UCR-IBS+: UCR with IBS-type symptoms, UCR-IBS-: UCR without IBS-type symptoms. Compared with HC, IBS, CDR-IBS+, $^*P<0.05$.

Table 3. The significant differences of microbial distribution of taxa (phylum and genus) in patients with inflammatory bowel disease in remission with IBS-type symptoms

	IBS	CDR-IBS^{+}	CDR-IBS^{-}	UCR-IBS^{+}	UCR-IBS^{-}	CDR-IBS^{+} *vs*UCR-IBS^{+}	CDR-IBS^{-} *vs*UCR-IBS^{-}	CDR-IBS^{+} *vs* IBS	UCR-IBS^{+}*vs* IBS	CDR-IBS^{+}*vs* CDR-IBS^{-}	UCR-IBS^{+} *vs*UCR-IBS^{-}
Bacteroidetes											
Butyricimonas	⬆b		⬇a		⬇a				a		
Odoribacter			⬇b		⬇a						
Firmicutes											
Enterococcus		⬆b	⬆b	⬆a	⬆a			⬆a			
Clostridium		⬆c					b				
Lachnospira			⬇a				b				
Faecalibacterium			⬇b				a			b	
Megasphaera		⬆b	⬆b	⬆a	⬆a						
Ruminofilibacter				⬆c		b			c		
Gemmiger			⬇b								
Proteobacteria											
Sutterella			⬇a	⬇a							
Desulfovibrio			⬇b	⬇b	⬇a				a		
Actinobacteria											
Actinomyces		⬆a	⬆c	⬆a			b				
Fusobacteria											
Fusobacterium	⬆b		⬆c				b		b		
Verrucomicrobia											
Akkermansia			⬇a	⬇b		a					

⬆ and ⬇ relative to controls. CD: Crohn's disease, CDR: Crohn's disease in remission, CDR-IBS^{+}: CDR with IBS-type symptoms, CDR-IBS^{-}: CDR without IBS-type symptoms. UC: ulcerative colitis, UCR: ulcerative colitis in remission, UCR-IBS^{+}: UCR with IBS-type symptoms, UCR-IBS^{-}: UCR without IBS-type symptoms. [a] $P<0.05$; [b] $P<0.001$; [c] $P<0.001$.

Figures

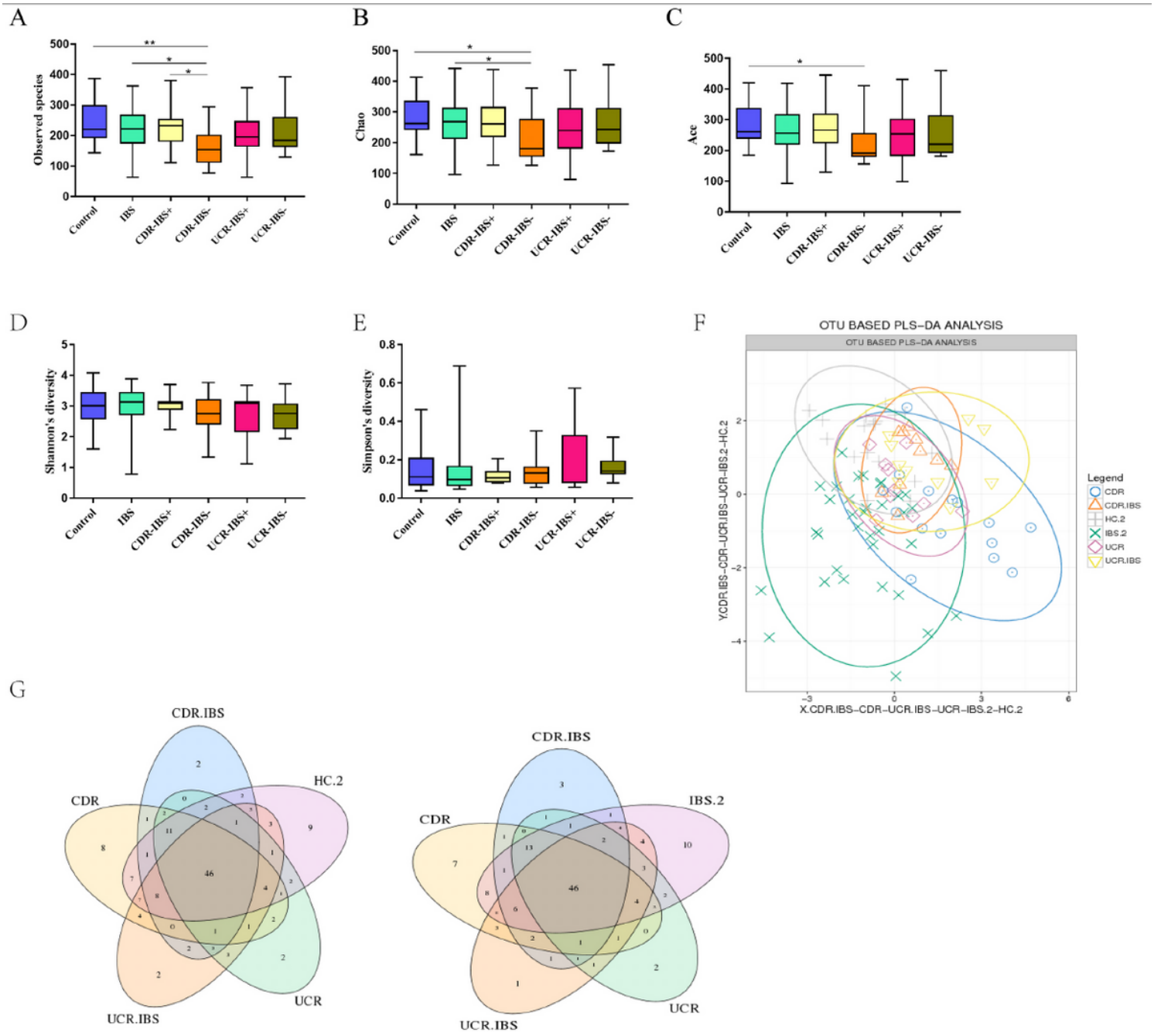

Figure 1

Alpha diversity analysis including community richness (Observed species, Chao, Ace) and diversity (Shannon, Simpson) and bacteria community for each group. (A) Observed species; (B)Chao; (C) Ace; (D) Shannon; (E) Simpson; (F) partial least-squares discriminant analysis (PLS-DA) plots; (G) Venn plots. *P<0.05. CD: Crohn's disease, CDR: Crohn's disease in remission, CDR-IBS+: CDR with IBS-type symptoms, CDR-IBS-: CDR without IBS-type symptoms. UC: ulcerative colitis, UCR: ulcerative colitis in remission, UCR-IBS+: UCR with IBS-type symptoms, UCR-IBS-: UCR without IBS-type symptoms.

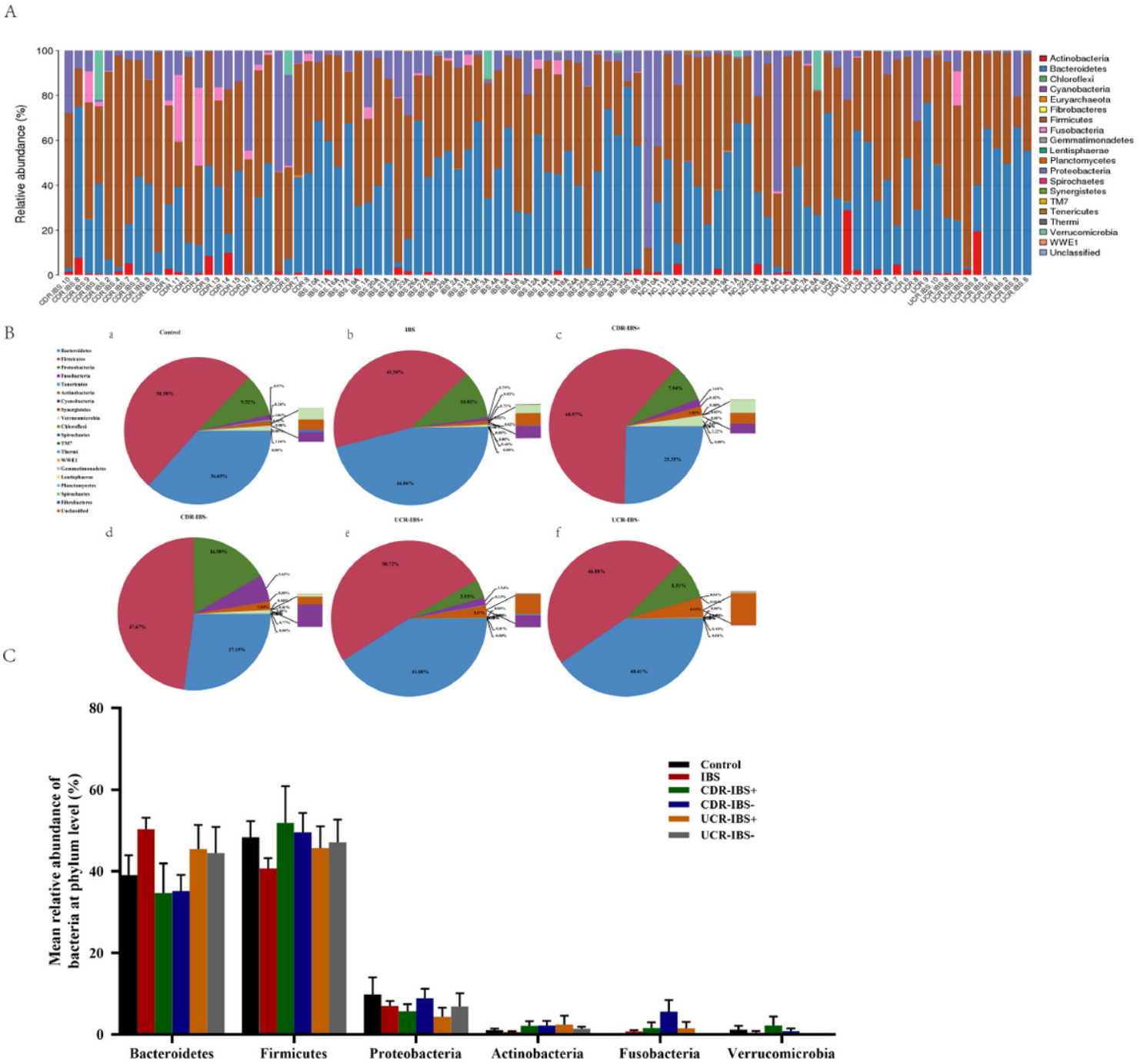

Figure 2

Taxonomic composition of bacteria in phylum level. (A) Individually; (B) Integrated chart for different groups, as followed: a: Control, b: IBS, c: CDR-IBS+, d: CDR-IBS-, e: UCR-IBS+, f: UCR-IBS-. (C) The boxplots shown the phylum abundance of the 6 most bacterial among the six groups. CD: Crohn's disease, CDR: Crohn's disease in remission, CDR-IBS+: CDR with IBS-type symptoms, CDR-IBS-: CDR without IBS-type symptoms. UC: ulcerative colitis, UCR: ulcerative colitis in remission, UCR-IBS+: UCR with IBS-type symptoms, UCR-IBS-: UCR without IBS-type symptoms.

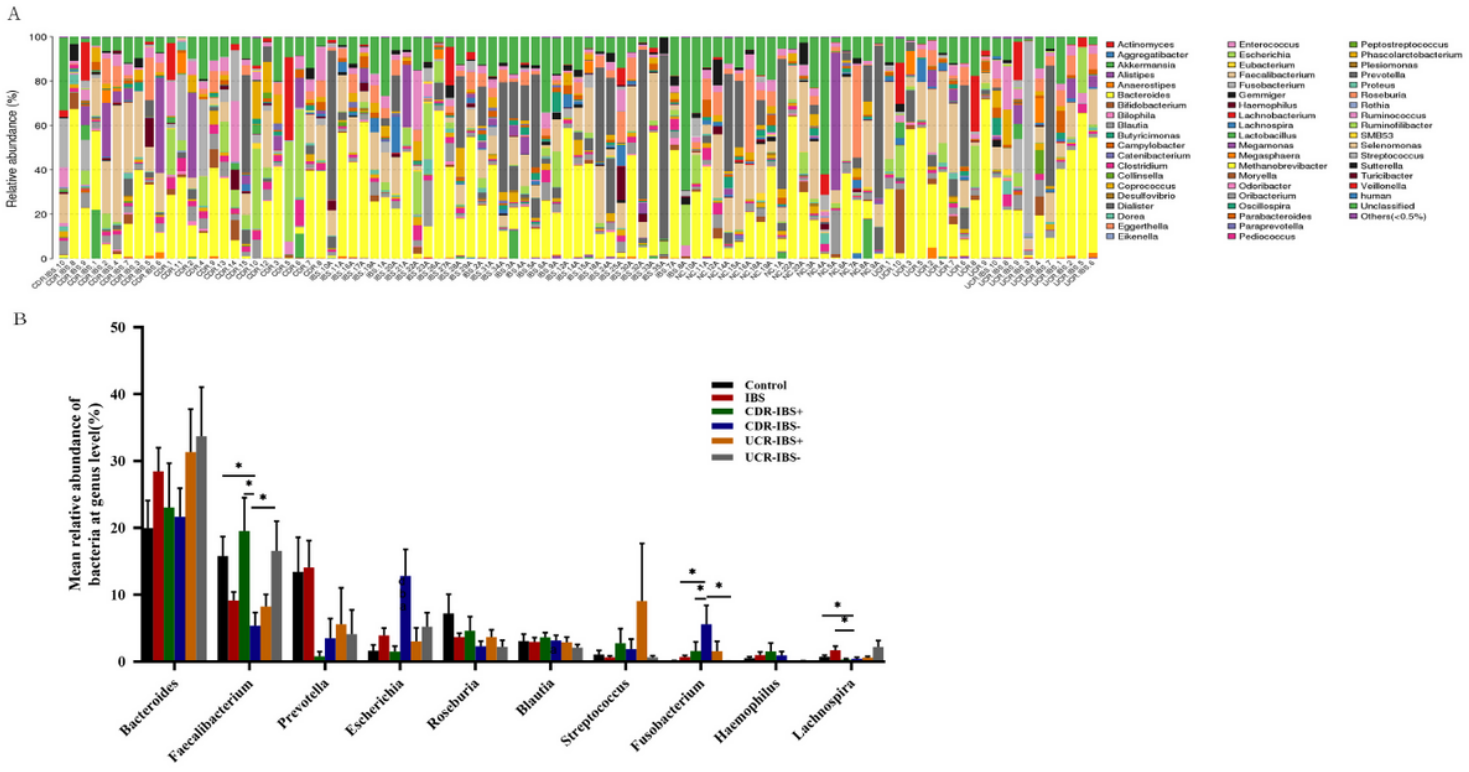

Figure 3

Analysis of taxonomic composition in genus level. (A) individually; (B) the boxplot indicated the most abundance genera bacteria in different groups including Control, IBS, CDR-IBS+, CDR-IBS-, UCR-IBS+, UCR-IBS-. *P<0.05. CD: Crohn's disease, CDR: Crohn's disease in remission, CDR-IBS+: CDR with IBS-type symptoms, CDR-IBS-: CDR without IBS-type symptoms. UC: ulcerative colitis, UCR: ulcerative colitis in remission, UCR-IBS+: UCR with IBS-type symptoms, UCR-IBS-: UCR without IBS-type symptoms.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigure1.pdf