Fecal microbiota profile in patients with inflammatory bowel disease in Taiwan

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Abstract

Background: Inflammatory bowel disease (IBD) is a chronic inflammatory disease associated with complicated interaction between immune, gut microbiota, and environmental factors in a genetically vulnerable host. Dysbiosis is often seen in patients with IBD. We aimed to investigate the fecal microbiota in patients with IBD and compared them with a control group in Taiwan.

Methods: In this cross-sectional study, we investigated fecal microbiota in 20 patients with IBD and 48 healthy controls. Fecal samples from both IBD patients and controls were analyzed by the next-generation sequencing method and relevant software.

Results: The IBD group showed lower bacterial richness and diversity compared with the control group. The principal coordinate analysis also revealed the significant structural differences between the IBD group and the control group. These findings were consistent whether the analysis was based on an operational taxonomic unit or amplicon sequence variant. However, no significant difference was found when comparing the composition of fecal microbiota between ulcerative colitis (UC) and Crohn's disease (CD). Further analysis showed that *Lactobacillus, Enterococcus,* and *Bifidobacterium* were dominant in the IBD group, whereas *Faecalibacterium* and *Subdoligranulum* were dominant in the control group at the genus level. When comparing UC, CD, and control group, *Lactobacillus, Bifidobacterium,* and *Enterococcus* were identified as dominant genera in the UC group. *Fusobacterium* and *Escherichia_Shigella* were dominant in the CD group.

Conclusion: Compared with the healthy control, the IBD group showed dysbiosis with a significant decrease in both richness and diversity of gut microbiota.

Keywords: Colitis, ulcerative; Dysbiosis; Gastrointestinal microbiome; High-throughput nucleotide sequencing; Inflammatory bowel diseases

1. INTRODUCTION

Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), is a chronic progressive inflammatory disorder that affects the gastrointestinal (GI) tract.[1,2] The prevalence of IBD increases globally.[3] Although the incidence of IBD is low in Taiwan, the prevalence of IBD kept raising in the past decades.[4] The urge of understanding the pathogenesis and to facilitate the treatment strategy of IBD is growing. The most accepted hypothesis of IBD pathogenesis is an abnormal immune response against the gut microbiota that is triggered by the external environment in a genetically vulnerable host.[2,5–7]

Dysbiosis of the gut microbiota alters host-microbiota interaction and the host immune system. Growing evidence has shown that dysbiosis is associated with several diseases, such as IBD, metabolic syndrome, and cardiovascular disease.[7,8] The composition of microbiota in IBD is altered compared with that in healthy subjects.[9,10]

The alteration of gut microbiota in IBD patients was found in many studies. Among them, a decrease of *Firmicutes* and an increase of *Proteobacteria* were reported mostly.[7,11–14] By fermenting resistant starch or indigestible carbohydrates, intestinal bacteria produce short-chain fatty acids (SCFAs). SCFAs are major anions in the colon, mainly as acetate, propionate, and butyrate. Butyrate is the energy source for colonic epithelial cells. The levels of SCFAs are significantly decreased in IBD and may be crucial in intestinal and immune homeostasis.[15,16]

Our study aims to investigate the fecal microbiota in patients with IBD and compare them with a healthy control group in Taiwan and to estimate the relationship between the microbiota and IBD.

2. METHODS

2.1. Patients

Patients diagnosed with IBD were enrolled in this study. The diagnosis of IBD was based on medical history, clinical evaluation,

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Conflicts of interest: Dr Yi-Hsiang Huang, Dr Ming-Chih Hou, and Dr Fa-Yauh Lee, editorial board members at Journal of the Chinese Medical Association, have no roles in the peer review process of or decision to publish this article. The other authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

Journal of Chinese Medical Association. (2021) 84: 580-587.

Received December 29, 2020; accepted March 8, 2021.

doi: 10.1097/JCMA.0000000000000532.

typical endoscopic, and histological findings, and the exclusion of an infectious etiology. Detailed clinical and laboratory data, including smoking, alcohol drinking, and the underlying comorbidities, such as diabetes, dyslipidemia, and medication including mesalazine, steroid, azathioprine, and biological agents were recorded at out-patient clinical departments. Anthropometric measurements (including body height and weight, waist circumference, body mass index [BMI], and blood pressure [BP]) were taken by experienced nursing staff. Blood tests including complete blood count, differentiated count, C-reactive protein, and stool routine were measured. Mayo score for UC and CD activity index (CDAI) for CD were calculated.[17,18]

Patients who had severe cardiovascular, pulmonary, hepatic, or renal disease; who had GI tract surgery; and who have taken proton pump inhibitors (PPIs), histamine-2 receptor antagonists, nonsteroidal anti-inflammatory drugs (NSAIDs), antibiotics, or probiotics within 4 weeks of sample collection were excluded.

Forty-eight subjects for the vaccination of hepatitis B virus/hepatitis A virus or for the physical checkup without prior IBD history and no surgery history for GI tract were recruited from the out-patient department as the control group with the same exclusion criteria as the IBD group.

This study complied with the standards of the Declaration of Helsinki and current ethical guidelines and was approved by our hospital's Institutional Review Board (approval no. 2018-07-013B). All enrolled subjects had signed the informed consent.

2.2. Stool bacterial genomic DNA extraction and PCR amplification

Fresh stool samples were collected. Bacterial genomic microbial DNAs were extracted for direct use in 16S rRNA gene sequencing.[17,18] The amounts and quality of isolated genomic DNA were determined with NanoDrop ND-1000 (Thermo Scientific, Wilmington, DE, USA). Genomic DNA was stored at –80°C before 16S rRNA sequencing. One microliter of sample DNA (10 pg-500 ng) was used as a template in a polymerase chain reaction (PCR) reaction for bacteria 16S rRNA variable region V3-V4. The primer set for the reaction was chosen with 341F_V3_illumina (5'-CCTACGGGNGGCWGCAG-3') and 805R_V4_illumina (5'-GACTACHVGGGTATCTAATCC-3').[17] PCR consisted of an initial denaturation at 94°C for 2 minutes, 30 cycles of 92°C for 20 seconds, 55°C for 30 seconds, and 68°C for 1 minute for amplification, 68°C for 1 minute to finish replication on all templates, and stored at 4°C. Dual-indexes (barcodes) were used for each sample before sequencing and next-generation sequencing was performed by the Illumina MiSeq Desktop Sequencer following the standard protocol.[18]

2.3. Data processing and statistical analysis

The raw sequencing reads were assembled using FLASH v.1.2.7.[19] The quality of reads was assessed by QIIME 1.9.1 pipeline and low-quality reads ($Q < 20$) were truncated.[20] Operational taxonomic unit (OTU) was clustered at 97% sequence identity using the UPARSE function in the USEARCH v.7 pipeline.[21,22] The RDP classifier (v.2.2) algorithm was used to annotate the taxonomy of each 16S rRNA gene sequence based on the information of Silva Database v.132,[23–25] with a confidence threshold of 80%.

The α-diversity indices evaluating gut microbial community richness (the observed OTUs and Chao1 indices) and community diversity (the Shannon and Simpson indices) were calculated using QIIME pipeline. Principal coordinate analysis (PCoA) based on Bray-Curtis distance was conducted using the R package. (v.2.15.3).[26] Analysis of similarities (ANOSIM) and permutational multivariate analysis of variance (PERMANOVA) were used to determine whether the community structures significantly vary among and within groups.

Welch's *t*-test was performed using the STAMP software (v2.1.3).[27] Statistically significant differences in the relative abundances of taxa were recognized and calculated using the linear discriminant analysis (LDA) effect size (LEfSe) analysis.[28] In this study, taxa with LDA score (log 10) >4 was considered significant.

In comparison, we also processed the reads with QIIME 2 v.2019.10 pipeline.[29] Deblur was used for denoising.[30] The amplicon sequencing variants (ASVs) were identified and the taxonomic features were classified by the information of Silva Database v.132. α-diversity and β-diversity were also evaluated between the IBD group and the control group, between the UC and CD groups as well.

All data were expressed as means ± SD. If some parameters were not normally distributed, nonparametric analysis was used. Results were compared between groups depending on the type of data analyzed using the Fisher's exact, Student's *t*, or nonparametric Mann-Whitney *U* tests. Statistical analyses were performed using Sample Power release 2.0 and SPSS for Windows version 14.0 (both by SPSS Inc., Chicago, IL, USA). All *p* values were two-tailed, and a *p* value of <0.05 was considered statistically significant.

3. RESULTS

3.1. Baseline clinical characteristics of study participants

The characteristics of 14 patients with UC, 6 patients with CD, and 48 healthy controls are demonstrated in the Table 1. The mean age was 49.8 ± 14.1 years in the IBD group and 49.9 ± 11.6 in the controls. Among the 14 patients with UC, nine patients were in remission (Mayo score 0-2), four patients had active disease with mild inflammation (Mayo score 3-5), and one patient had active disease with moderate inflammation (Mayo score 6-10). For the six patients with CD, five patients were in remission (CDAI < 150) and one patient had moderate inflammation (CDAI = 300) when entered the study. In the 20 study subjects, 10 patients had taken azathioprine, five had received biological agents (including one CD patient for concurrent rheumatoid arthritis) at the data and sample collection. Only one patient with UC did not receive any medication for disease control at the time of stool collection.

3.2. The microbiota composition in patients with IBD and healthy controls

After 16S rRNA gene sequencing and quality filtering, 5.4 million reads from a total of 6.5 million pair-end reads were obtained. A total of 835 OTUs were identified from 68 stool samples.

The α-diversity was estimated. The richness of microbiota was estimated by observed OTUs and Chao1 indices, and the evenness was evaluated by Shannon and Simpson index. Compared with the control group, the IBD group had significantly lower richness (observed OTUs, $p = 0.02$; Chao1, $p = 0.03$). The IBD group was also less evenness than the control (Shannon, $p < 0.01$; Simpson, $p < 0.01$) (Fig. 1).

The overall structure of the fecal microbiome among the two groups was evaluated. PCoA showed a separation between the control group and the IBD group, which revealed significant differences in bacterial genera abundance (Fig. 2). Significant intercommunity differences among the two groups were demonstrated by ANOSIM ($R = 0.3344$, $p = 0.001$) and PERMANOVA analyses ($p < 0.001$).

Phyla *Bacteroidetes*, *Firmicutes,* and *Proteobacteria* consisted >90% of stool microbiota in both control and IBD groups. Although the IBD group had a higher percentage of *Firmicutes* (41.5% vs. 37.9%) and a lower proportion of *Bacteroidetes*

Table 1

Baseline clinical characteristics of study subjects

	IBD (n = 20)	Control (n = 48)	*p*	UC (n = 14)	CD (n = 6)	*p*
Women/men, n (%)	3/17	19/29	<0.01	2/12	1/5	0.68
Age, y	49.8 ± 14.1	49.9 ± 11.6	0.96	50.1 ± 11.1	48.8 ± 11.2	0.51
BMI, kg/m^2	21.5 ± 9.4	23.2 ± 2.8	0.38	20.0 ± 10.3	26.2 ± 3.82	0.27
CRP, mg/dL				0.49 ± 0.58	0.65 ± 0.29	0.57
WBC, /μL	6266.7 ± 1822.4	5313.2 ± 1267.9	0.03	6407.7 ± 2040.6	5900.0 ± 1179.0	0.61
Hb, g/dL	13.6 ± 2.2	13.7 ± 1.30	0.80	13.31 ± 2.54	14.3 ± 1.15	0.41
Mayo score for UC, n (%)				n = 14		
0-2				9 (64.3)		
3-5				4 (28.6)		
6-10				1 (7.1)		
>10				0 (0)		
CDAI for CD, mean ± SD (range)					78.5 ± 111.9 (11-300)	
Medication						
Mesalazine, n (%)	18 (90)					
Steroid, n (%)	2 (10)					
Immunotherapy, n (%)	10 (50)					
Biologic agents, n (%)	5 (25)					
Enema, n (%)[a]	9 (45)					

Data are expressed as the mean ± SD.

BMI = body mass index; CD = Crohn's disease; CDAI = Crohn's disease activity index; CRP = C-reactive protein; Hb = hemoglobin; IBD = inflammatory bowel disease; UC = ulcerative colitis; WBC = white blood cell.

[a]One patient received enema with steroid, eight patients received enema with 5-aminosalicylic acid.

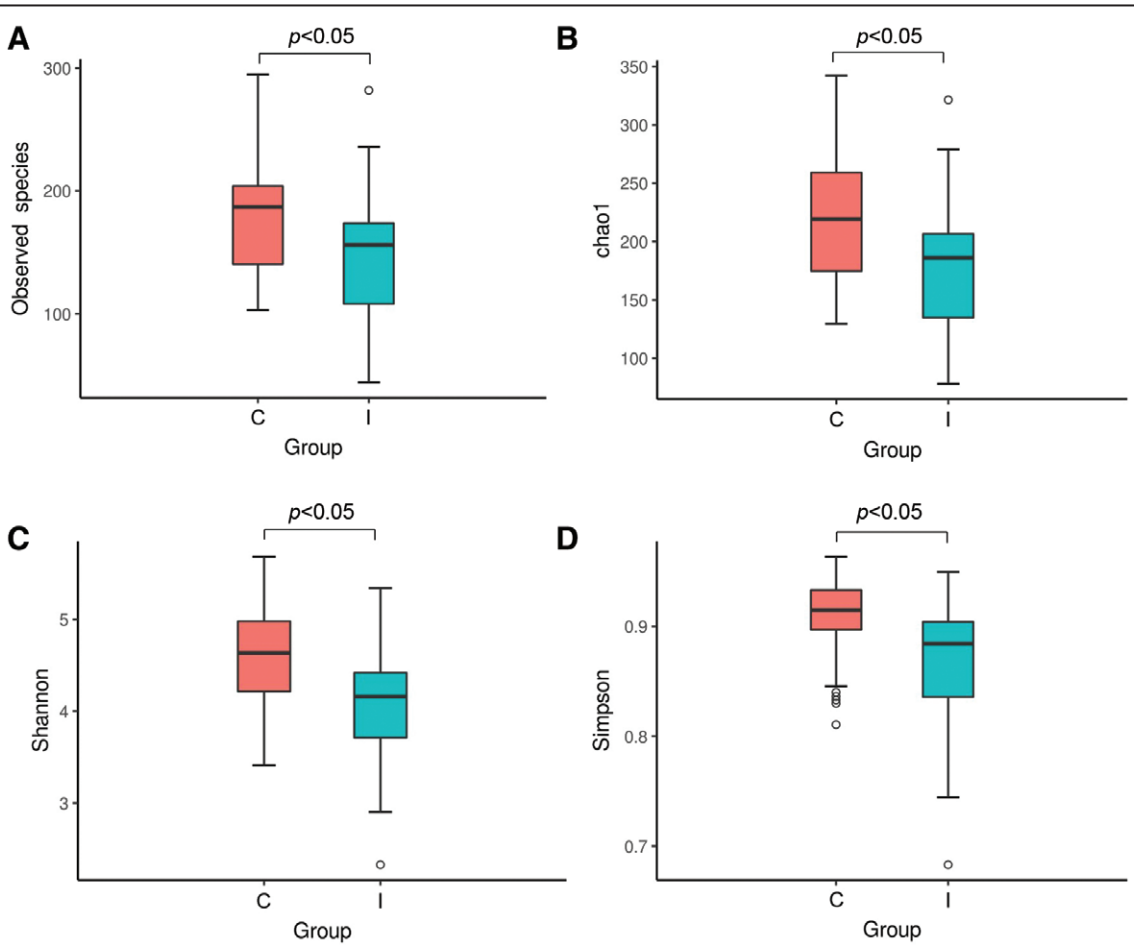

Fig. 1 The diversity of fecal microbiota between patients with inflammatory bowel disease (IBD) and controls. Observed operational taxonomic units revealed a significant difference between the IBD group and the control group ($p = 0.02$, A). Chao1 estimated a significant difference between the IBD group and the control group ($p = 0.03$, B). The control group showed higher bacterial diversity, as estimated by the Shannon diversity index and Simpson index, when compared with the IBD group ($p < 0.01$, C and D). The boxes (containing 50% of all values) showed the median (horizontal line across the middle of the box) and the interquartile range, whereas the blackspots represented the 10th and the 90th percentiles. C = control; I = IBD

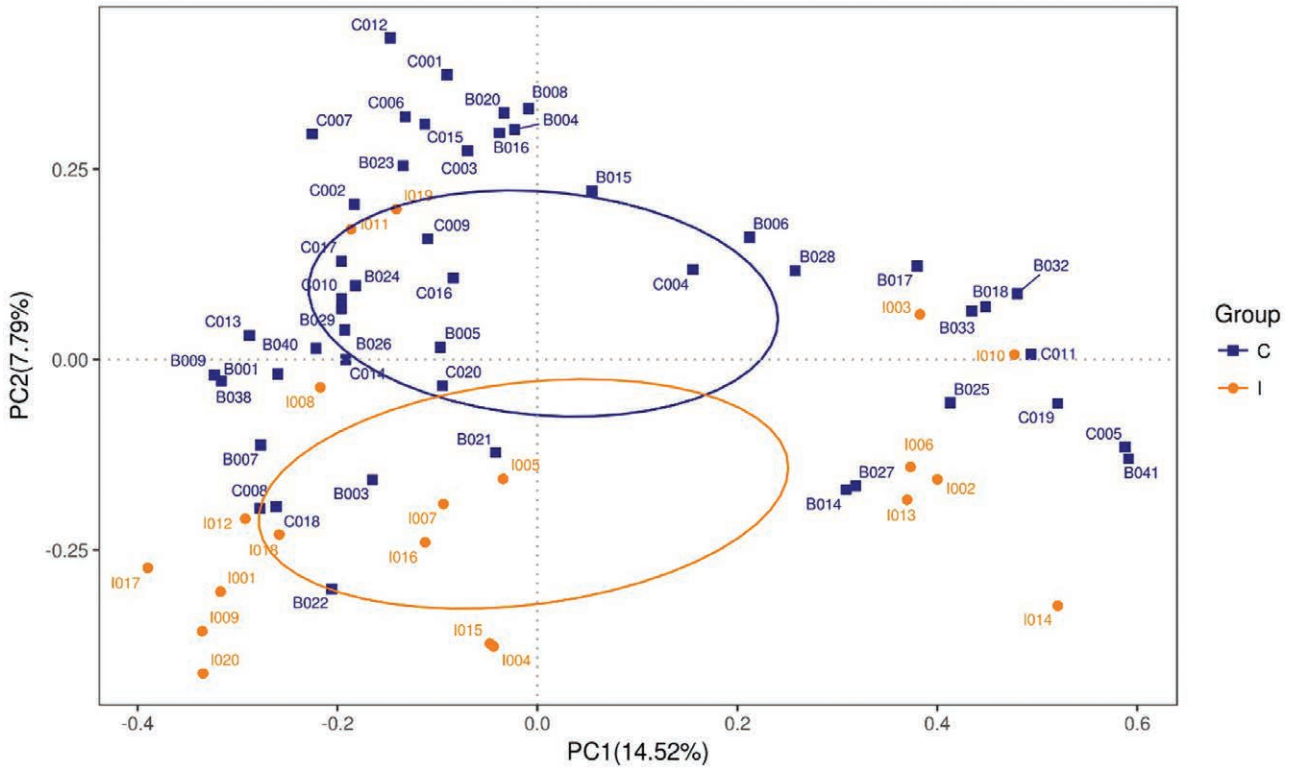

Fig. 2 Principal coordinate analysis (PCoA) of bacterial genera abundance. Gut microbial genera composition of the control and the inflammatory bowel disease groups was analyzed with PCoA. Analysis of similarities revealed significant differences in the structures of both groups (R = 0.3344, p = 0.001). Permutational multivariate analysis of variance analyses also showed significant differences between the two groups (p < 0.001). The axes represent the three dimensions explaining the greatest proportion of variance in the communities. Each symbol represents a sample.

(40.9% vs. 51.3%) compared with the control group, the *Firmicutes to Bacteroidetes* ratio was not statistically different between the two groups.

There were nine dominant classes were found in both groups, including *Bacteroidia*, *Clostridia*, *Negativicutes*, *Gammaproteobacteria*, *Bacilli*, *Fusobacteriia*, *Verrucomicrobiae*, *Actinobacteria*, *Coriobacteriia*, and *Deltaproteobacteria*. Bacterial class *Bacilli* belonging to *Firmicutes* (5.5% vs. 0.2%, p = 0.03) and *Actinobacteria* belonging to *Actinobacteria* (2.4% vs. 0.5%, p < 0.01) were significantly higher in the IBD group when compared with the control group. In the contrast, bacterial class *Bacteroidia* belonging to *Bacteroidetes* (40.9% vs. 51.3%, p = 0.04) and *Deltaproteobacteria* belonging to *Proteobacteria* (0.2% vs. 0.7%, p < 0.01) were significantly lower in the IBD group when compared with the control group.

In genus level, *Faecalibacterium* (2.6% vs. 7.6%, p < 0.01), *Subdoligranulum* (0.1% vs. 1.9%, p < 0.01), *Parabacteroides* (1.6% vs. 3.2%, p = 0.03), *Ruminococcaceae UCG-002* (0.4% vs. 1.3%, p < 0.01), and *Paraprevotella* (0.1% vs. 0.9%, p = 0.03) were significant lower in the IBD group when compared with the controls. *Bifidobacterium* (2.3% vs. 0.5%, p < 0.01), *Ruminococcus gnavus* group (1.1% vs. 0.2%, p < 0.01), *Streptococcus* (1.0% vs. 0.2%, p = 0.02), and *Blautia* (0.9% vs. 0.3%, p = 0.01) were significant higher in the IBD group compared with the control group. Analysis of composition of microbiomes (ANCOM) in genus level revealed that *Lactobacillus, Sellimonas*, and *Bifidobacterium* were dominant in the IBD group and *Subdoligranulum* was dominant in the control group. *Bifidobacterium longum* subsp. *longum* was significantly increased in the IBD group when compared with controls.

The discriminant analysis by using the LEfSe method was applied to recognize the key taxa accountable for the difference between the two groups. The recognized taxa were emphasized on the cladogram along with their LDA scores. *Lactobacillus* and *Veillonella* were identified as dominant genera in the stool microbiome of the IBD group, whereas genera *Faecalibacterium* was dominant in the control group (Fig. 3). Genera *Bifidobacterium, Streptococcus, Blautia, Enterococcus*, *Proteus,* and *Helicobacter* were more abundant in the IBD group than in the controls, yet the LDA scores did not reach 4.0. To be recorded, *Proteus mirabilis* was only recognized in the stool samples of patients with IBD, but the abundance was low. In contrast, genera *Akkermansia*, *Ruminococcaceae_UCG_002,* and *Ruminococcaceae_UCG_003* were more dominant in the control group, but with LDA scores <4.0.

3.3. The microbiota composition analysis with ASV

A total of 2034 ASV were identified from 68 stool samples with a total frequency of 976 619. The mean frequency per feature was 480.1. Similar to the analysis based on OTU clusters, the IBD group had a lower richness and lower evenness compared with the control group (observed OTUs, p < 0.01; Shannon, p < 0.01). The β-diversity between the two groups also had a significant difference in Unweighted UniFrac distance (p = 0.03) and Bray-Curtis distance (p < 0.01). Furthermore, PCoA presented a significant separation between the control group and the IBD group, which revealed significant differences in bacterial genera abundance (Fig. 4A). ANCOM revealed that *Lactobacillus and Subdoligranulum* were the most dominant genera in the IBD group and the control group, respectively. The LEfSe method discovered that *Lactobacillus*, *Enterococcus,* and *Bifidobacterium*

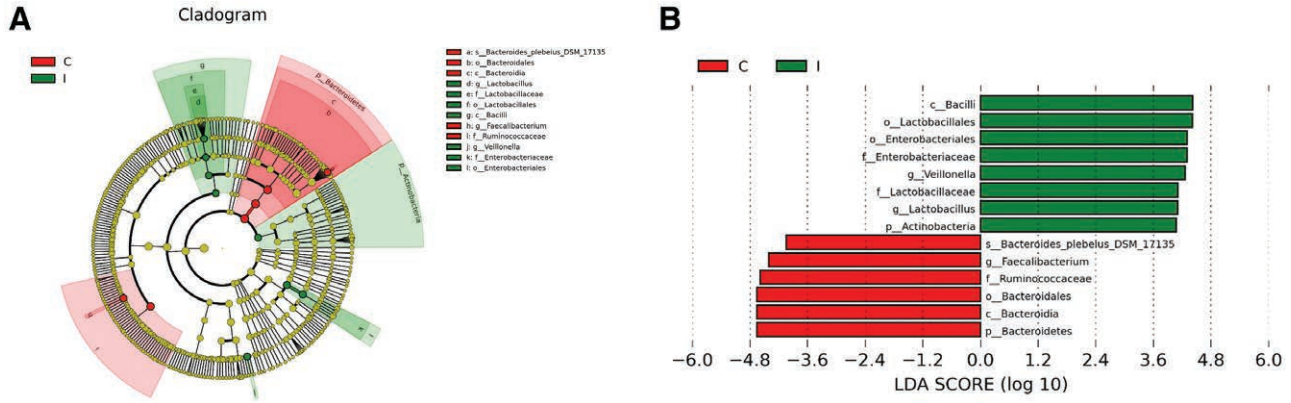

Fig. 3 Known taxa abundance reported by LEfSe in the bacterial community. Cladogram showed taxa with the higher differences in relative abundances between the control and the inflammatory bowel disease groups. The circle sizes in the cladogram plot were proportional to the bacterial abundances. From the inside to the outside, the circles represented the phylum, class, order, family, and genus. Only taxa with a linear discriminant analysis (LDA) score of >4 and $p <$ 0.05 in the Wilcoxon signed-rank test are shown (A). A logarithmic LDA-score cutoff of 4.0 was used to identify significant taxonomic differences between the fecal microbiomes of controls and patients with IBD (B).

were dominant genera in the IBD group, whereas genera *Faecalibacterium* and *Subdoligranulum* were dominant in the control group (Fig. 5A, B). Yet, genus *Veillonella* was not recognized to be prominent in the IBD group in the analysis with ASV.

Additionally, the α-diversity of the CD, UC, and control groups was analyzed. The CD group remained significantly lower α-diversity compared with the control group (observed OTUs, $p <$ 0.01; Shannon, $p <$ 0.01). However, the α-diversity revealed no significant difference between the UC and control group (observed OTUs, p = 0.08; Shannon, p = 0.06), and between the CD and UC group (observed OTUs, p = 0.06; Shannon, p = 0.32).

The β-diversity between the CD group and the control group also had a significant difference in Unweighted UniFrac distance ($p <$ 0.01) and Bray-Curtis distance ($p <$ 0.01). Similar results were found between the UC group and the control group (Unweighted UniFrac distance, $p <$ 0.01; Bray-Curtis distance, $p <$ 0.01). Yet, the β-diversity showed no significant difference between the CD group and the UC group. PCoA revealed a significant separation between the control group and the CD group, and also between the control group and the UC group (Fig. 4B).

By using the LEfSe method, *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* were identified as dominant genera in the UC group. *Fusobacterium* and *Escherichia_Shigella* were dominant in the CD group. *Faecalibacterium* and *Subdoligranulum* were dominant in the control group (Fig. 5C, D).

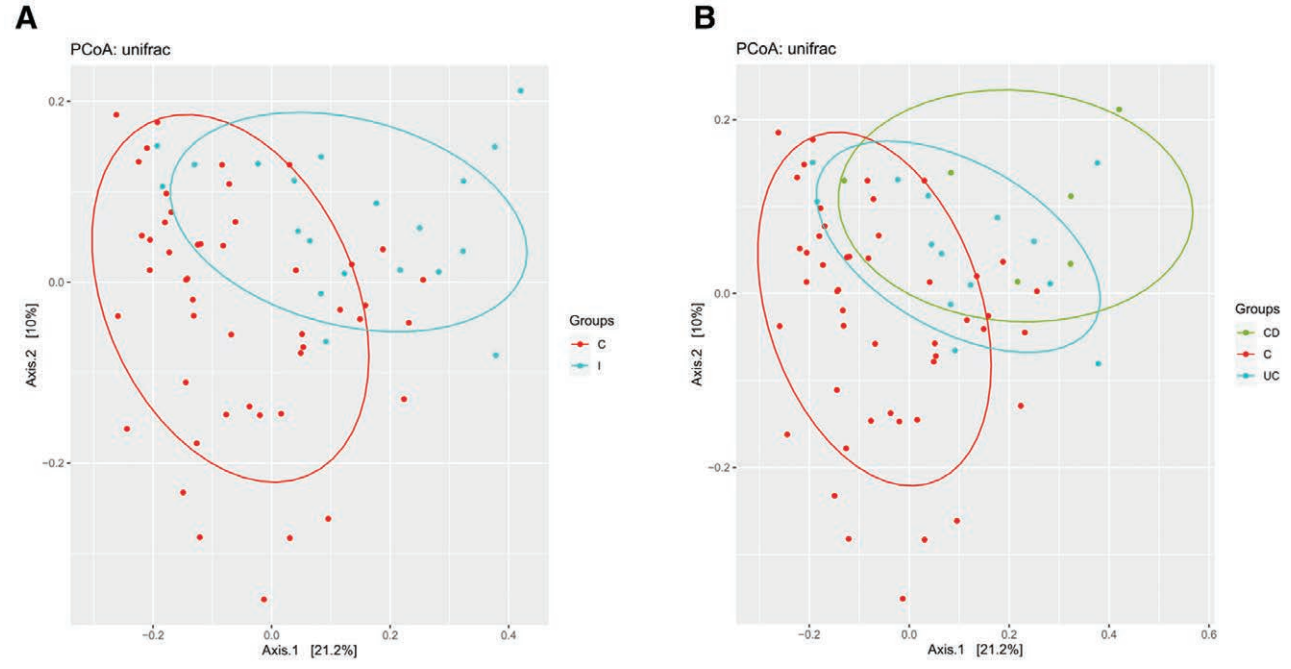

Fig. 4 Principal coordinate analysis (PCoA) of bacterial genera abundance. Based on the base of amplicon sequencing variant by using QIIME2, comparison between the inflammatory bowel disease group and the control group showed significant differences (A). PCoA revealed significant separation between the control group and the Crohn's disease (CD) group, and also between the control group and the ulcerative colitis (UC) group (B).

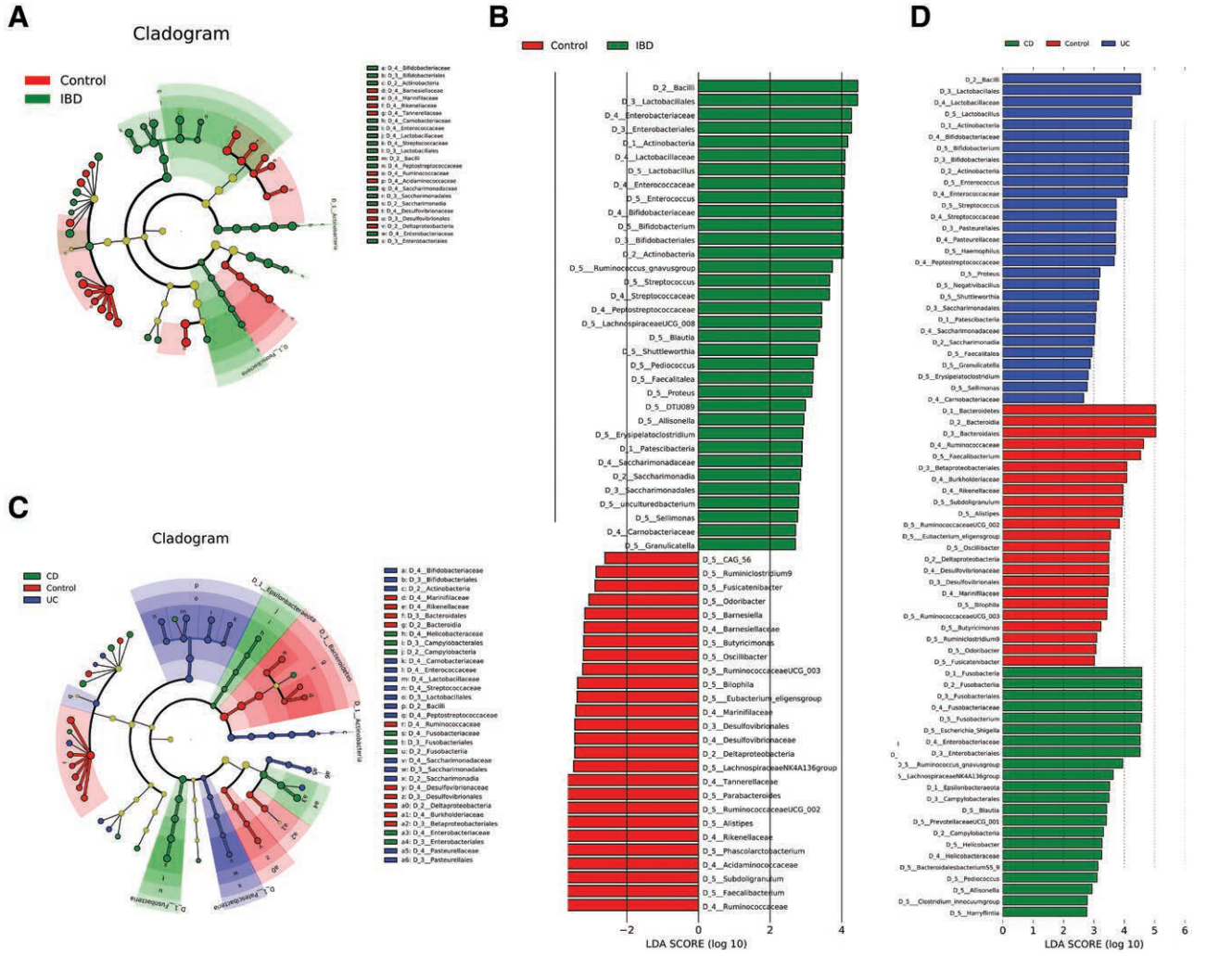

Fig. 5 Known taxa abundance reported by LEfSe in the bacterial community. The circle sizes in the cladogram plot were proportional to the bacterial abundances. From the inside to the outside, the circles represented the phylum, class, order, family, and genus. Cladogram showed taxa with the higher differences in relative abundances between the control and the inflammatory bowel disease (IBD) groups. Taxa with a linear discriminant analysis (LDA) score of >2 and $p < 0.05$ in the Wilcoxon signed-rank test are shown (A). A logarithmic LDA-score cutoff of 2.0 was used to identify significant taxonomic differences between the fecal microbiomes of controls and patients with IBD (B). Cladogram comparing control group, ulcerative colitis (UC), and Crohn's disease is shown. Taxa with an LDA score of >2 and $p < 0.05$ in the Wilcoxon signed-rank test are shown (C). A logarithmic LDA-score cutoff of 2.0 was used to identify significant taxonomic differences between the fecal microbiomes of controls and patients with IBD (D).

4. DISCUSSION

In our study, we found that the IBD group had a significant reduction in both richness and diversity of gut microbiota compared with the control group. The composition of gut microbiota was significantly different between the IBD patients and healthy controls. Our findings were similar to previous studies.[13,14]

In healthy individuals, the gut microbiota and the host present a symbiotic relationship. The epithelial barrier could accommodate the commensal microbiota, whereas the epithelial cells defy the invading microorganisms by secreting antimicrobial peptides. The binding of lipopolysaccharides (LPS) to CD14 and the toll-like receptor is essential for the activation of the proinflammation pathway.[31,32] The intestinal macrophages usually lack CD14 and can tolerate the presence of LPS.[32,33] Through CCR2-dependent recruitment, Ly6C^{hi}-monocyte could migrate to the intestinal mucosa and differentiate inflammatory macrophage.[33,34] Accumulation of inflammatory macrophages can lead to inflammatory response to microbiota.

The development of IBD is associated with environmental exposures in genetically susceptible individuals that may induce abnormal immune responses toward gut microbiota and cause intestinal inflammation. Through genome-wide association studies, hundreds of IBD-associated loci were found successively.[13,35,36] Those gene loci involved in the epithelial barrier function, innate immunity, cytokine signaling, autophagy, and the interleukin (IL)-23-Th17 pathway.[13,36,37] The disruption of susceptibility genes is also associated with an impaired immune response to bacterial ligands and metabolites, and uprising inflammation.[13,38,39] The dysfunction of the epithelial barrier is thought to be an early event in the pathogenesis of IBD.[40,41] Barrier dysfunction allowed the bacteria to invade the mucosa, hence trigger the inflammatory response. Furthermore, the secretion of proinflammatory cytokines such as tumor necrosis factor-α (TNFα), IL-1β, and IL-18 may result in disruption of the gut epithelial barrier.[42]

Imbalance of microbial diversity within the gut is found in patients with IBD. The gut microbial composition of IBD

has been studied, and diverse results were found. At the phylum level, a decrease of *Actinobacteria* and *Firmicutes* in CD patients was frequently reported, but the change was not consistent in UC patients.[14] Increased abundance in *Proteobacteria* in IBD patients was noted.[13,14] At the family level, a decrease of *Ruminnococcaceae* had been found in IBD patients.[13,43] In a study of treatment-naïve pediatric CD patients, the CD patients had an increase of *Enterobacteriaceae, Pasteurellacae, Veillonellaceae*, and *Fusobacteriaceae*, and a decrease of *Erysipelotrichales, Bacteroidales*, and *Clostridiales* compared with controls.[44] In the present study, a decrease of *Ruminnococcaceae* and an increase of *Enterobacteriaceae* and *Veillonellaceae* were found in the IBD patients, which were practically consistent with previous studies.[13,43,44] We found a significant decrease in α-diversity in the IBD group compared with the controls.

Butyrate-producing bacteria include family *Lachnospiraceae*, *Ruminococcaceae,* and *Erysipelotrichaceae*.[45,46] Decrease of butyrate-producing species such as *Faecalibacterium prausnitzii* and *Roseburia hominis* in both UC and CD patients were also found.[15,47,48] Butyrate activates target cells via G-protein-coupled receptors, participates in the maintenance of intestinal barrier, influences the production of proinflammatory cytokines, and modulates immune response.[38,45,49,50] Reduction of butyrate and butyrate-producing bacteria probably deteriorates the disrupted mucosal barrier and unconfined inflammation in IBD. In the present study, we identified that the IBD group had decrease abundance in *Faecalibacterium* and *Subdoligranulum* compared with the control group, both genera have capabilities to produce butyrate.[45] Contrarily, we found that *Bifidobacterium* and *Blautia* were increased in the IBD group. *Blautia* is also known as a butyrate producer.[45] *Bifidobacterium* participates in the production of acetate and lactate, both could be converted to butyrate.[46] However, the concentration of butyrate or other bacterial metabolite was not exanimated in this study. Whether the change of bacterial composition in the gut of IBD patients affects the butyrate concentration and the pathogenesis could not be proved in this study.

In the present study, we found that *Lactobacillus, Bifidobacterium,* and *Enterococcus* were dominant in the IBD group. *Lactobacillus* and *Bifidobacterium* were assumed to be probiotics. However, the change of *Lactobacillus* and *Bifidobacterium* in IBD patients was conflicted.[9,43,44,51–54] There were studies from the United States and Europe that suggested the abundance of *Lactobacillus* and *Bifidobacterium* had decreased in IBD.[43,44,51] In a Japanese study, *Bifidobacterium* was found to be decreased in active CD patients and increased in healthy patients and IBD patients with remission or maintenance phase.[53] Furthermore, Sha et al[54] found the difference of *Lactobacillus* between the IBD patients and healthy controls was insignificant. In the present study, the majority of the enrolled patients were UC patients with mild or quiescent disease activity. About 90% of the enrolled IBD patients took mesalazine. A previous report revealed that the increased concentration of mucosal 5-aminosalicylic acid (5-ASA) was positively associated with an increased abundance of several mucosal microbiota, including *Bifidobacterium, Ruminococcus*, and *Blautia*.[55] Whether the increased abundance of *Lactobacillus* and *Bifidobacterium* in the IBD group is linked to disease activity, therapeutic drugs for IBD or is a regional feature of patients in Taiwan is uncertain. A study involving a larger number of patients and comparing microbiota status and their medications in these IBD patients with different disease activities during their long-term follow-up is ongoing.

It is our strength that we used different tools to analyze the data of 16s rRNA sequencing.[20,29] No significant conflict was found while evaluating the microbial composition of the IBD and control group. The dominant genera identified in each group were generally similar. However, the calculated LDA scores were different among *Veillonella*, *Bifidobacterium*, and *Akkermansia*. The differences may be contributed to the filtering and denoising done by QIIME2.

There were several limitations to this study. First, the included IBD patients were heterogeneous, including 14 UC patients and 6 CD patients. The disease activity and the treatment they received were also different though most of them were under the stable condition of the disease. The composition of gut microbiota may be varied in the patient who had remitted or active IBD. However, because of the small number of included IBD patients, subgroup analysis for disease activity and treatment were not performed. Second, the composition of gut microbiota is easily affected by environmental factors such as antibiotics, diet, and obesity.[6,56] The diet patients consumed before collecting stool samples were not recorded. Also, the medication patients had taken was basically according to their medical records through the drugs bought in the counter or taken from other clinics was reviewed and medication with PPI, histamine-2 receptor antagonists, NSAIDs, antibiotics, or probiotics within 4 weeks of sample collection was prohibited. Third, the control group of this study had a higher BMI and a higher rate of females when compared with the IBD group. Fourth, the metatranscriptomic data, metabolomics, and metabolite measurements such as SCFA were not performed in this study. Fifth, biomarkers involving proinflammatory processes such as LPS or CD14 were not assessed in this study.

In conclusion, the IBD group had a significant reduction in richness and diversity of gut microbiota when compared with the control group. The composition of gut microbiota was significantly different between the IBD patients and healthy controls. *Lactobacillus* and *Bifidobacterium* were found to increase in the IBD group. Whether these genera had an impact on inflammation and influence of IBD should be further assessed.

ACKNOWLEDGMENTS

This study was funded in part by the grants from Taipei Veterans General Hospital (VN 108-04, V108D41-001-MY3-3) and Ministry of Science and Technology of Taiwan (MOST 108-2314-B-010-050, MOST 109-2314-B-075-034) to JCL. But they did not involve in the conduct of the research, study design, data collection, analysis and interpretation of data, writing the manuscript, and in the decision to submit the article for publication. The authors express their gratitude to Mrs. Pui-Ching Lee (Department of Medicine, Taipei Veterans General Hospital) for her help in figure editing.

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