Comparison of mucosa-associated microbiota in Crohn's disease patients with and without anti-tumor necrosis factor-α therapy

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Most studies on the gut microbiome of Crohn's disease have been conducted using feces, instead of intestinal mucus to analyze the mucosa-associated microbiota. To investigate the characteristics of mucosa-associated microbiota in Crohn's disease patients and the effect of anti-tumor necrosis factor (TNF)-α therapy on mucosa-associated microbiota, we analyzed microbiota in Crohn's disease patients using brushing samples taken from terminal ileum. The recruited subjects were 18 Crohn's disease patients and 13 controls. There were 10 patients with anti-TNF-α therapy in Crohn's disease group. Crohn's disease patients had significantly reduced α-diversity in Shannon index compared to the controls. The comparative analysis of the taxonomic composition at the genus level between the Crohn's disease group and the controls indicated that butyrate-producing bacteria were less abundant in the Crohn's disease group compared to the controls. There were no differences in the diversity between the patients taking anti-TNF-α therapy and the patients without. The comparative analysis of the taxonomic composition at the genus level between the two groups indicated that some of anti-inflammatory bacteria were less abundant in the anti-TNF-α therapy group than the other. Reduction of specific bacteria producing anti-inflammatory molecules, especially butyrate-producing bacteria may play important roles in the pathophysiology of Crohn's disease.

***Key Words*: Crohn's disease, mucosa-associated microbiota, anti-TNF-α therapy, butyrate-producing bacteria, fecal microbiota transplantation**

Crohn's disease (CD) is one of two types of inflammatory bowel disease (IBD) and is a relapsing inflammatory disease, mainly affecting the gastrointestinal tract (GI) caused by multiple pathophysiology.(1) Although, the exact cause of CD remains unknown, commensal microbiome is considered playing important roles.(2) The gut microbiota and its metabolites regulate the host inflammatory conditions, and the composition change of gut microbiota is confirmed to be associated with various inflammatory diseases including CD. The previous studies investigating mucosal bacteria using biopsy samples indicated high concentrations of mucosal bacteria in IBD patients.(3) There are a number of recent papers reporting microbial alterations in IBD patients, such as reduced bacterial diversity, decreased taxa within the phylum Firmicutes and increased Gammaproteobacteria, etc.(4)

Although many of these studies used fecal samples which is predominantly resident in the lumen and can be collected easily, it is important to target bacteria adhering to the intestinal epithelium that is called mucosa-associated microbiota (MAM). There is increasing attention about more stable MAM, and the evaluating MAM in precise and accurate manners is recognized as a clinically important trial to characterize and understand the host-microbe interactions and their roles. We previously reported the difference in the bacterial community profile of MAM using brush samples during endoscopic procedures from fecal samples. Brushing during colonoscopy procedure instead of using feces samples might be useful to analyze MAM.(5) Moreover, current studies support an anti-inflammatory role for the short-chain fatty acids, especially butyrate produced by bacterial fermentation of dietary fiber. Although an association of IBD with gut microbiota and its metabolites has been reported,(6–9) a few clinical studies have examined MAM and few studies have investigated the effect of treatment on MAM.

Fecal microbiota transplantation (FMT) is recently reported and suggested to be safe and affective to correct the dysbiosis.(10) Current therapies in CD, such as anti-tumor necrosis factor (TNF)-α, anti-interleukin (IL)-12p40 antibody, and anti-integrin antibodies are generally effective, but each therapy induces a sustained remission in a part of patients.(11,12) These therapies target effector immune responses, however the data about the effect on the gut microbiota is still lacking. There is possibility that anti-TNF-α therapy may influence changes in the gut microbiota. This study aimed to investigate the MAM profile of Japanese patients with CD using brushing samples comparing to controls and compared MAM between CD patients taking anti TNF-α therapy and patients without it.

Materials and Methods

Ethics. Ethical approval was obtained from Kawasaki Medical School Ethic and Medical Research Committee (no. 3087). Written informed consent was achieved from each research subject before enrollment. All patients were enrolled at the Division of Gastroenterology of Kawasaki Medical School Hospital.

Patients and Sample collection. Thirteen non-IBD healthy controls undergoing routine medical checkups who did not receive any medication and complain of any gastrointestinal symptoms and 18 CD patients were enrolled. Brush samples were taken from the normal appearing mucosa of terminal ileum

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using an endoscopic microbiology brush (COOK, Bloomington, IN) after usual preparation for colonoscopy using polyethylene glycol (PEG). It has been reported that there was no difference in microbial diversity by biopsy sites from terminal ileum to rectum in patients with Crohn's disease,[13] and the mucus layer in the active lesions is reduced in thickness or is absent.[2,14] Therefore, we took brushing samples from the endoscopic normal mucosa in the nearby active lesions of the terminal ileum to minimize invasion.

DNA extraction and 16S rRNA sequencing. DNA extraction, preparation of library of amplicons encoding 16S rRNA gene, and sequencing were performed as described previously.[15] The samples were profiled by high-throughput amplicon sequencing with dual-index barcoding using the Illumina Miseq platform (Illumina, San Diego, CA). The V3–V4 regions of the gene encoding 16S rDNA (460 bp) were tailed PCR amplified.[16] PCR amplicons were purified using SPRI select beads (Beckman Coulter, Miami, FL). DNA concentration of purified amplicons were measured using a Quantus Fluorometer and the QuantiFluor dsDNA System (Promega, Madison, WI) and approximately equal amount of their DNA were pooled. The pooled sample was sequenced using MiSeq Reagent Kit V3 (600 cycle) (Illumina) on the MiSeq system according to the manufacturer's instructions. Sequence data were analyzed using the Quantitative Insights into Microbial Ecology (QIIME) pipeline (ver. 1.8.0).

Bioinformatics analysis. Processing of sequence data, including quality filter, chimera check, operational taxonomic unit (OTU) definition and taxonomy assignment, was performed using QIIME ver. 1.9.0, USEARCH ver. 9.2.4, UCHIME ver. 4.2.40 and VSEARCH ver. 2.4.3. Singletons were removed. The RDP classifier ver. 2.10.2 with the Greengenes database (published May, 2013) was used for taxonomy assignment of the acquired OTUs (97% sequence similarity).

α-Diversity and β-diversity. The observed species, Chao1, and Shannon indices were calculated by the phyloseq package of R software. β-Diversity was estimated using the UniFrac metric to calculate the distances between the samples using QIIME ver. 1.9.1. It was visualized by principal coordinate analysis (PCoA) using R software and statistically analyzed using permutational multivariate analysis of variance (PERMANOVA) by QIIME ver. 1.9.1.

Statistical analysis. Values were presented as mean ± SD or median and 25–75% range whichever was appropriate depending on whether the data were normally distributed or non-normally distributed. The category data ware presented as counts with percentage and analyzed by chi-square test. The continuous were tested by Mann-Whitney *U* test for comparison between two groups. The statistical analyses were made using SPSS (ver. 25 for Windows, IBM Japan, Ltd., Tokyo, Japan). *P* value of <0.05 was regarded as statistically significant.

Results

The mean age was older in controls (55.8 years old) than in the CD group (46.9 years old) and the percentage of men was higher in the CD group (67%) than in the controls (38%), but the differences were not significant between the two groups. The mean Crohn's Disease Activity Index (CDAI) in the CD group was 99.1, with a minimum of 28 and a maximum of 261. Twelve patients (67%) had asymptomatic remission (0 to 149), four (22%) had mildly to moderately active CD (150 to 220), and two (11%) had moderately to severely active CD (221 to 450). The percentage of types of disease in the CD group was 39% for ileitis, 50% for ileocolitis and 11% for colitis. Ten (56%) CD patients were taking 5-ASA or salazopyrin, and eight (44%) patients taking anti-TNF-α therapy. The mean CDAI of CD patients with anti-TNFα therapy was 98, with a minimum of 28 and a maximum of 261. Six patients (75%) had asymptomatic remission (0 to 149) and two (25%) had moderately to severely active CD (221 to 450). The mean CDAI of CD patients without anti-TNFα therapy was 100, with a minimum of 39 and a maximum of 177. Six patients (60%) had asymptomatic remission (0 to 149) and four (40%) had mildly to moderately active CD (150 to 220). Seven patients (39%) in the CD group had longitudinal erosions or ulcers in the terminal ileum, of which five were taking anti-TNFα therapy. eleven patients (61%) had no active lesions at the terminal ileum, and five of them had ulcer scars.

Comparison between the CD patients and controls. Shannon index was significantly lower in MAM of the CD group compared to the controls (Fig. 1). There was no difference in β-diversity between the CD group and controls (Fig. 2). The comparative analysis of the taxonomic composition at the genus level between the two groups indicated that sum of the relative abundance of major butyrate-producing bacterial genera, namely *Butyricicoccus*, *Butyricimonas*, *Butyrivibrio*, *Coprococcus*, *Faecalibacterium*, *Megasphaera*, *Oscillospira*, and *Roseburia* was significantly less abundant in the CD group. Moreover, the abundance of 6 genera was significantly different between the CD group and controls. The genera *Coprococcus*, *Roseburia*, others belonging to the family *Lachnospiracea*, *Ruminococcus*, *Oscillospira*, and others belonging to the family *Ruminococcaceae* were significantly less abundant in the CD group compared to the controls (Table 1).

Comparisons between the TNF-α therapy group and the other CD patients. There was no significant difference in the clinical parameters between the two groups (Table 2). There were no significant differences in the α-diversity indices between the two groups (Fig. 3). There was no difference in β-diversity between the two groups (Fig. 4).

The comparative analysis of the taxonomic composition at the genus level between the two groups indicated that the abundance of 4 genera was significantly different between the TNF-α therapy group and the other CD patients. The genera *Bacteroides*, *Parabacteroides*, *Oscillospira*, and *Eubacterium* belonging to the family *Erysipelotrichaceae* were less abundant in the TNF-α therapy group than in the other CD group (Table 3).

Discussion

In the present study, the reduced bacterial diversity was confirmed in CD patients compared to healthy controls, as previously reported.[17,18] A systematic review including 48 studies from 45 articles that compared gut microbiota in patients with IBD compared to healthy controls was recently reported. In this review, the most common findings were decreased α-diversity in CD patients compared to controls.

The results reported different profiles of gut microbiota in IBD are inconsistent, and the consistent finding of decreased amount of *Faecalibacterium prausnitzii* in IBD was found only in studies using fecal samples.[19] In our brushing samples, the 5 genera including *Coprococcus*, *Roseburia*, *Ruminococcus* were significantly less abundant in the CD group compared to the controls, and this finding is similar to another previous Japanese study investigating MAM.[20] Most of these genera are butyrate-producing bacteria, and butyrate has an anti-inflammatory effect through the production of IL-10 from dendritic cells and induction of regulatory T cells. The decrease in butyrate-producing bacteria in CD can result in a decrease in butyric acid production in the intestinal tract, which may lead to inflammation. In addition, butyrate is a major source of energy in the colonic epithelium and is metabolized by β-oxidation. This activates mitochondrial metabolism, which increases the oxygen consumption of colonic epithelial cells and decreases the oxygen concentration in the barrier, resulting in an increase in obligate anaerobic bacteria such as phylum Firmicutes. In addition, the hypoxic

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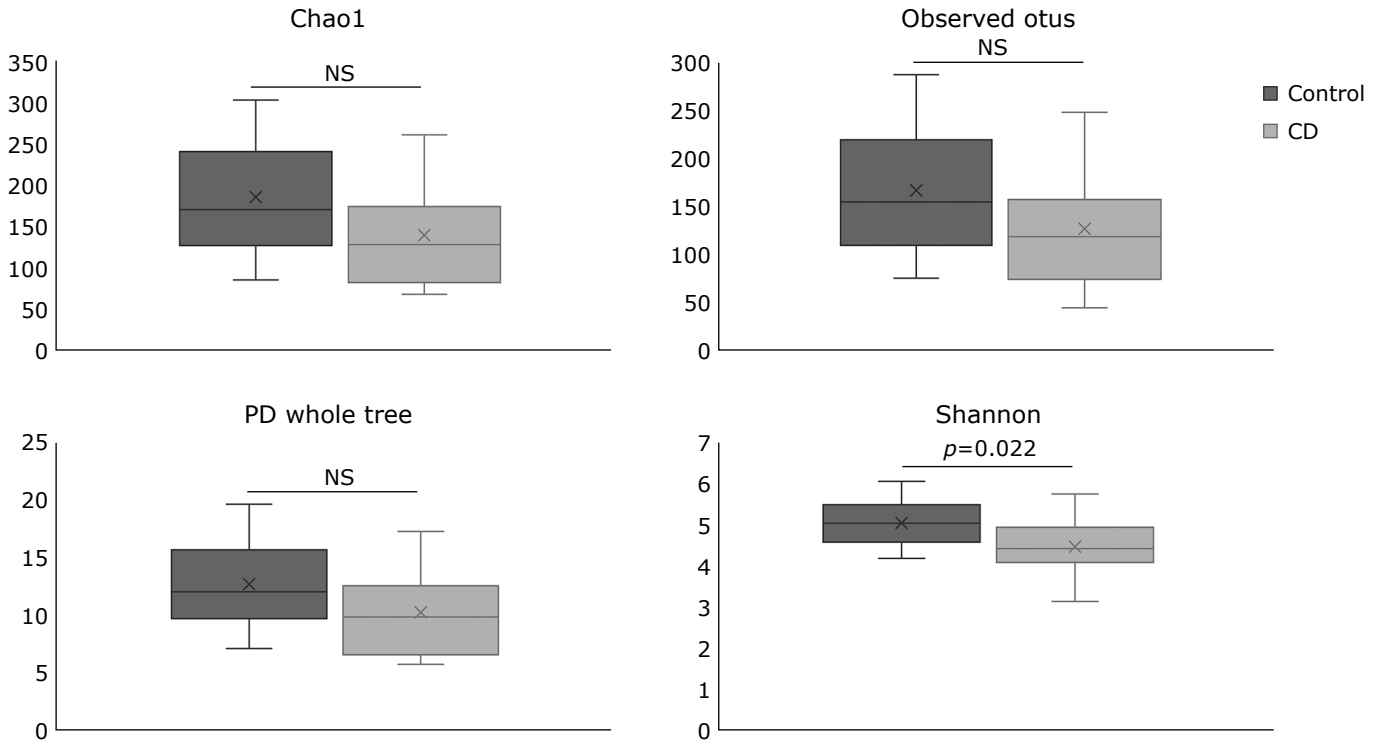

Fig. 1. Comparative analyses of α-diversity indices between the CD patients and the controls. Box and whisker plots are shown where the box contains 50% of the data, the bar represents the median value, and the whiskers show the range from the maximum to minimum. X indicates the mean of sample. Statistical comparisons between groups were made using the Mann-Whitney *U* test.

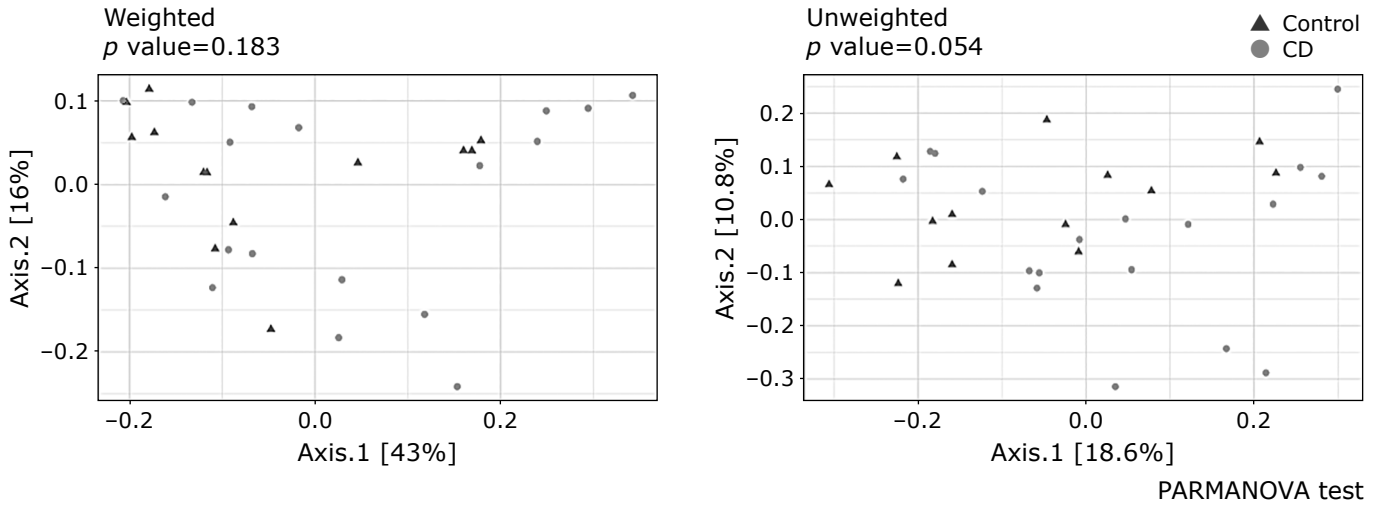

Fig. 2. Comparative analyses of β-diversity between the CD patients and the controls.

response activates the hypoxia-inducible factor (HIF)-1 pathway, which renews the expression of tight junction components between epithelial cells.[21]

There are few studies investigating microbiota to anti-TNF-α therapy, although there are increased number of studies investigating fecal microbiota in fecal microbiota transplantation or cancer therapy. In our study, there was no difference in the diversity of MAM between CD patients with and without anti-TNF-α therapy. However, interestingly, the taxonomic composition was different between the two groups. The 4 genera *Bacteroides*, *Parabacteroides*, *Oscillospira*, *Eubacterium* belonging to family *Erysipelotrichaceae* were less abundant in the treatment group. The previous meta-analysis indicated that lower level of *Bacteroides* in the gut microbiota is associated with IBD especially in patients with active disease.[22] *Bacteroides* and *Parabacteroides* have been reported to have anti-inflammatory effects in the intestinal epithelium,[23,24] they are known to reinforce the epithelial barrier and ameliorate inflammation by producing anti-inflammatory molecules such as polysaccharide A (PSA), sphingolipids and outer membrane vesicles (OMV). *Oscillospira* has also anti-inflammatory effect by producing butyric acid.[21] About *Eubacterium*, *Eubacterium*

Table 1. Comparison of relative abundance of bacterial genera between the Crohn's disease group and the controls

Bacteria	Control Median (25–75 percentile)	Crohn's disease Median (25–75 percentile)	*p* value*
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__*Lachnospiraceae*; Other	0.00160 (0.00017–0.00239)	0.00003 (0.00000–0.00078)	0.028
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__*Lachnospiraceae*; g__	0.06474 (0.02951–0.08716)	0.02364 (0.00535–0.04674)	0.025
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__*Lachnospiraceae*; g__*Coprococcus*	0.02747 (0.01464–0.04785)	0.00146 (0.00012–0.01754)	0.002
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__*Lachnospiraceae*; g__*Roseburia*	0.01051 (0.00647–0.04116)	0.00007 (0.00000–0.01124)	0.006
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__*Ruminococcaceae*; g__	0.01433 (0.00976–0.04230)	0.00137 (0.00004–0.01498)	0.01
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__*Ruminococcaceae*; g__*Oscillospira*	0.00737 (0.00593–0.02058)	0.00477 (0.00057–0.00712)	0.028
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__*Ruminococcaceae*; g__*Ruminococcus*	0.01603 (0.00834–0.02969)	0.00431 (0.00181–0.00760)	0.016
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__[*Mogibacteriaceae*]; g__	0.00259 (0.00110–0.00475)	0.00044 (0.00000–0.00184)	0.046
Sum of major known butyrate- producing bacteria**	0.13660 (0.74985–0.22074)	0.04241 (0.01254–0.09584)	0.013

The relative abundance of bacterial genera was compared between the two groups. **P* values by Mann-Whitney *U* test. **Sum of relative abundance of *Butyricicoccus*, *Butyricimonas*, *Butyrivibrio*, *Coprococcus*, *Faecalibacterium*, *Megasphaera*, *Oscillospira*, and *Roseburia*.

Table 2. Demographic and basic characteristics of the Crohn's disease patients

	Without anti TNF-α antibody	With anti TNF-α antibody	*p* value
Number of patients	10	8	
Age	50.5	42.4	0.408
Gender (M:F)	4:06	2:06	0.502
CDAI Mean (range)	100.0 (39–177)	98.0 (28–261)	0.515
Type of disease Ileitis/Ileocolitis/Colitis	5 (50%)/4 (40%)/1 (10%)	2 (25%)/5 (63%)/1 (13%)	0.552
5-ASA, SASP	5 (50%)	5 (63%)	0.596
PSL	0 (0%)	0 (0%)	—
AZA, 6-MP	0 (0%)	2 (25%)	0.094

CDAI, Crohn's Disease Activity Index; 5-ASA, 5-aminosalicylic acid; SASP, salicylazosulfapyridine; PSL, prednisolone; AZA azathioprine; 6-MP, 6-mercaptopurine.

species belong to the Firmicutes bacteria have been shown to be frequent and stable members of both the normal fecal and mucosa associated bacterial community promoting a healthy gut environment, and the previous studies reported loss of *Eubacterium* species in IBD patients.[19] Therefore, microbiome producing anti-inflammatory molecules seems to decrease in the patients with TNF-α therapy.

If anti-TNF-α therapy reduces CD activity and changes gut bacterial composition, anti-inflammatory bacteria such as butyrate-producing bacteria would be expected to increase in the group receiving anti-TNF-α therapy, but they decreased in this study. The previous Japanese study indicated no significant differences in α-diversity and no clear distinction in microbial communities between active and inactive mucosa in CD patients without detection of specific taxa which significantly associated with the active lesions of CD.[20] Forbes *et al.*[25] conducted a large number of MAM study conducted by using biopsy samples of IBD patients and demonstrated that MAM does not change in the inflamed mucosa. There is a recent report using fecal samples taken from 15 CD patients including 5 patients with remission 14 weeks after the initiation of anti-TNF-α therapy. The study indicated no significant difference in the overall gut microbiota composition and no specific bacterial markers for achieved remission.[26] In this study, there was no difference in CDAI at the time of sample collection between the group with anti-TNF-α therapy and the group without. However, the anti-TNF-α therapy probably had introduced to the patients with more severe initial activity. The decrease in butyrate-producing bacteria in the anti-TNF-α therapy group may not be due to the effect of the therapy, and the reduction of anti-inflammatory bacteria seems to be possible cause to the induction. The further prospective studies investigating the changes of MAM after anti-TNF-α therapy are required.

There are limitations in this case-control study. First, the number of patients is small and more than half of CD patients are in remission. Therefore, the larger number of subjects including patients with active phase is required to examine the nature of the pathogenesis of CD. The study is not cohort and duration, types of anti-TNF-α therapy and the timing of sample collection were different among the patients. The data before anti-TNF-α therapy in the same patients are lacking. Moreover, the information on dietary intake, especially precise volume of elemental diet is lacking, and patients were taking different medicines, especially 5-ASA, which may affect MAM. In addition, PEG preparation also affects microbiota compositions and detection by removing luminal resident bacteria. However, our data using brushing samples possibly represent more intensely mucosa attached bacteria, which might play an important role of host-microbe interactions contributing to the CD pathophysiology.

In conclusion, we indicated the reduced bacterial diversity of

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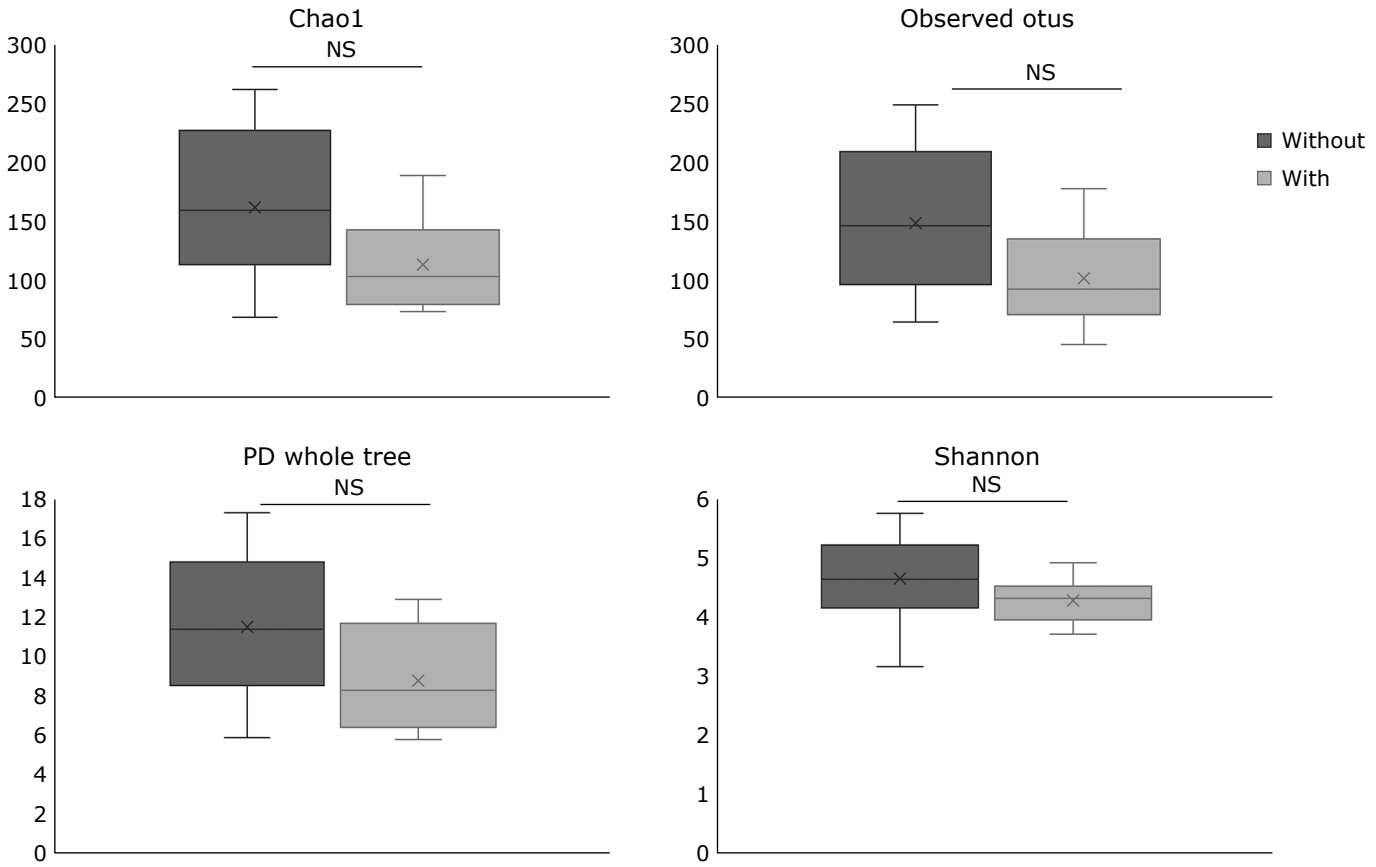

Fig. 3. Comparative analyses of the α-diversity indices between the CD patients treated with and without anti TNF-α therapy. Box and whisker plots are shown where the box contains 50% of the data, the bar represents the median value, and the whiskers show the range from the maximum to minimum. X indicates the mean of sample. Statistical comparisons between groups were made using the Mann-Whitney *U* test.

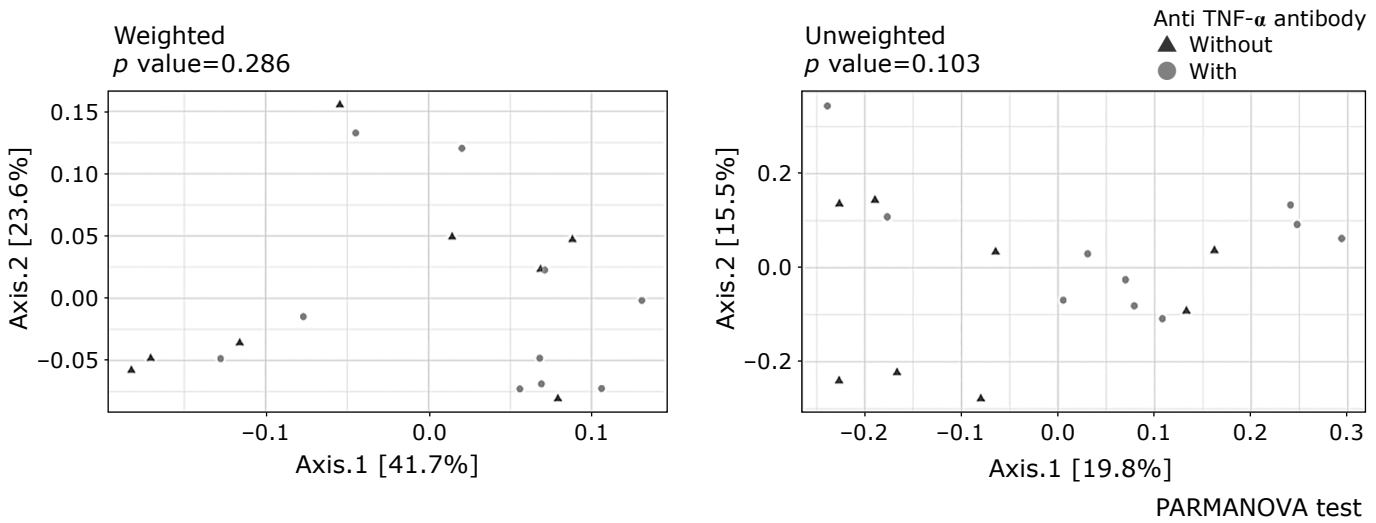

Fig. 4. Comparative analyses of the β-diversity indices between the CD patients treated with and without anti TNF-α therapy.

MAM and reduction of specific bacteria especially butyrate -producing bacteria in the MAM of CD patients compared to the controls. The loss of specific bacteria producing anti-inflammatory molecules was also confirmed in the CD patients taking anti-TNF-α therapy. Reduction of specific bacteria producing anti-inflammatory molecules, especially butyric acid may play important roles in the pathophysiology of CD. The further studies are required to be confirmed the association of specified microbial structure with anti-TNF-α therapy.

Table 3. Comparisons of relative abundance of bacterial genera between the CD patients treated with and without anti TNF-α therapy.

Bacteria	Without Median (25–75 percentile)	With Median (25–75 percentile)	*p* value
k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__*Bacteroidaceae*; g__*Bacteroides*	0.08811 (0.05721–0.11166)	0.05053 (0.01465–0.06263)	0.043
k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__*Porphyromonadaceae*; g__*Parabacteroides*	0.01846 (0.00140–0.03785)	0.00013 (0.00000–0.00994)	0.012
k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__*[Odoribacteraceae]*; g__*Butyricimonas*	0.00046 (0.00000–0.00168)	0.00000 (0.00000–0.00000)	0.034
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__*Eubacteriaceae*; g__*Pseudoramibacter_Eubacterium*	0.00005 (0.00000–0.00142)	0.00000 (0.00000–0.00000)	0.012
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__*Ruminococcaceae*; g__*Oscillospira*	0.00670 (0.00430–0.01160)	0.00113 (0.00002–0.00523)	0.034
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__*Veillonellaceae*; g__*Phascolarctobacterium*	0.00349 (0.00006–0.00702)	0.00000 (0.00000–0.00000)	0.003
k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__*Erysipelotrichaceae*; g__[*Eubacterium*]	0.01040 (0.00371–0.02265)	0.00044 (0.00010–0.00056)	0.004
k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Neisseriales; f__*Neisseriaceae*; g__*Neisseria*	0.00000 (0.00000–0.00003)	0.00089 (0.00017–0.00307)	0.009

The relative abundance of bacterial genera was compared between the two groups. *P* values by Mann-Whitney *U* test.

Author Contributions

AS: study concept and design, study supervision
HM: sample collection, critical revision of the manuscript for important intellectual content
OH: sample collection, critical revision of the manuscript for important intellectual content
YH: critical revision of the manuscript for important intellectual content
MO: sample collection
TM: sample collection
EU: sample collection, critical revision of the manuscript for important intellectual content
MK: critical revision of the manuscript for important intellectual content
RI: analysis and interpretation of data
YN: study supervision

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Abbreviations

CD	Crohn's disease
CDAI	Crohn's Disease Activity Index
FMT	fecal microbiota transplantation
GI	gastrointestinal tract
IBD	inflammatory bowel disease
IL	interleukin
MAM	mucosa-associated microbiota
TNF	tumor necrosis factor

Conflict of Interest

No potential conflicts of interest were disclosed.

References

1 Kaser A, Zeissig S, Blumberg RS. Inflammatory bowel disease. *Annu Rev Immunol* 2010; **28**: 573–621.
2 Sartor RB, Wu GD. Roles for intestinal bacteria, viruses, and fungi in pathogenesis of inflammatory bowel diseases and therapeutic approaches. *Gastroenterology* 2017; **152**: 327–339.
3 Swidsinski A, Ladhoff A, Pernthaler A, *et al*. Mucosal flora in inflammatory bowel disease. *Gastroenterology* 2002; **122**: 44–54.
4 Kostic AD, Xavier RJ, Gevers D. The microbiome in inflammatory bowel disease: current status and the future ahead. *Gastroenterology* 2014; **146**: 1489–1499.
5 Matsumoto H, Kuroki Y, Higashi S, *et al*. Analysis of the colonic mucosa associated microbiota (MAM) using brushing samples during colonic endoscopic procedures. *J Clin Biochem Nutr* 2019; **65**: 132–137.
6 Kinnebrew MA, Buffie CG, Diehl GE, *et al*. Interleukin 23 production by intestinal CD103(+)CD11b(+) dendritic cells in response to bacterial flagellin enhances mucosal innate immune defense. *Immunity* 2012; **36**: 276–287.
7 Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell* 2005; **122**: 107–118.
8 Mortha A, Chudnovskiy A, Hashimoto D, *et al*. Microbiota-dependent crosstalk between macrophages and ILC3 promotes intestinal homeostasis. *Science* 2014; **343**: 1249288.
9 Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol* 2009; **9**: 313–323.
10 Laurell A, Sjöberg K. Prebiotics and synbiotics in ulcerative colitis. *Scand J Gastroenterol* 2017; **52**: 477–485.
11 Gomollón F, Dignass A, Annese V, *et al*. 3rd European Evidence-based Consensus on the Diagnosis and Management of Crohn's Disease 2016: Part 1: Diagnosis and medical management. *J Crohns Colitis* 2017; **11**: 3–25.
12 Matsuoka K, Kobayashi T, Ueno F, *et al*. Evidence-based clinical practice guidelines for inflammatory bowel disease. *J Gastroenterol* 2018; **53**: 305–353.
13 He C, Wang H, Liao WD, *et al*. Characteristics of mucosa-associated gut microbiota during treatment in Crohn's disease. *World J Gastroenterol* 2019; **25**: 2204–2216.
14 Okumura R, Kurakawa T, Nakano T, *et al*. Lypd8 promotes the segregation of flagellated microbiota and colonic epithelia. *Nature* 2016; **532**: 117–121.
15 Hayashi A, Mikami Y, Miyamoto K, *et al*. Intestinal dysbiosis and biotin deprivation induce alopecia through overgrowth of *Lactobacillus murinus* in mice. *Cell Rep* 2017; **20**: 1513–1524.

doi: 10.3164/jcbn.21-41

16 Klindworth A, Pruesse E, Schweer T, *et al*. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res* 2013; **41**: e1.
17 Nagalingam NA, Lynch SV. Role of the microbiota in inflammatory bowel diseases. *Inflamm Bowel Dis* 2012; **18**: 968–984.
18 Frank DN, Robertson CE, Hamm CM, *et al*. Disease phenotype and genotype are associated with shifts in intestinal-associated microbiota in inflammatory bowel diseases. *Inflamm Bowel Dis* 2011; **17**: 179–184.
19 Pittayanon R, Lau JT, Leontiadis GI, *et al*. Differences in gut microbiota in patients with vs without inflammatory bowel diseases: a systematic review. *Gastroenterology* 2020; **158**: 930–946.e1.
20 Nishino K, Nishida A, Inoue R, *et al*. Analysis of endoscopic brush samples identified mucosa-associated dysbiosis in inflammatory bowel disease. *J Gastroenterol* 2018; **53**: 95–106.
21 Kelly CJ, Zheng L, Campbell EL, *et al*. Crosstalk between microbiota-derived short-chain fatty acids and intestinal epithelial HIF augments tissue barrier function. *Cell Host Microbe* 2015; **17**: 662–671.
22 Zhou Y, Zhi F. Lower level of *Bacteroides* in the gut microbiota is associated with inflammatory bowel disease: a meta-analysis. *Biomed Res Int* 2016; **2016**: 5828959.
23 Hiippala K, Kainulainen V, Suutarinen M, *et al*. Isolation of anti-inflammatory and epithelium reinforcing *Bacteroides* and *Parabacteroides* spp. from a healthy fecal donor. *Nutrients* 2020; **12**: 935.
24 Matsutani M, Matsumoto N, Hirakawa H, *et al*. Comparative genomic analysis of closely related *Acetobacter pasteurianus* strains provides evidence of horizontal gene transfer and reveals factors necessary for thermotolerance. *J Bacteriol* 2020; **202**: e00553.
25 Forbes JD, Van Domselaar G, Bernstein CN. Microbiome survey of the inflamed and noninflamed gut at different compartments within the gastrointestinal tract of inflammatory bowel disease patients. *Inflamm Bowel Dis* 2016; **22**: 817–825.
26 Vatn S, Carstens A, Kristoffersen AB, *et al*. Faecal microbiota signatures of IBD and their relation to diagnosis, disease phenotype, inflammation, treatment escalation and anti-TNF response in a European Multicentre Study (IBD-Character). *Scand J Gastroenterol* 2020; **55**: 1146–1156.