Gut Pathogens

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Dynamic changes in the gut microbiota composition during adalimumab therapy in patients with ulcerative colitis: implications for treatment response prediction and therapeutic targets

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Abstract

Background While significant research exists on gut microbiota changes after anti-tumor necrosis factor-alpha (anti TNF-α) therapy for ulcerative colitis, little is known about the longitudinal changes related to the effects of anti TNF-α. This study aimed to investigate the dynamics of gut microbiome changes during anti TNF-α (adalimumab) therapy in patients with ulcerative colitis (UC).

Results The microbiota composition was affected by the disease severity and extent in patients with UC. Regardless of clinical remission status at each time point, patients with UC exhibited microbial community distinctions from healthy controls. Distinct amplicon sequence variants (ASVs) differences were identified throughout the course of Adalimumab (ADA) treatment at each time point. A notable reduction in gut microbiome dissimilarity was observed only in remitters. Remitters demonstrated a decrease in the relative abundances of *Burkholderia-Caballeronia-Paraburkholderia* and *Staphylococcus* as the treatment progressed. Additionally, there was an observed increase in the relative abundances of *Bifidobacterium* and *Dorea*. Given the distribution of the 48 ASVs with high or low relative abundances in the pre-treatment samples according to clinical remission at week 8, a clinical remission at week 8 with a sensitivity and specificity of 72.4% and 84.3%, respectively, was predicted on the receiver operating characteristic curve (area under the curve, 0.851).

Conclusions The gut microbiota undergoes diverse changes according to the treatment response during ADA treatment. These changes provide insights into predicting treatment responses to ADA and offer new therapeutic targets for UC.

Keywords Microbiome, Ulcerative colitis, Tumor necrosis factor inhibitor

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Background

Inflammatory bowel disease (IBD), which includes Crohn's disease (CD) and ulcerative colitis (UC), is a chronic inflammatory disorder whose incidence has been increasing over time, expanding from Western countries to different regions around the world. Recently, the prevalence of the disease has increased in many Asian countries, including South Korea, Japan, and China, with urbanization and a Westernized lifestyle [1–6]. Notably, a Korean population-based study revealed that the incidence and prevalence of UC had gradually increased. The mean annual incidence rates of UC increased significantly from 0.29 and 0.06 per 100,000 inhabitants in 1986–1990 to 5.82 and 2.44, respectively, in 2011–2014 [7].

The pathophysiology of IBD remains unclear; however, abnormal interaction between mucosal immune response and gut microbiome in genetically susceptible individuals has been suggested as a key pathophysiology [8]. With the development of bacteria controlling and sequencing technology, the role of the gut microbiome has been highlighted in the past decade, and dysbiosis of intestinal microbiota has been suggested to induce an imbalance of mucosal immunity, which contributes to the increasing incidence of UC [9, 10]. Recent attempts were made based on these studies to identify the changes in the microbial profile associated with the treatment response and predict the efficacy of biologic therapy [11–13].

Adalimumab (ADA) is a fully human IgG1 monoclonal antibody directed against tumor necrosis factor-alpha (TNF-α) that inhibits the activity of cytokines by blocking the interaction of TNF-α with its p55 and p75 cell surface receptors [14]. ADA has been approved for use in patients with moderate to severe CD and UC who have shown unsuccessful outcomes following conventional therapy with corticosteroids and/or immunomodulators [15, 16]. As its efficacy and safety in patients with UC have been demonstrated in previous studies, including Western and Asian areas [17, 18], ADA has gained importance in treating moderately to severely active UC. However, little is known about the relationship between the gut microbiome and ADA treatment in patients with UC.

We demonstrated the efficacy and safety of ADA for induction and maintenance therapy in patients with moderately to severely active UC in our previous prospective, observational, multicenter study [19]. The clinical outcomes of ADA were similar to those of other real-world studies [15, 16, 20]. To better understand the association between clinical outcomes and gut microbiome, we analyzed fecal samples collected longitudinally during treatment. We hypothesized that the gut microbiome would exhibit different changes based on the clinical response of patients with UC undergoing treatment with ADA. Furthermore, we assumed that we could utilize them to predict the prognosis of patients or identify bacteria with the potential to serve as new therapeutic targets for UC. Therefore, we evaluated the changes in the fecal microbiome by analyzing 16S rRNA microbiome profiles using longitudinal patient stool samples collected before and after ADA treatment.

Methods

Participants and study design

This prospective, observational, multicenter study was conducted at 17 academic hospitals in Korea between June 2015 and September 2018. Adult patients with moderately to severely active UC (Mayo score [14] 6–12 with an endoscopic subscore ≥ 2) who failed conventional therapy, including 5-aminosalicylic acid, corticosteroids, and azathioprine/6-mercaptopurine or previous anti-TNF-α agents other than ADA, were recruited. The patients received subcutaneous injections of ADA (160 mg at week 0, 80 mg at week 2, and 40 mg every other week from week 4). Fecal samples were collected from patients at designated time points (week 0, 8, and 56) after ADA therapy initiation. Prior to stool collection, all the participants were asked to refrain from taking antibiotics or probiotics for a period of 4 weeks prior to sample collection, which could affect the gut bacterial composition, while maintaining their usual diet. Disease severity and clinical response were assessed using the Mayo score. Clinical response was defined as a decrease in the Mayo score from baseline by ≥ 3 points and $\geq 30\%$ with an accompanying decrease in rectal bleeding subscore of ≥ 1 point or an absolute rectal bleeding subscore of 0 or 1. Clinical remission was defined as a Mayo score ≤ 2 with no individual subscore exceeding 1 point. This study was performed in accordance with the principles of the Declaration of Helsinki and approved by the Institutional Review Board of Chung-Ang University Hospital (IRB No. C2015020). Written informed consent was obtained from each participant before inclusion into the study.

Microbiome analysis

DNA isolation

Fecal samples taken before (week 0) and after ADA administration at weeks 8 and 56 were immediately transported on ice and stored at -80 °C without freeze-drying. DNA was extracted using a FastDNA SPIN kit for bacterial DNA (MP Biomedicals, Irvine, CA, USA) according to the manufacturer's instructions.

16S rRNA gene amplification

To detect the bacterial 16S rRNA gene, we performed polymerase chain reaction (PCR) amplification of the

V3–V4 region using gene-specific sequences with Illumina adapter overhang nucleotide sequences [21]. Amplification was performed using KAPA HiFi HotStart reagent with 5 ng/μL sample DNA and 0.5 μM of each primer per reaction. The PCR amplification profile included an initial step of 95 °C for 3 min followed by 25 cycles of 95 °C (30 s), 55 °C (30 s), and 72 °C (30 s), and a final extension cycle for 5 min at 72 °C. Then, PCR products were purified using Sera-MagTM Select (29,343,052; GE Healthcare, Chicago, IL, USA) reagent prior to elution in Tris–EDTA buffer (Sigma-Aldrich, Burlington, MA, USA). The cleaned amplicons were attached to dual indices and Illumina sequencing adapters using a Nextera XT Index Kit. Indexing was performed with 5 μL PCR amplicon per reaction, and 5 μL each of N7 Nextera XT Index Primer 1 and S5 Nextera XT Index Primer 2 and 25 μL of KAPA HiFi HotStart reagent on a thermal cycler using the following program: 95 °C for 3 min followed by 8 cycles of 95 °C (30 s), 55 °C (30 s), and 72 °C (30 s). After cycling, the reactions were incubated for 5 min at 72 °C. After the indexing PCR and further clean-up with Sera-MagTM Select, the 16S libraries were quantified using Qubit 2.0 (Invitrogen, Carlsbad, CA, USA). Equimolar pools of 16S libraries were sequenced on an Illumina MiSeq platform using the paired-end 300-cycle MiSeq Reagent Kit V3 (Illumina, San Diego, CA, USA).

16S rRNA gene microbiome analysis

The 16S rRNA gene sequences were processed and analyzed using Quantitative Insights Into Microbial Ecology 2 (QIIME2) [22]. Briefly, divisive amplicon denoising algorithm version 2 (DADA2 1.12.1) was used for quality-filtered, trimmed, error correction, exact sequence inference, chimera removal with default parameter and "–p-trunc-len-f 279 –p-trunc-len-r 206", and merged paired-end sequences and generate the amplicon sequence variant (ASV) table [23]. We aligned the denoised sequences in MAFFT [24], filtered the alignments, and constructed a phylogeny using the "align-to-tree-mafft-fasttree" pipeline in QIIME 2. Taxonomic classification was performed using a sklern-based classifier using the SILVA 132 database. ASVs assigned to the chloroplast (class level) and mitochondria (family level) were excluded from further analysis. For rarefaction, the ASV count was normalized to a depth of 2332 per sample. The rarefied ASV table was used for α-diversity analysis (Shannon's diversity, Faith's phylogenetic diversity, and Simpson evenness), and principal coordinates analysis was conducted on the Unifrac unweighted distance matrices. Using the QIIME1 script (compare_categories.py), analysis of similarities (ANOSIM) was performed to evaluate the differences in the bacterial community composition among groups. In R v.4.0.2, the statistical tests for comparing alpha diversity and the relative abundance of the specific ASVs were conducted using the Wilcoxon test and t-test and visualized using ggplot2. The nucleotide sequences of *Bifidobacterium* assigned ASVs were aligned using MUSCLE and were used to construct a phylogenetic tree using the neighbor-joining method in MEGA X [25]. The evolutionary distances were computed using the Tamura 3-paramter method, and the variation rate among sites was modeled using a gamma distribution. The phylogenetic tree of *Bifidobacterium* ASVs was visualized using Interactive Tree of Life v6 (iTOL) [26].

We conducted linear discriminant analysis effect size (LEfSe) to identify specific ASV explaining variation between groups [27]. For the statistical test incorporated in LEfSe, the Kruskal–Wallis test among groups was performed at the 0.05 significance level, and the threshold of the logarithmic linear discriminant analysis (LDA) score for different ASVs was set at 2.0. The area under the receiver operating characteristic (ROC) curve (AUC) was used to predict clinical remission in patients with UC based on microbiome data using the ROC function in the Epi package (http://bendixcarstensen.com/Epi/).

Results

Study population and clinical outcomes

This study included 131 patients with moderately to severely active UC, who were administered ADA, and 40 healthy controls (HC). The mean age of the HC was 40.6 years, and 42.5% were men. Table 1 summarizes the baseline clinical characteristics of patients with UC. The mean age of the patients with UC was 44.7 years (range: 18–74 years), and 35.1% of the patients were men. Statistical analysis showed no significant difference in the proportion of males between healthy controls (HC) and patients with UC, indicating that the gender variable did not significantly influence the microbiome findings. The baseline mean Mayo and endoscopic subscores were 8.7 and 2.5, respectively. The clinical response rates were 52.1% (29/146) and 37.7% (36/146) at weeks 8 and 56, respectively. The clinical remission rates were 24.0% (35/146) and 22.0% (32/146) at weeks 8 and 56, respectively [19]. Clinical outcomes were assessed for 146 patients, while fecal samples for microbiome analysis were available from 131 patients.

Analysis of the gut microbiota between HC and patients with UC at baseline

DNA was extracted and sequenced from 244 samples (99, 100, and 45 samples at 0, 8, and 56 weeks, respectively) of 131 patients with UC and 40 samples of HC. The results on diversity showed a significant reduction in Shannon diversity and Faith's phylogenetic diversity in patients

Table 1 Baseline demographic and clinical characteristics of participants

Characteristics	Patients with UC (n = 131)
Age (years)	44.7 ± 14.9
Male sex, no (%)	46 (35.1)
Body weight (kg)	63.5 ± 12.6
BMI (kg/m^2)[a]	22.6 ± 3.7
Age at diagnosis (years)	38.8 ± 14.5
Duration of disease (months)	52.1 ± 49.6
Mayo score	8.7 ± 1.4
Endoscopic subscore	2.5 ± 0.5
Disease location	
Proctitis	24 (18.3)
Left-sided colitis	58 (44.3)
Extensive colitis	49 (37.4)
Fecal calprotectin (mg/kg)	
Mean ± SD[b]	892.8 ± 628.1
C-reactive protein (mg/dL)	
Mean ± SD	4.6 ± 11.4
Albumin (g/dL)	
Mean ± SD	3.7 ± 0.6
Concomitant medication (overlapped), n (%)	
5- aminosalicylates	110 (84.0)
Azathioprine/6-Mercaptopurine	61 (46.6)
Systemic corticosteroid	41 (31.3)
Prior anti-TNF[c] therapy, n (%)	33 (25.2)
1 medication	32 (97.0)
2 medications and above	1 (3.0)

BMI: body mass index; SD: standard deviation; TNF: tumor necrosis factor

with UC compared to HC, while there was no significant difference in Simpson's evenness. This suggests that UC is associated with both a loss of species diversity and a reduction in phylogenetic richness. However, the impact of UC may not extend to the dominance structure of the bacterial communities. Principal component analysis of beta diversity showed significantly different clustering between the HC and UC groups (ANOSIM, R = 0.369, $P = 0.001$) (Fig. 1A). LEfSe was used to identify important bacterial taxa that contributed to classifying HC and patients with UC. ASVs related to the Bacilli, *Peptostreptococcaceae*, *Lactobacillus,* and *Bifidobacterium* were predominant in patients with UC (Fig. 1B).

No significant differences were observed in alpha diversity and beta diversity based on the severity and extent of the disease (see figure, Supplementary Data Content 1). To determine the differentially abundant ASVs in HC and patients according to the severity and extent of UC, we conducted a LEfSe analysis. The 20 ASVs showing higher abundance in patients with severe UC included ASVs belonging to Bacilli, *Sporosarcina, Streptococcus thermophilus* TH1435, *Pediococcus*, and *E. coli*. Extensive colitis bacteria are characterized by a high abundance of ASVs, including *Blautia* (ASV5214), *Lactobacillus* (ASV3095), *Peptostreptococcus* (ASV6142), and Bacilli (ASV2551) (see figure, Supplementary Data Content 2).

A significant difference was observed between patients with high (≥ 500 mg/kg) and low (< 500 mg/kg) [28, 29] FC levels (R = 0.092, $P = 0.001$). Baseline fecal samples were stratified based on high and low ADA drug levels (trough level, serum ADA drug level of 5 ug/mL), showing no significant differences in the gut microbiome between these groups (R = 0.031, $P = 0.098$) (see figure, Supplementary Data Content 3) [28, 29].

Dynamics and diversity of microbes throughout the course of ADA treatment

LEfSe analysis revealed significant differences in bacteria at each time point during the 56-week ADA treatment period (see figure, Supplementary Data Content 4).

To examine the dynamics and diversity of microbes throughout the course of ADA treatment, we classified samples based on the attainment of clinical remission at each time point. The distribution of samples is presented in the Supplementary Table (see table, Supplementary Data Content 5). Baseline samples were divided according to the attainment of clinical remission at week 8.

The bacterial diversity of HC was higher than that of all other groups, regardless of the time point and remission. The principal coordinate analysis plot revealed distinct gut microbiome differences between HC and remitters at week 8 (R = 0.184, $P = 0.001$), and significant differences were also observed between HC and remitters at week 56 (R = 0.208, $P = 0.001$) (Fig. 2A). Unlike non-remitters, baseline dissimilarities significantly decreased in remitters at week 8, with levels lower than those at week 56 (Fig. 2A). The dissimilarities between remitters were significantly different, whereas no significant difference was observed among non-remitters (Fig. 2A and B). Furthermore, a notable reduction in dissimilarities was observed among remitters at week 8 when compared to non-remitters at the same time point (Fig. 2C and D).

After 56 weeks of ADA treatment, the gut microbiota composition of patients who achieved clinical remission showed distinct differences compared to that of HC. Figure 3 shows significantly different genera between 56-week remitters and HC, as confirmed by LEfSe analysis.

We explored the distinctive microbes identified in remitters at each time point and examined the changes in their abundance. In the baseline samples with remission at week 8, we noted an increase in *Burkholderia-Caballeronia-Paraburkholderia, Staphylococcus,* and *Murdochiella*; Lachnospiraceae UCG-008 in the remitters

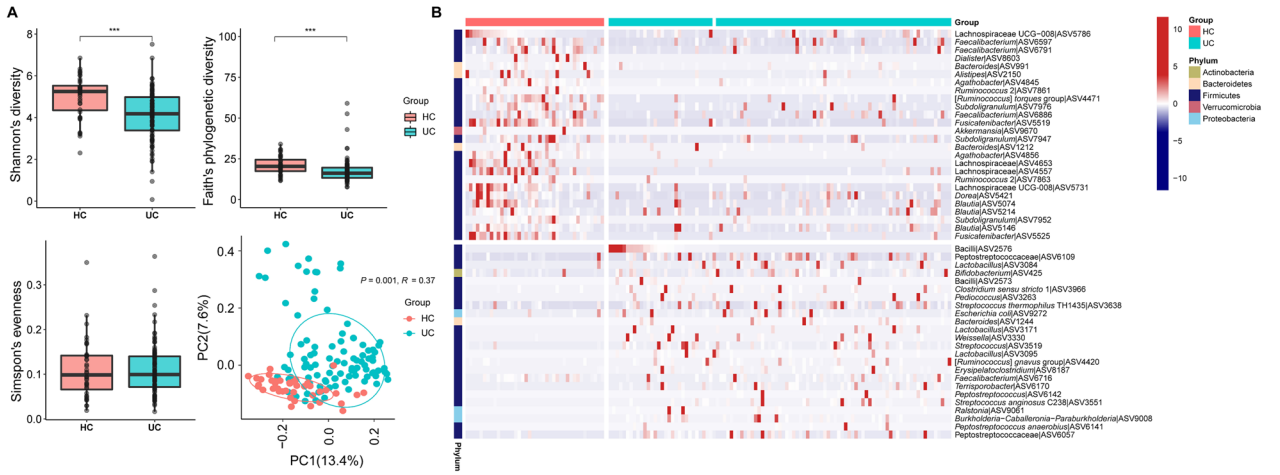

Fig. 1 Characterization of the gut microbiome of healthy controls (HC) and patients with ulcerative colitis (UC) at baseline (week 0). **A** Biodiversity was calculated using Shannon's diversity, Faith's phylogenetic diversity, and Simpson's evenness indices. Principal coordinate analysis (PCoA) plot of the microbiome profile of all participants was conducted using Unifrac unweighted distance matrix. The statistical significance of alpha diversity was tested using the non-parametric Wilcoxon rank sum test (*$P < 0.05$; **$P < 0.01$, ***$P < 0.001$), and the PCoA was evaluated using the analysis of similarities (ANOSIM) test. **B** Heatmap showed the significantly different amplicon sequence variants (ASVs) obtained from the linear discriminant analysis effect size (LEfSe) analysis. Only linear discriminant analysis scores > 3.2 are shown in this figure. Relative abundance was normalized to a Z-score,to show relative changes across the samples. Blue on the heat map indicates low abundance and red indicates high abundance. The row represents the taxonomic classification level from phylum to species of ASV, and the column is each sample

at week 8, and *Bifidobacterium, Dorea, [Ruminococcus] torques* group, and Lachnospiraceae FCS020 in the remitters at week 56 (Fig. 4A). Notably, decreased relative abundances were found in *Burkholderia-Caballeronia-Paraburkholderia* and *Staphylococcus* with time, and increased relative abundances of *Bifidobacterium* and *Dorea* in the remitters (Fig. 4B). However, in the non-remitters, the relative abundances of these four genera remained consistent across each time point, except for *Burkholderia-Caballeronia-Paraburkholderia*, which exhibited the highest abundance at baseline and the lowest at week 56.

Potential biomarker predicting clinical remission to ADA treatment

To predict clinical remission following ADA treatment at week 8 using the gut microbiome, we compared different ASVs between remitters and non-remitters. We compared the ASV tables of baseline samples with and without remission at week 8 (Fig. 5A). The baseline samples of remitters at week 8 showed a higher abundance of 40 ASVs, including *Sporosarcina* (ASV2803), *Bacteroides* sp. (ASV1298, ASV1490), *Enterobacter* (ASV9330, ASV9332), *Prevotella bivia* DSM 20514 (ASV2051), [*Eubacterium*] sp. (ASV6247, ASV6259), and *E. coli* (ASV9259), than those of non-remitters. On the other hand, they showed a lower abundance of 8 ASVs, including *Bifidobacterium* (ASV236, ASV396, and ASV509), *Blautia* (ASV5128), *Enterococcus* (ASV2914 and ASV2922), *Anaerostipes* (ASV5000), and Lachnospiraceae (ASV4860).

Considering the 48 ASVs with high or low relative abundances in the baseline samples of patients in clinical remission at week 8, we identified the distribution of these ASVs. The mean relative abundance of positive ASVs at week 0 for a patient was divided by the mean relative abundance of negative ASVs (Fig. 5B). The log value was higher for remitters than for non-remitters. The log ratio of positive ASVs/negative ASVs for predicting remission at week 8 was 0.348, with a sensitivity of 65.5% and specificity of 91.4% on the ROC curve (AUC, 0.851; Fig. 5C). Similarly, we attempted to determine positive and negative ASVs and evaluate the effect of ADA on clinical remission at week 56 using baseline and week-8 samples (see figure, Supplementary Data Content 6). However, a prediction model was not obtained (data not shown).

Discussion

Dysbiosis is defined as an altered diversity, composition, and structure of the intestinal microbiota, which can be caused by a spectrum of chronic inflammation and may lead to the development of IBD [30, 31]. The understanding and control of the gut microbiota is the key to overcoming IBD. However, despite the critical role of anti-TNF-α therapy in the treatment of UC, limited knowledge exists regarding the longitudinal changes in the gut microbiome following anti-TNF-α therapy.

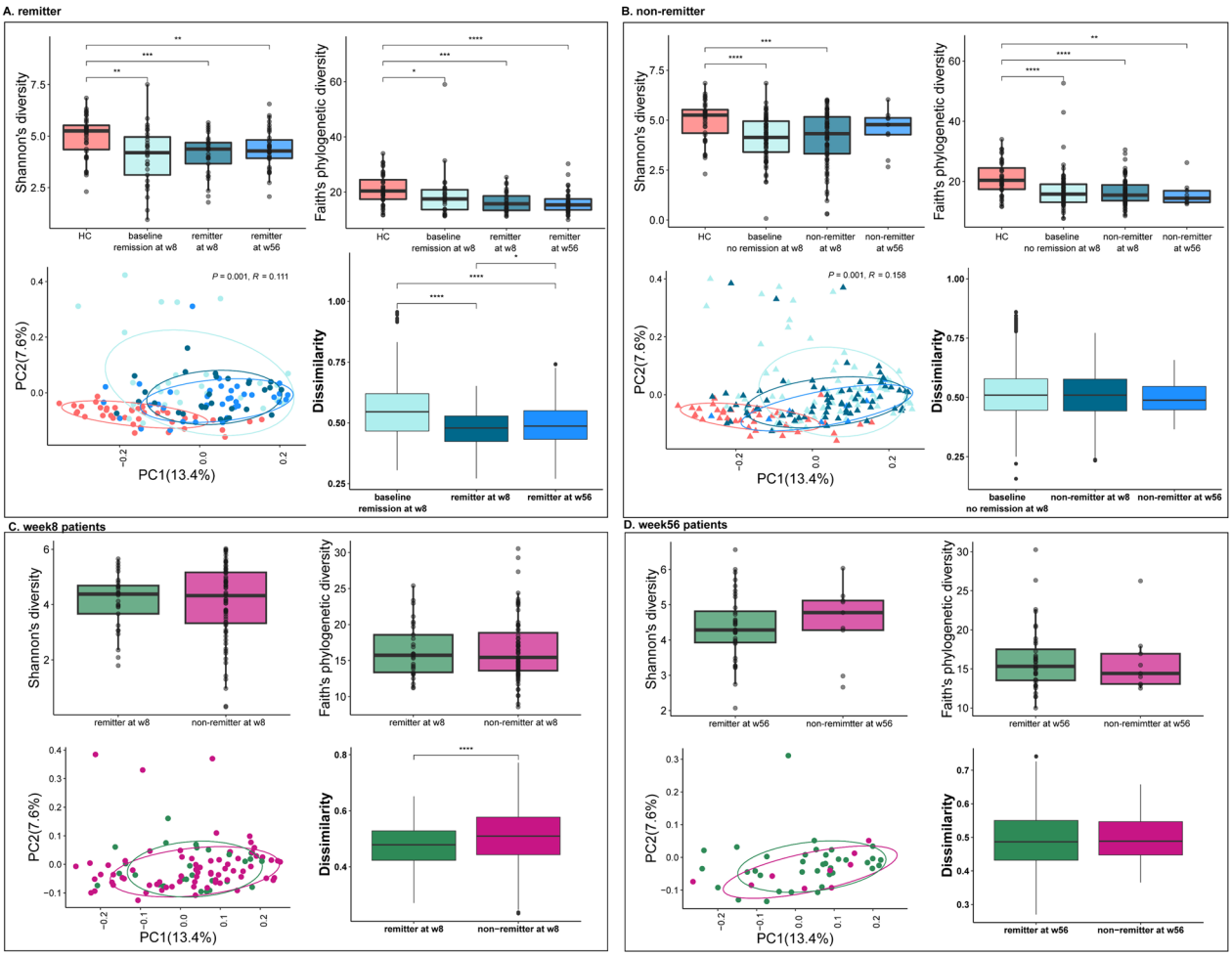

Fig. 2 Diversity and dissimilarity of the gut microbial community in each time point (week 0, 8, and 56) after adalimumab treatment to patients with ulcerative colitis (UC). Characterization of the gut microbiomes of remitters (**A**) and non-remitters (**B**) at each time point. Biodiversity was calculated using Shannon's diversity and Faith's phylogenetic diversity indices. Richness indices showed no significant difference based on the time point in patients with UC who showed clinical remission. Beta diversities comparing healthy controls (HC) and remitters (**A**) or non-remitters (**B**) at each time point and dissimilarities between the groups were calculated using Unifrac unweighted distance matrices. The comparison of dissimilarities between remitters was significantly different but not for non-remitters. Comparison of the gut microbiome of remitters and non-remitters at weeks 8 (**C**) and 56 (**D**)

While the restoration of gut diversity has been previously noted with anti-TNF therapy [12], a comprehensive understanding of the distinctions in the gut microbiome linked to the clinical responses during anti-TNF-α therapy is still lacking. We conducted a longitudinal analysis of changes in the gut microbiome in patients with UC before and after ADA treatment, followed by a description of these changes in relation to clinical response in the present study.

In patients with UC, notable variations in the microbial community structure were observed when compared to those in the HC, as evidenced by distinct features in Shannon's diversity and beta diversity. However, alpha diversity comparisons based on disease severity or extent did not reveal significant differences. Nonetheless, the composition of gut microbes varies according to the severity or extent of the disease. Three ASVs assigned to *Lactobacillus*, *Streptococcus sp.*, and class Bacilli were consistently identified as the predominant ASVs in patients with UC when comparing both HC and patients with UC based on the extent of the disease and disease severity. In particular, Bacilli (ASV2551) and *Streptococcus* (ASV3437, ASV3508, and ASV3519) were consistently present in patients with severe disease and extensive colitis. Previous studies related to UC also reported the enrichment of *Lactobacillus* and *Streptococcus* in patients with UC [32, 33], while several species within *Lactobacillus* and *Streptococcus* are categorized as

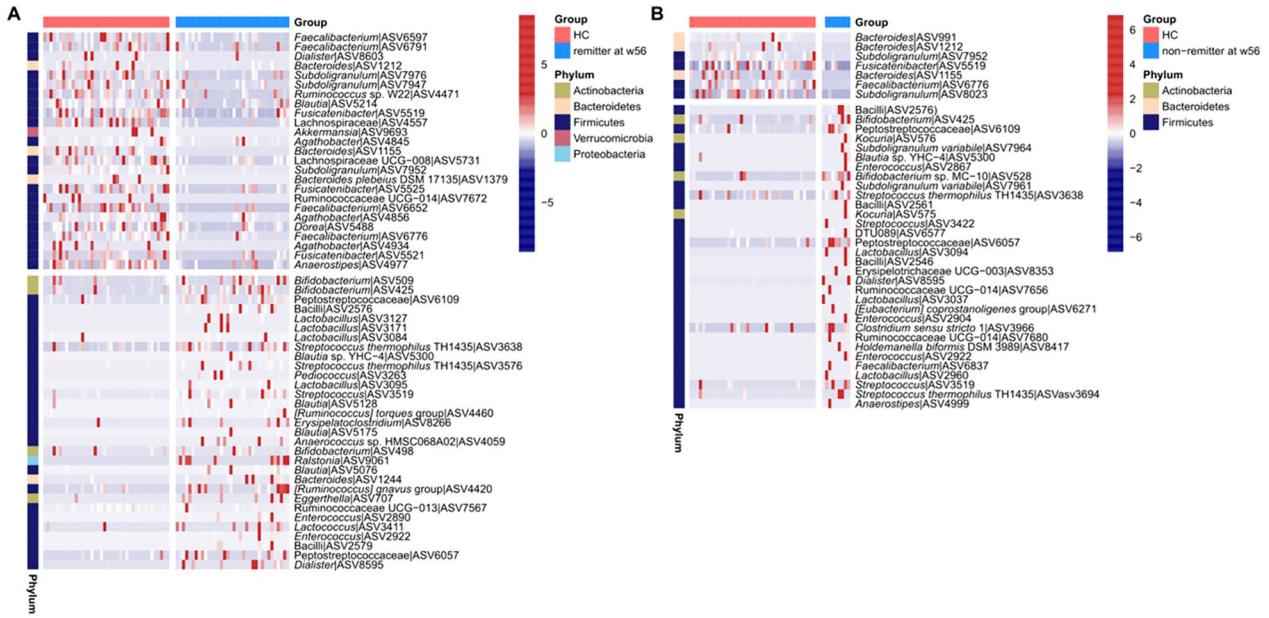

Fig. 3 Unique gut microbiome of patients with ulcerative colitis (UC) treated with adalimumab (ADA) at 56 weeks. Heatmap showing different gut microbiomes between healthy controls (HC), remitters (**A**), and non-remitters (**B**) who were treated with ADA and showed clinical remission or no remission at week 56 at the amplicon sequence variants (ASV) level. Relative abundance was normalized to a Z-score, and blue (lower) or red (higher) on the Z-score bar represents the calculated relative abundance. Each row represents the ASV, and the column represents each sample

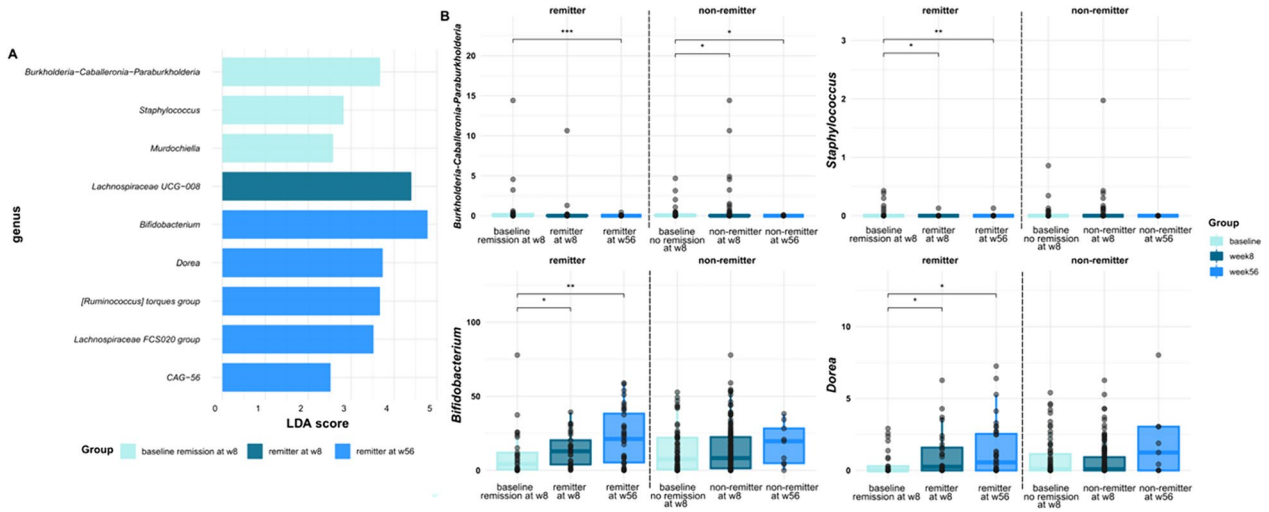

Fig. 4 Distinctive microbes identified in remitters at each time point and changes in their abundance. **A** The x-axis is the linear discriminant analysis score from a linear discriminant analysis effect size (LEfSe) analysis, and the y-axis represents each significantly different genus. **B** The relative abundances of continuously increased or decreased genera over time in the gut microbiome of remitters and non-remitters. Two genera, *Burkholderia-Caballeronia-Paraburkholderia* and *Staphylococcus*, decreased in remitters, and two genera, *Bifidobacterium* and *Dorea*, increased in remitters with a change of time point. The x-axis indicates the group, including baseline, week 8, and week 56, and the y-axis indicates the relative abundance of each genus

lactic acid bacteria [34]. Specific highly virulent strains of *Streptococcus* species have been considered potential risk factors for systemic inflammatory diseases, including UC [35, 36]. Additionally, certain *Lactobacillus* species are proposed to be linked with extensive disease involvement and heightened disease activity [37]. Although no clear evidence supports the association of a specific type of gut bacteria with UC development, these

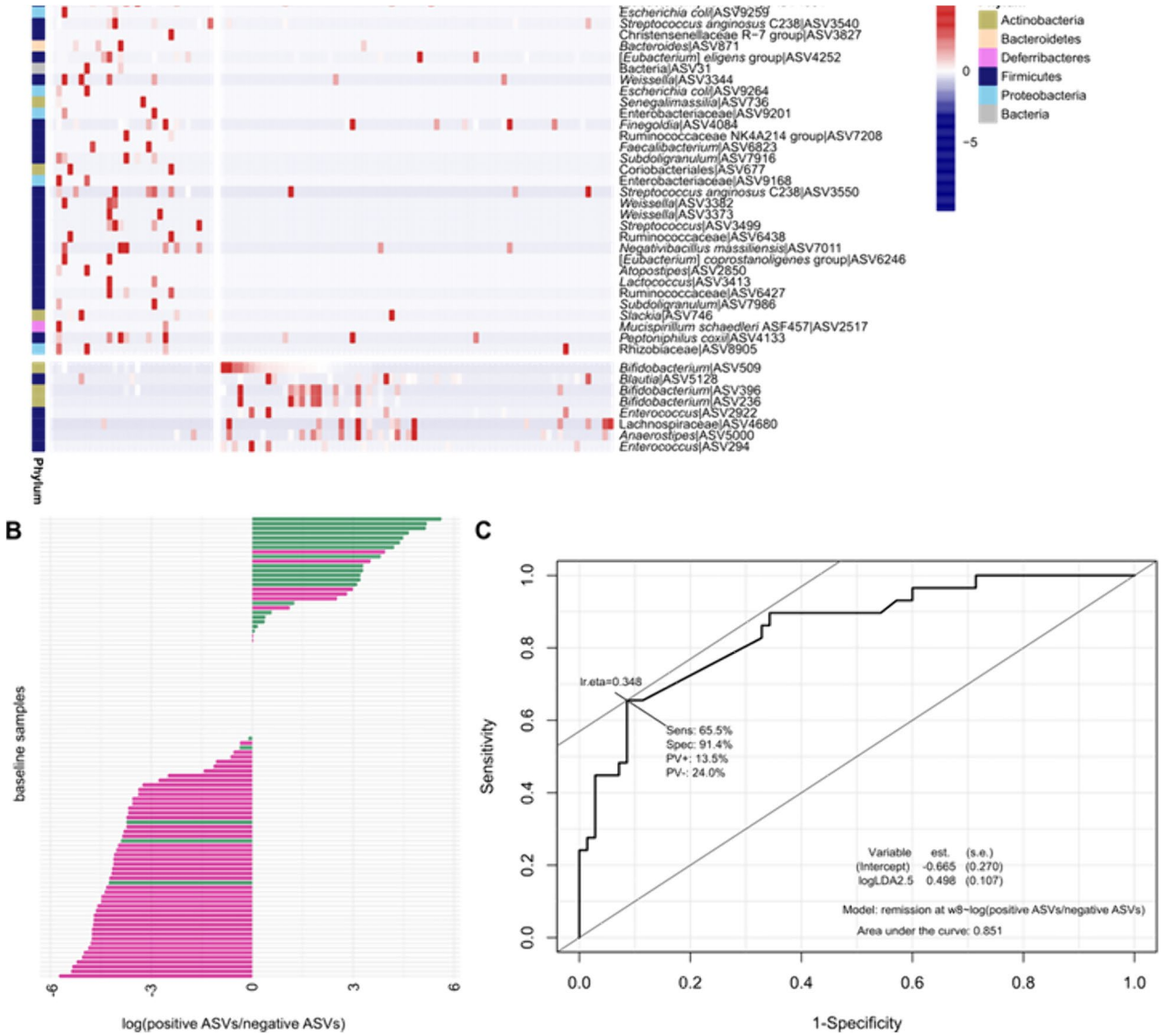

Fig. 5 Amplicon sequence variants (ASVs) as biomarkers for predicting clinical remission at week 8. **A** Different 48 ASVs were identified by comparing baseline samples of week-8 remitters vs non-remitters (Linear discriminant analysis score 2.0). **B** Bar graph of the positive and negative ASVs related to remission to adalimumab (ADA) treatment in patients with ulcerative colitis (UC). The positive and negative ASVs were included in the 48 different ASVs shown in **A**. The positive ASVs were the ASVs highly found in the samples with a clinical remission shown in the upper part of the heat map, and the negative ASVs were found in the samples of non-remitters shown at the bottom of the heat map. Each bar represents the value obtained by the log ratio of the average relative abundance of positive ASVs/average relative abundance of negative ASVs. **C** The receiver operating characteristic curve (ROC-curve) based on the log ratio of Avg. relative abundance of positive ASVs/Avg. relative abundance of negative ASVs. Using the ROC function in the Epi package in the R v4.0.2, the ROC curve was plotted with the area under the curve (AUC)

findings suggest that as the disease progresses, the gut environment may change to favor the colonization and expression of certain bacteria. Thus, the changing gut environment should be considered with the progression of the disease through further research.

In our study, despite patients with UC achieving clinical remission at 8 or 56 weeks after ADA treatment, their overall microbial diversity did not recover to the levels observed in the HC group. When comparing the gut microbiota composition of patients who reached clinical remission at 56 weeks with HC using LEfSe analysis, a notable difference in the abundance of various bacterial species was observed between the two groups. However, dissimilarity significantly decreased in patients who achieved clinical remission compared to before treatment, and notably, at 8 weeks of treatment, remitters

showed significantly lower dissimilarity compared to non-remitters. The dissimilarity is a measure used to quantify how distinct one microbial community is from another in terms of composition, structure, or function. These findings suggest that clinical remission with anti-TNF-α therapy does not result in the transformation of the gut microbiota composition to resemble that of HC; instead, patients seem to maintain their distinct gut microbial community.

The genus-level analysis showed a significant decrease in *Burkholderia-Caballeronia-Paraburkholderia* and *Staphylococcus* and significant increase in *Bifidobacterium* and *Dorea* from baseline to week 56 in patients with UC who showed clinical remission. A previous study reported that a higher proportion of the *Burkholderiales* order could be a biomarker of clinical response to anti-TNF treatment [38]. Further research is warranted on these taxa in patients with UC treated with anti-TNF agents. A low relative abundance of *Bifidobacterium* and *Dorea* in patients with active UC was consistent with the findings of previous studies [39, 40], and a high relative abundance of *Staphylococcus* in patients with UC was observed in a previous study that revealed *S. aureus* infection in the gut during IBD [41]. *Bifidobacterium* is the well-known butyrate-producing bacteria in the human gut and showed lower abundance in patients with active UC than in the remitters [42, 43]. Although a simple increase or decrease in specific bacteria may not fully reflect the overall gut microbiome status of patients with UC, our study provided a specific list of gut microbes for patients who achieved clinical remission through ADA treatment and suggested the evidence of the correlation between ADA treatment and gut microbes. We considered that the changes in the gut microbiome composition observed in patients who achieved remission through ADA treatment could be applied for exploring therapeutic targets for the treatment of UC. In addition, our findings on the longitudinal changes in gut microbiota among remitters align with results from studies conducted in other regions, suggesting that certain microbial changes during anti-TNF-α therapy may be universal [12, 13, 38]. However, some differences were noted, which could be attributed to regional variations in diet, genetics, and environmental factors. These results underscore the importance of considering regional differences when developing microbiome-based therapies and highlight the potential for personalized treatment strategies based on individual microbiome profiles.

In the present study, we identified a notable difference in the abundance of each gut microbe at the ASV level between baseline samples showing clinical remission and those showing no remission. ASVs belonging to *Sporosarcina*, *Bacteroides* spp., *Enterobacter*, and *Prevotella bivia* DSM 20514 were higher in baseline samples of week-8 remitters. ASVs assigned to taxa, including *Bifidobacterium*, *Blautia*, *Enterococcus*, and Lachnospiraceae, were less common in baseline samples of remitters. In addition, the log ratio of positive to negative ASVs was higher in remitters than in non-remitters based on the ROC curve analysis of baseline samples for predicting the response to ADA treatment. This result shows the importance of analyzing ASV levels to identify key microbes associated with an active member of the UC gut. The ratio of positive to negative ASVs could be a key factor for evaluating the effectiveness of ADA treatment in patients with UC.

Our study has several limitations. First, the smaller number of samples at 56 weeks could introduce bias into the longitudinal analysis. Additionally, the majority of samples collected at 56 weeks were from patients who demonstrated treatment efficacy at that time point. Second, this study may not account for all potential confounding factors that could influence the gut microbiome, such as dietary habits or lifestyle factors. As this research is a longitudinal investigation, continuously monitoring and accounting for dietary or lifestyle changes over the study period was challenging. Third, while this study contributes to understanding the microbial community dynamics influenced by anti-TNF treatment, the specific mechanisms and causal relationships between microbial changes and treatment outcomes were not elucidated. Lastly, the duration of the study, up to 56 weeks post-treatment, might not capture the long-term effects or changes that could occur beyond this timeframe. Considering these limitations, future research with larger and more diverse cohorts, longer follow-up durations, and consideration of potential confounding factors would provide a more comprehensive understanding of the effects of ADA therapy on the gut microbiome in patients with UC.

Conclusion

This study demonstrated that the composition of gut microbiota can undergo continuous changes during the course of ADA treatment, and such changes may vary in direction based on the clinical response. Furthermore, when reaching clinical remission, the gut bacteria were found to create a new environment distinct from that of healthy individuals, establishing a balance within it. Additionally, the ratio of positive to negative microbes in baseline samples can serve as a predictor for clinical remission. These findings help us to understand the flow of changes in the microbial community induced by anti-TNF treatment and suggest the possibility of personalized treatment through this flow in patients with UC.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13099-024-00637-5.

Additional file 1.

Acknowledgements
Not applicable.

Author contributions
Study concept and design: CHC, WJS. Acquisition of data: SYS, HJK KL, SJP. Statistical analysis and interpretation of data: HNO, SYS, JK, JB, WK, WJS. Drafting of the manuscript: HNO, SYS, JK. Critical revision of the manuscript for important intellectual content: CHC, WJS. Study supervision: CHC, WJS. Final approval of the version: all authors.

Funding
This study was funded by AbbVie and the National Research Foundation of Korea (NRF) (Grant/Award Number: NRF-2017R1D1A1B03031924). WJS was supported by the Chung-Ang University research grant in 2021.

Availability of data and materials
The 16S rRNA gene sequence data from the present study has been archived at the NCBI Sequence Read Archive (SRA) under the BioProject accession number PRNJA952830.

Declarations

Ethics approval and consent to participate
This manuscript has not been published or presented elsewhere in part or in entirety and is not under consideration by another journal. This study was performed in accordance with the principles of the Declaration of Helsinki and approved by the Institutional Review Board of Chung-Ang University Hospital (IRB No. C2015020). Written informed consent was obtained from each participant before inclusion into the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 15 February 2024 Accepted: 16 August 2024
Published online: 26 August 2024

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