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ORIGINAL ARTICLE

Discovery of fecal microbial signatures in patients with ankylosing spondylitis

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ABSTRACT

Objectives: This study aimed to investigate the characteristics of the gut microbiota in Chinese patients with ankylosing spondylitis (AS) and healthy controls in Quanzhou aiming to explore the correlation between microbiome changes and AS activities.

Patients and methods: In this study, high-throughput sequencing of the gene of 16S ribosomal RNA (16S rRNA) in fecal samples from 40 AS patients and 40 healthy controls, for a total of 80 participants (70 males, 10 females; mean age 33.7±10.7 years; range, 15 to 58 years), was conducted between January 2018 and January 2019. Alpha and beta diversity were analyzed using the QIIME (Quantitative Insights Into Microbial Ecology) software, and differences were analyzed using Student's t-test, linear discriminant analysis coupled with effect size and Metastats. Finally, a correlation network was constructed using Pearson's analysis.

Results: The alpha index values of the AS group were not significantly different from those of the control group. At the genus level, eight genera, *Ruminiclostridium_9, Fusicatenibacter, Adlercreutzia, CAG-56, Intestinimonas, Lachnospira, Bacteroides*, and *Pseudoflavonifractor*, were significantly enriched in patients with AS, whereas the abundance of *uncultured_bacterium_f_Saccharimonadaceae, Prevotella_7, uncultured_bacterium_f_Enterobacteriaceae, Cronobacter, Prevotellaceae_NK3B31_group,* and *Weissella* were significantly decreased in patients with AS. In addition, disease-related gut microbial communities were detected in patients with AS.

Conclusion: We found differences in the gut microbiome between the patients with AS and controls and identified potential disease activity-related bacterial communities.

Keywords: Ankylosing spondylitis , clinical indicators, gut microbiome, 16S rRNA gene.

Ankylosing spondylitis (AS) is a chronic inflammatory autoimmune disease that is characterized by sacroiliitis and spondylitis, which may lead to severe bony ankylosis.[1,2] The current prevalence rate in the Chinese Han population ranges from 0.2 to 0.54%.[3] Among the various genetic risk factors associated with AS, human leukocyte antigen (HLA)-B27 is dominant, as it is present in up to 90% of patients with AS.[4] In addition to family history, repetitive trauma at joint structures, osteoporosis, and intestinal inflammation are also believed to increase the risk of AS.[5,6] HLA-B27 may predispose individuals to AS by changing the gut microbiome,[7] and studies have shown that about half of patients with AS have chronic inflammation of the intestinal mucosa.[1] Furthermore, intestinal inflammation can make sacroiliitis inflammation more severe.[8]

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The gut microbiota is critical for the host defense system. Dysbiosis in the gut may lead to disease,[9] and an intestinal imbalance can directly or indirectly drive chronic gut inflammation by inducing autoimmunity.[10] Many studies have shown that 5 to 10% of patients with AS progress to subclinical gut inflammation, and the microbiome can mediate gut function.[11,12] An increasing number of studies have demonstrated a link between gut dysbiosis and bowel inflammation. Therefore, it is logical to consider that gut dysbiosis may play an important role in the pathogenesis of AS. Recently, specific variations in the gut microbial profiles of patients with AS from various regions have been studied using different methods.[13-16]

The 16S ribosomal RNA (16S rRNA) gene consists of 10 conserved regions that are common to bacteria and nine highly variable regions that are species-specific.[17] Thus, sequencing of the 16S rRNA gene can distinguish between bacterial genera and species. With the development of next-generation sequencing, the gut microbiota, which plays an important role in immunity and disease, can now be more deeply investigated.

In this study, we investigated the changes in the gut microbiota using fecal samples to identify specific markers of AS and to reveal their association with clinical indicators of AS.

PATIENTS AND METHODS

The study was conducted with 40 AS patients recruited from the Department of Ankylosing Spondylitis, Quanzhou Orthopedics-Traumatological Hospital, between January 2018 and January 2019 and 40 age-and sex-matched healthy volunteers; thus, a total of 80 participants (70 males, 10 females; mean age 33.71±10.7 years; range, 15 to 58 years) were included in the study. All patients fulfilled the modified New York criteria for AS. The healthy volunteers were recruited from the Medical Examination Center of Quanzhou Orthopedics-Traumatological Hospital. Clinical questionnaire survey showed that none of the controls had a history of gastrointestinal tract disorders, such as inflammatory bowel disease, rheumatic disease, or any other systemic disease.

Participants who underwent treatment with antibiotics or anti-inflammatory drugs or any yogurt products with live cultures within the last four weeks and those who had constipation or diarrhea prior to sampling were excluded. The antibiotics in our questionnaires included beta-lactams, aminoglycosides, tetracyclines, macrolides, chloramphenicol, and lincomycin. No close relationships were found among the study participants. These results were based on clinical questionnaires.

Collection and measurement of clinical indicators

The clinical information of the participants (sex, age, and clinical manifestations) was collected. The HLA-B27 gene type, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) levels were assessed. The Bath Ankylosing Spondylitis Functional Index (BASFI), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), and Ankylosing Spondylitis Disease Activity Score (ASDAS) were calculated.[18,19] Additionally, spinal computed tomography (CT) scans of all participants were obtained. The demographic and disease characteristics of the patients and healthy controls are shown in Table 1.

Sample collection and bacterial genomic deoxyribonucleic acid (DNA) extraction

A fresh fecal sample was collected from each participant and transported to the laboratory in ice boxes within 2 h. The samples were stored at -80°C until DNA extraction using the Power Fecal DNA Isolation Kit (Mobio Laboratories, Carlsbad, CA, USA). The DNA samples were stored at -80°C until analysis.

High-throughput sequencing of the 16S rRNA gene

The quality and quantity of the extracted DNA were evaluated using a NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis. The V3-V4 hypervariable region of the 16S rRNA gene was amplified using the V3-V4 region primers 338F, 5′-ACTCCTACGGGAGGCAGCA-3′ and 806R, 5′-GGACTACHVGGGTWTCTAAT-3′ with barcodes.[20,21] For polymerase chain reaction, 200 ng of fecal DNA was used as the template along with the ExTaq enzyme mixture (TaKaRa

Table 1. Population characteristics

	HC group (n=40)		AS group (n=40)	
Characteristic	n	Mean±SD	n	Mean±SD
Age (year)		33.7±11.0		33.7±10.6
Male	92.50		87.50	
Duration of disease (year)		N.A.		7.95±7.31
CRP (mg/L)		N.A.		32.81±30.53
ESR (mm/h		N.A.		31.38±25.29
BASDAI (score)		N.A.		2.06±0.79
ASDAS (score)		N.A.		2.82±0.91
BASFI (score)		N.A.		3.13±10.38
HAL-B27 positive	N.A.		100	
CT grade of sacroiliitis		N.A.		2.90±0.90

HC: Healthy controls; AS: Ankylosing spondylitis; SD: Standard deviation; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; BASDAI: Bath ankylosing spondylitis disease activity index; ASDAS: Ankylosing spondylitis disease activity score; BASFI: Bath ankylosing spondylitis functional index; HLA-B27: Human leukocyte antigen B27; CT: Computed tomography; Continuous, normally distributed variables between two groups were analyzed by Student's t-test. The Mann-Whitney test was applied for data that was continuous but nonnormally distributed. Category variables were tested by the chi-square test.

Bio Inc., Shiga, Japan), and the polymerase chain reaction products were gel-purified using a gel extraction kit (Qiagen GmbH, Hilden, Germany).

The MiSeq Reagent Kit V2 (Illumina Inc., San Diego, CA, USA) was used for library construction. The concentration and quality (including insert sizes) of the DNA library were estimated using a Qubit 2.0 fluorometer (Life Technologies, Carlsbad, CA, USA) and a Fragment Analyzer (Agilent Technologies, Inc., Santa Clara, CA, USA), respectively. All samples were sequenced on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA).

Quality control and data processing

Paired-end reads (250 bp in length) were merged using Flash version 1.2.7 software and then processed using the QIIME (Quantitative Insights into Microbial Ecology) package.[22] In brief, low-quality regions (with a mean quality score <20) and those with more than two ambiguous bases were excluded from the original paired-end raw reads.

Bioinformatics analysis

Bioinformatics analysis was performed using the Biomarker biocloud platform (www.biocloud.net/). A sequence with ≥97% similarity was defined as an operational taxonomic unit (OTU).[23] Taxonomy was assigned to all OTUs by searching the Silva database[24] (http://www.arb-silva.de.) using QIIME. Subsequent analyses were conducted using normalized data.

Statistical analysis

Data were analyzed using the online data analysis platform (http://www.biocloud.net/). Intergroup statistical analysis was performed using Student's t-test, and the correlation between the gut microbiota and clinical indicators was assessed using Pearson's correlation analysis. All data are expressed as the mean ± standard error, and statistical significance was defined as a p value less than 0.05.

RESULTS

Population characteristics

The mean age was 33.7±10.6 years and 33.7±11.0 years in the AS and control groups, respectively. Clinical indicators were measured for subjects in the AS group, and the mean values were calculated as follows: the duration of disease

was 7.9±7.3 years, CRP was 32.81±30.53 mg/L, ESR was 31.38±25.29 mm/h, BASDAI score was 2.06±0.79, ASDAS score was 2.82±0.91, BASFI score was 3.13±10.38, and the CT grade of sacroiliitis was 2.90±0.90. All patients were HLA-B27 positive (Table 1).

Diversity profile of the gut microbiota

From the sequencing analysis of the 80 samples, we obtained 12,902,183 raw reads; after filtering, 8,294,983 clean reads remained. We identified a total of 395 OTUs, including five unique OTUs in AS patients and eight unique OTUs in the control group. The rarefaction curves were close to saturation, indicating that the number of sequenced reads was large enough to reflect the bacterial diversity of the samples. Analysis of Shannon-Wiener and rank-abundance curves showed that the sequences were abundant and reflected the majority of the gut microbiota in the study subjects. In addition, the coverage estimates for the AS and control groups were 99.93% and 99.95%, respectively; thus, both groups showed good coverage rates.

For analysis of the diversity and richness of the AS and control groups, multiple alpha diversity indices were assessed using the number of observed OTUs (Figure 1). The results showed no significant difference in the assessed alpha diversity index values (ACE [abundance-based coverage estimator], Chao1, Shannon, and Simpson) between the AS and control groups (Figures 1a-d). Principal coordinate analysis of the Jaccard distance matrix intuitively indicates differences among all individuals. The microbiota in the AS and HC groups did not cluster well along the principal coordinates (Figure 1e). Moreover,

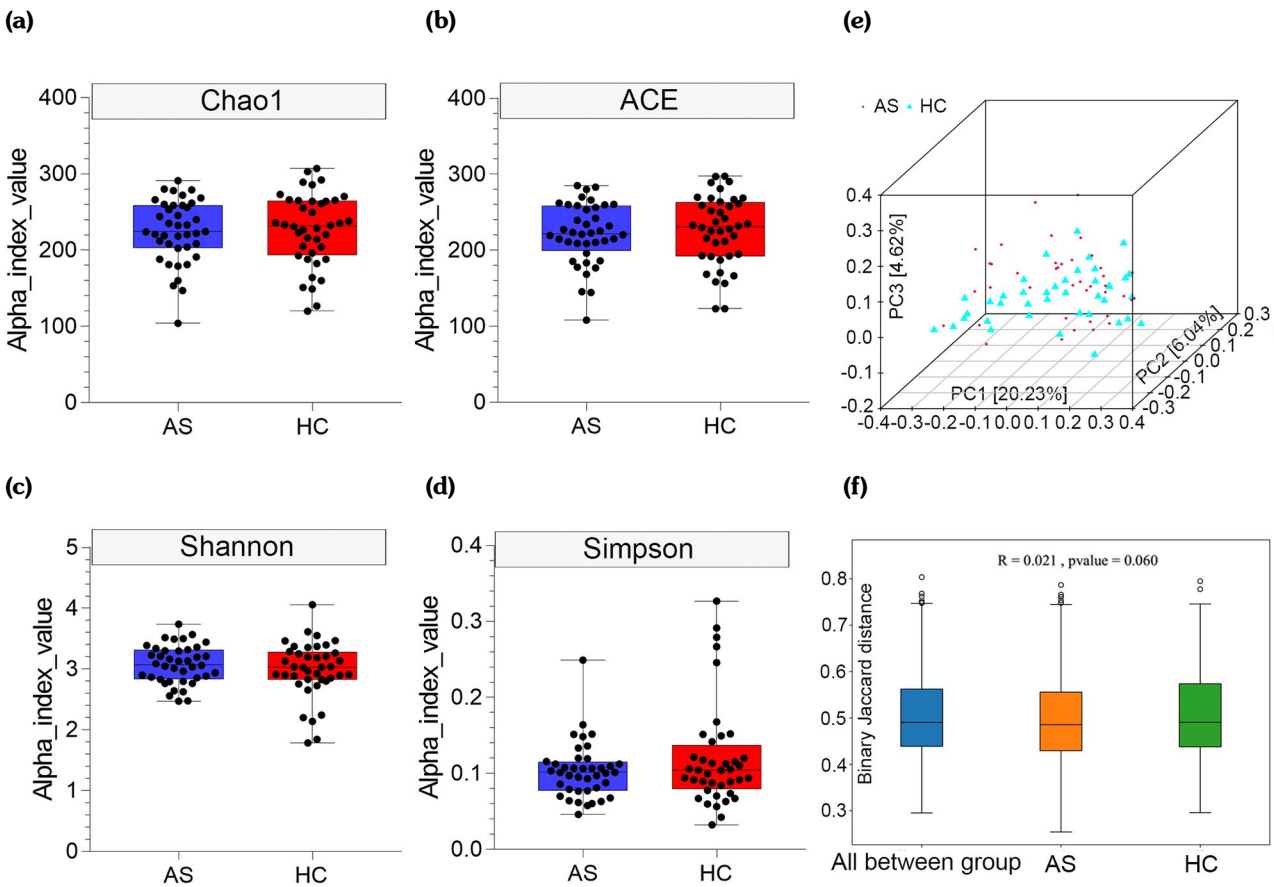

Figure 1. Analysis of DNA sequence data, microbial diversity index and bacterial community structures. **(a)** Chao1 index. **(b)** ACE index. **(c)** Shannon index. **(d)** Simpson index. **(e)** PCoA analysis by using Jaccard distance. Points with different color represent different groups. The distance between the two points represent the difference of RM. **(f)** ANOSIM analysis.
AS: Patients with ankylosing spondylitis; HC: Healthy controls; ACE: Abundance-based coverage estimator.

ANOSIM (analysis of similarities) revealed no difference in the microbiota composition between the two groups (R^2=0.021, p=0.060, Figure 1f).

Alteration of the bacterial composition in AS

The bacterial composition and structure in the AS and control groups were assessed at different taxonomic levels. At the phylum level, Firmicutes (AS, 57.08%; control, 58.53%) was the most abundant in all samples, followed by Bacteroidetes (AS, 32.05%; control, 26.06%). Linear discriminant analysis coupled with effect size (LEfSe) difference analysis showed that Bacteroidetes was significantly enriched, while *Proteobacteria*, *Actinobacteria*, and *Patescibacteria* were lower in the AS group than in the control group (Figure 2a).

At the family level, *Ruminococcaceae* (AS, 24.65%; control, 21.69%), *Bacteroidaceae* (AS, 24.19%; HC, 18.55%), and *Lachnospiraceae* (AS, 21.15%; control, 20.28%) were the three most abundant families in all samples (Figure 2b). The abundances of *Enterobacteriaceae*, *Leuconostocaceae*, and *Saccharimonadaceae* were lower in the AS group than in the control group, whereas *Bacteroidaceae* was significantly enriched in the AS group compared to the control group.

The predominant genera in all samples were *Bacteroides* (AS, 24.19%; control, 18.55%), *Faecalibacterium* (AS, 16.59%; control, 12.83%), *Escherichia-Shigella* (AS, 4.07%; control, 5.22%), and *Agathobacter* (AS, 5.15%; control, 3.43%) (Figure 2c). Additionally, an

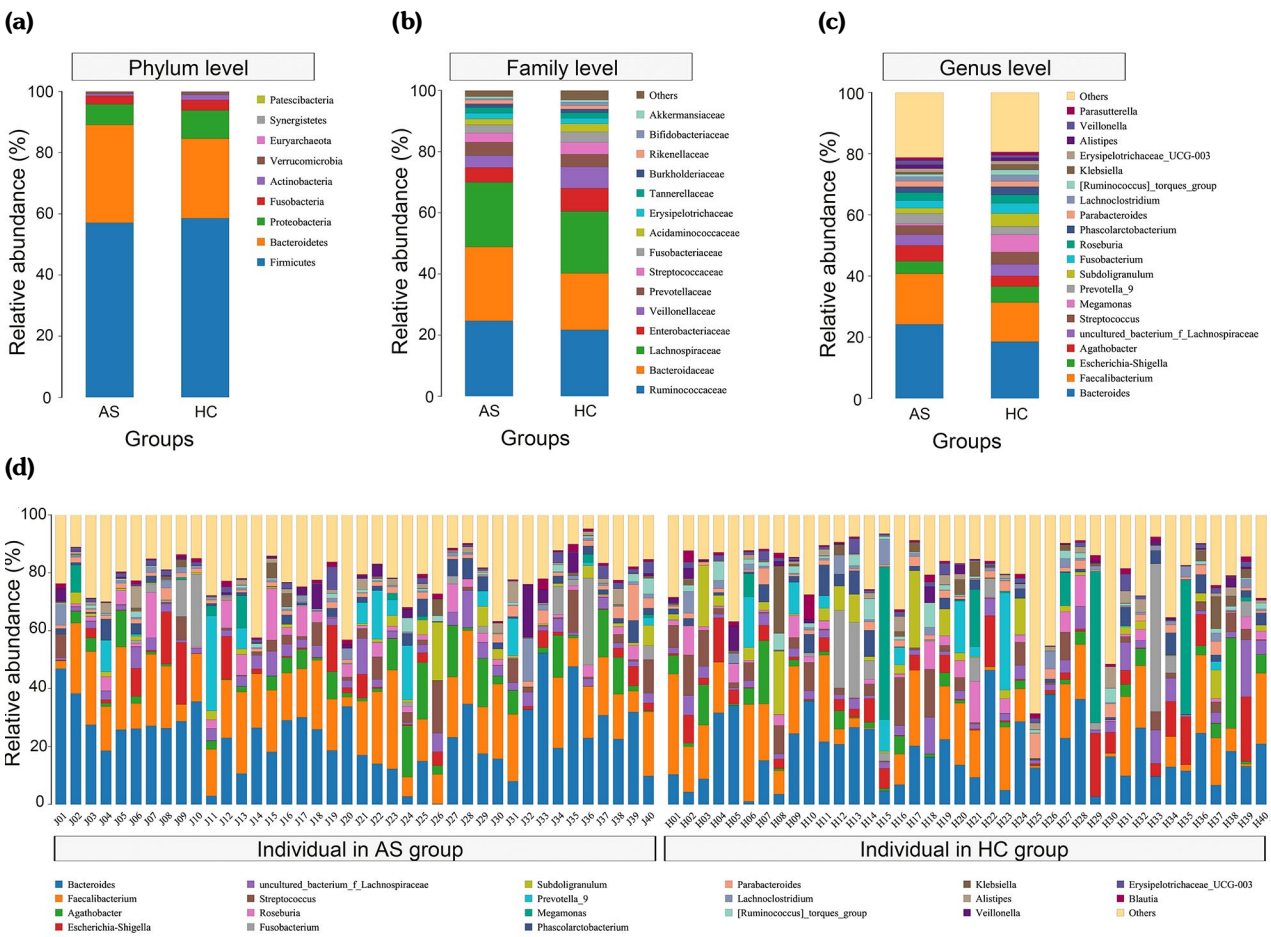

Figure 2. Alteration of composition of bacterial community in AS and HC. Each bar represents relative abundance of each bacterial taxon. Taxa distributions **(a)** at Phylum level, **(b)** at Family level and **(c)** at Genus level. **(d)** Relative abundance of genera in individual for all 80 samples.

AS: Patients with ankylosing spondylitis; HC: Healthy controls.

analysis of the relative abundance of genera in all 80 individuals was conducted, and the results showed that the genera in individuals from the same group also differed from each other (Figure 2d).

Microbial signatures in AS

Both Metastats and LEfSe were conducted at the genus level to reveal the microbial signatures in AS, which identified 26 and 25 genera, respectively, that had the potential to be microbial signatures in AS.

The Metastats analysis showed significantly higher relative abundance of *Aeromonas, Ruminiclostridium_9, Fusicatenibacter, Adlercreutzia, CAG-56, Ruminococcus_1, Intestinimonas, Negativibacillus, Lachnospira, Bacteroides, Faecalibacterium, Ruminococcaceae_UCG-009, Bilophila, and Pseudoflavonifractor* in patients with AS than those in the control group ($p<0.05$), and significantly lower abundance of *Rikenellaceae_RC9_gut_group, uncultured_bacterium_f_Saccharimonadaceae, Prevotella_7, uncultured_*

Table 2. Relative abundance of AS patients and healthy controls at genus level analyzed by Metastats analysis

	AS patients	Healthy controls		
Genus	Mean±SD	Mean±SD	*p* value	Q value
Aeromonas	0.03±0.03	0.00±0.00	0.000999001	0.045288045
Ruminiclostridium_9	0.16±0.03	0.04±0.02	0.000999001	0.045288045
Rikenellaceae_RC9_gut_group	0.00±0.00	0.01±0.01	0.000999001	0.045288045
uncultured_bacterium_f_Saccharimonadaceae	0.02±0.00	0.05±0.01	0.001998002	0.045288045
Prevotella_7	0.00±0.00	0.50±0.32	0.001998002	0.045288045
uncultured_bacterium_f_Enterobacteriaceae	0.03±0.01	0.53±0.23	0.001998002	0.045288045
Cronobacter	0.01±0.00	0.06±0.02	0.002997003	0.058227487
Fusicatenibacter	0.51±0.16	0.15±0.03	0.003996004	0.067932068
Prevotellaceae_NK3B31_group	0.00±0.00	0.04±0.02	0.004995005	0.075480075
Rothia	0.08±0.02	0.37±0.12	0.005994006	0.081518482
Adlercreutzia	0.02±0.01	0.00±0.00	0.006993007	0.086458996
CAG-56	0.69±0.20	0.22±0.07	0.00999001	0.113220113
Ruminococcus_1	0.81±0.27	0.20±0.06	0.010989011	0.114961961
Intestinimonas	0.02±0.01	0.01±0.00	0.012987013	0.126159555
Cloacibacillus	0.01±0.00	0.10±0.05	0.014985015	0.135864136
Negativibacillus	0.21±0.05	0.07±0.02	0.01998002	0.16983017
Lachnospira	0.96±0.29	0.36±0.10	0.028971029	0.228498774
Methanobrevibacter	0.00±0.00	0.10±0.07	0.032967033	0.228498774
Tyzzerella_3	0.03±0.01	0.29±0.13	0.032967033	0.228498774
Bacteroides	23.78±1.87	18.05±1.76	0.034965035	0.228498774
Faecalibacterium	17.38±1.31	12.89±1.73	0.036963037	0.228498774
Ruminococcaceae_UCG-009	0.02±0.01	0.01±0.00	0.036963037	0.228498774
Bilophila	0.27±0.07	0.13±0.02	0.038961039	0.230378317
[Ruminococcus]_torques_group	0.85±0.19	1.64±0.33	0.041958042	0.237762238
Pseudoflavonifractor	0.01±0.00	0.00±0.00	0.044955045	0.244555445
Weissella	0.03±0.01	0.10±0.04	0.04995005	0.261277184

AS: Ankylosing spondylitis.

bacterium_f_Enterobacteriaceae, Cronobacter, Prevotellaceae_NK3B31_group, Rothia, Cloacibacillus, Methanobrevibacter, Tyzzerella_3, and Weissella in patients with AS than those in the control group ($p<0.05$, Table 2).

Discriminant analysis was conducted using LEfSe to identify significant taxa variation, which distinguished each group (Figure 3). Patients with AS had an abundance of *Pseudoflavonifractor, uncultured_bacterium_f_Saccharimonadaceae, Intestinimonas, Allisonella, Lachnospiraceae_UCG_010, Adlercreutzia, Ruminiclostridium_9, Flavonifractor, Tyzzerella, Megasphaera, Catenibacterium, Fusicatenibacter, Lachnospira, CAG_56, Coprococcus_2*, and *Bacteroides*. In contrast, *Escherichia_Shigella, uncultured_bacterium_f_Enterobacteriaceae, Prevotella_7, Ruminiclostridium_6, Prevotellaceae_NK3B31_group, Weissella, Cronobacter, Candidatus_Stoquefichus*, and *uncultured_bacterium_f_*

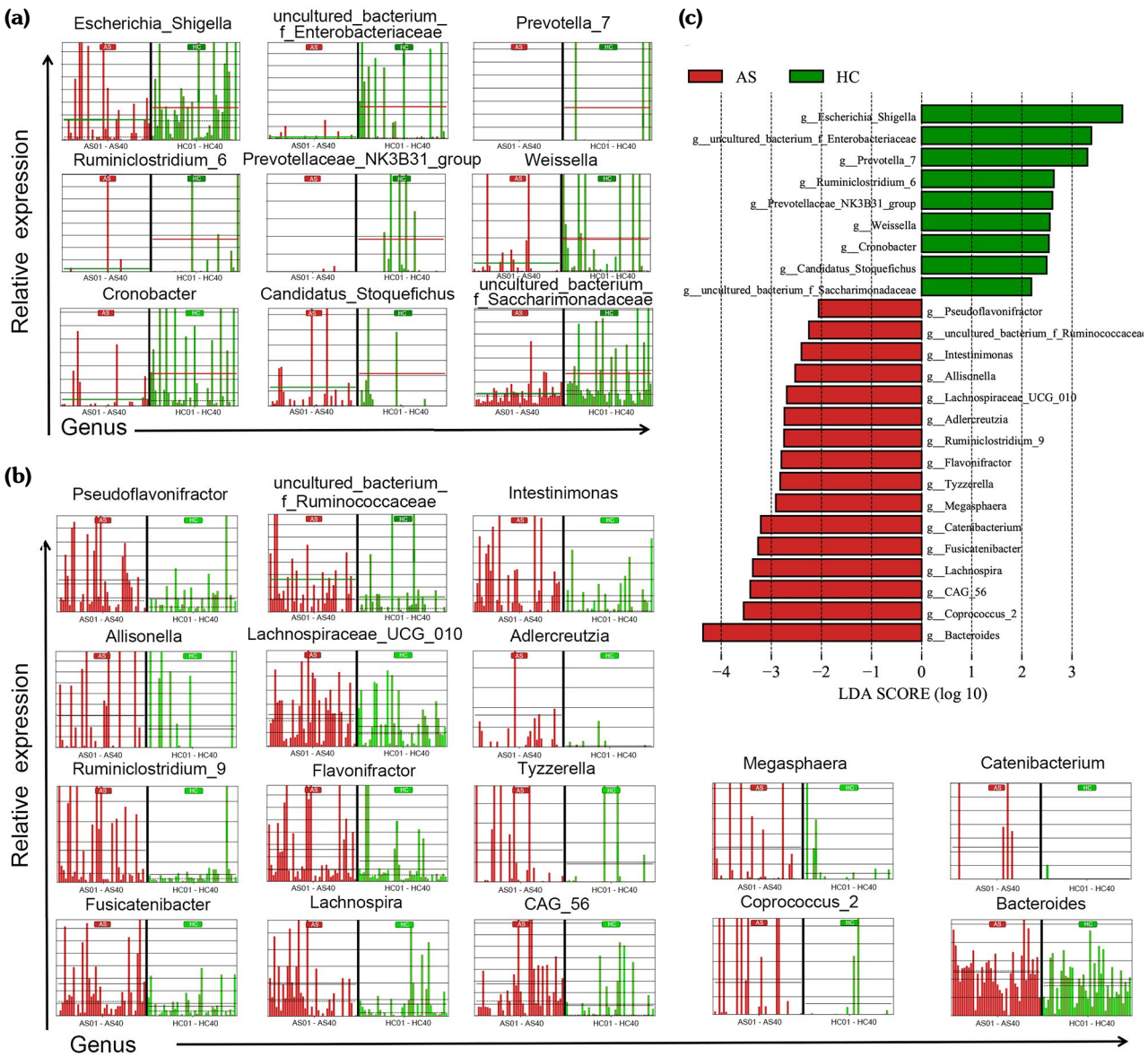

Figure 3. Identification of microbial signatures in groups investigated by using LEfSe at the genus level. **(a)** Significant decreased and **(b)** enriched in AS compared with HC; **(c)** LEfSe analysis of AS and HC. Only those attributed with a linear discriminant analysis threshold >2.0 were displayed.

LEfSe: Linear discriminant analysis coupled with effect size; AS: Patients with ankylosing spondylitis; HC: Healthy controls.

Saccharimonadaceae were less prevalent in the AS group.

Considered together, these analyses revealed that *Ruminiclostridium_9, Fusicatenibacter, Adlercreutzia, CAG-56, Intestinimonas, Lachnospira, Bacteroides, and Pseudoflavonifractor* were significantly enriched in AS, whereas *uncultured_bacterium_f_Saccharimonadaceae, Prevotella_7, uncultured_bacterium_f_Enterobacteriaceae, Cronobacter, Prevotellaceae_NK3B31_group,* and *Weissella* were significantly decreased in AS.

Correlations between clinical indicators and the gut microbiome in patients with AS

As shown in Figure 4 and Table 3, three genera were positively correlated with different clinical indicators. Roseburia was correlated with CT grade, age, disease duration, BASDAI, ASDAS, and ESR (r=0.44, p<0.001; r=0.37, p=0.03; r=0.36, p=0.01; r=0.39, p=0.01; r=0.29, p=0.01; and r=0.25, p=0.03, respectively). *Faecalibacterium* was positively correlated with CT grade, age, disease duration, CRP, and ESR (r=0.27-0.41, maximum p=0.03). *Uncultured_bacterium_f_Lachnospiraceae* was correlated with BASFI (r=0.16, p=0.01).

Unlike the AS group, as no clinical activity occurred in the control group, it was impossible to perform a correlation analysis. However, we could evaluate the relative abundance of clinical indicator-related genera between AS and control groups. The results showed no significant differences in these three genera, *Roseburia, Faecalibacterium,* and *uncultured_bacterium_f_Lachnospiraceae,* between the AS and control groups (p>0.05).

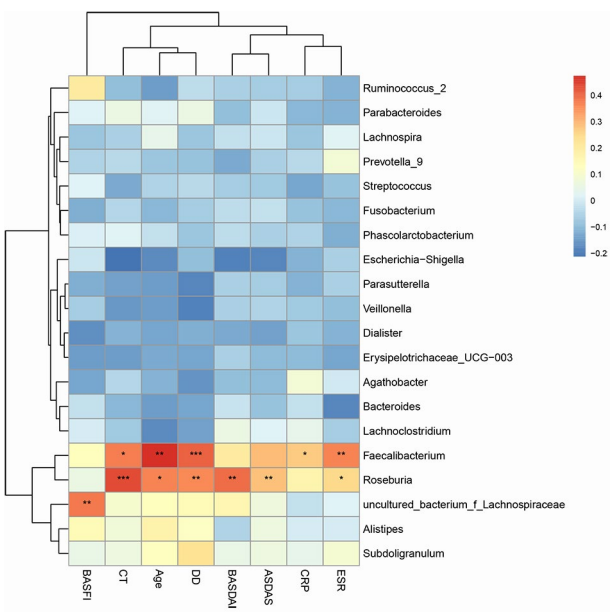

Figure 4. Correlation heat map between gut microbiota at genus level and clinical indicators in the AS group.

ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; ASDAS: ankylosing spondylitis disease activity score; BASDAI: Bath ankylosing spondylitis disease activity index; DD: Duration of disease; CT: Computed tomography grade of sacroiliitis; BASFI: Bath ankylosing spondylitis functional index; * P value <0.05; ** P value <0.01; *** P value <0.001.

DISCUSSION

In the present study, 16S rRNA gene high-throughput sequencing of human fecal DNA samples was used to analyze the gut microbiota alterations related to AS. We observed a relatively clear distinction in the gut microbiota between patients with AS and the controls. Correlations between clinical indicators and gut microbiota were analyzed using Pearson's coefficient.

The richness of the gut microbiota at the genus level in the AS and control groups was relatively inconsistent (Table 4).[15,16,21,25] The increase in *Ruminiclostridium_9* (#9, Table 4) observed in patients with AS was consistent with a previous study in the Chinese population, which included 10 cases of AS.[21] Ankylosing spondylitis is considered an inflammation-related disorder, and a previous study found that *Ruminiclostridium_6* was positively correlated with some proinflammatory factors, including plasma lipopolysaccharide, interleukin (IL)-6, tumor necrosis factor alpha (TNF-α), and IL-17A, in mice with diabetes mellitus.[26] The increased abundance of *Intestinimonas* (#10, Table 3) in AS and the dynamic distribution of *Bacteroides* (#7, Table 4) and *Prevotella_7* (#8, Table 4) were consistent with previous reports.[16,21,25] However, the changing trend in *Lachnospira* (#4, Table 4), which was enriched in patients with AS in our study, differed from that reported in other studies.[15,16] This was since the data came from different geographical regions and populations. Their genetic backgrounds, even recipes and dietary habits can have impacts on gut microbiota. Therefore, in clinical practice,

Table 3. Correlations between the disease activities and gut genera

Disease activities	Genera	r value	*p* value
BASDAI	Roseburia	0.39	0.01
ASDAS	Roseburia	0.29	0.01
BASFI	Uncultured_bacterium_f_Lachnospiraceae	0.16	0.01
Age	Roseburia	0.37	0.03
	Faecalibacterium	0.47	0.01
Disease duration	Roseburia	0.36	0.01
	Faecalibacterium	0.41	<0.001
C-reactive protein	Faecalibacterium	0.27	0.03
Computed tomography	Roseburia	0.44	<0.001
	Faecalibacterium	0.37	0.02
Erythrocyte sedimentation rate	Roseburia	0.25	0.03
	Faecalibacterium	0.37	0.01

BASDAI: Bath ankylosing spondylitis disease activity index; ASDAS: Ankylosing spondylitis disease activity score; BASFI: Bath ankylosing spondylitis functional index.

it is essential to investigate the disease in particular areas.

The number of bacteria is usually related to the immune response rather than an infection. Results from animal models revealed that *Bacteroides* could cause inflammation of the peripheral joints and intestines,[27,28] and previous studies showed that the development of irritable bowel disease and arthritis is related to the presence of *Bacteroides vulgaris*.[29,30] Our data confirmed that the abundance of *Bacteroides* was greater in patients with AS than in the controls. *Prevotella* spp. have proinflammatory properties and can release inflammatory mediators. Therefore, some *Prevotella* strains might be clinically important pathogens that could affect human health by promoting chronic inflammation.[31] However, a decrease in immunostimulatory *Prevotella* in the intestine may be closely related to human immunodeficiency, which is supported by the decrease in the abundance of *Prevotella* in the intestines of patients with AIDS[32] and in the lung tissue of patients with asthma and chronic obstructive pulmonary disease.[33] Although the remaining genera with abundance differences have not been previously reported (Table 4), these may also participate in the pathogenesis and development of AS by modulating the innate and adaptive immune systems.

C-reactive protein and ESR are two commonly used indicators of inflammation in human diseases,[34] including AS. C-reactive protein, which is produced by the liver and circulates in the bloodstream, is an acute-phase reactant and a biomarker that directly reflects inflammation.[35] It has been reported that CRP is positively correlated with *Bacteroides* and inversely correlated with *Ruminococcus, Helicobacter*, and *Parasutterella* in the Chinese population.[21] Our data showed, for the first time, that *Faecalibacterium* was correlated with CRP levels. Erythrocyte sedimentation rate might be a complementary indicator to CRP as a marker of systemic inflammation in disease. Three ESR-related genera,[21] *Bacteroides*, *Ruminococcus*, and *Parasutterella*, have been reported. However, our data also indicated that *Roseburia* and *Faecalibacterium* are novel ESR-related genera in AS. A recent study showed that *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium in the gut that is associated with a higher risk of postoperative recurrence of ileal Crohn's disease in humans.[36] In our study, the genera *Roseburia, Faecalibacterium*, and *uncultured_bacterium_f_Lachnospiraceae* were associated with clinical indicators of AS. Although significant relationships between clinical indicators and the

Table 4. Differences in AS patients and healthy controls at the genus level in recent published reports

Study	Year	Case region	Method	Enriched in AS	Decreased in AS	AS (n)	HC (n)	Ref
1	2019	Anhui, China	16 S rRNA	*Acetanaerobacterium;* *Anaeroplasma;* *Blautia;* *Coprobacillus;* *Dorea;#1* *Intestinibacter;#10* *Lactobacillus;#2* *Lautropia;* *Megamonas;* *Ralstonia;* *Streptococcus;#3* *Sutterella;*	*Akkermansia;* *Clostridium_IV;* *Clostridium_XIVb;* *Elusimicrobium;* *Kluyvera;* *Lachnospira;#4* *Mycoplasma;* *Parasutterella;#5* *Roseburia;* *Ruminococcus;#6*	103	104	16
2	2019	Guangzhou, China	16 S rRNA	*Alloprevotella;* *Comamonas;* *Collinsella;* *Dialister;* *Prevotella_2;#8* *Prevotella_9;#8* *Streptococcus;#3*	*Bacteroides;#7* *Eubacterium_ruminantium_Group;* *Lachnospira;#4* *Ruminococcus_gnavus_group;#6*	41	19	15
3	2019	The west of Sweden	GA-map™ Dysbiosis test	*Bifidobacterium sp;* *Dorea spp;#1* *Lactobacillus spp. 2;#2* *Lactobacillus spp;#2* *Pseudomonas spp;* *Streptococcus spp. 2;#3* *Streptococcus spp;#3* *Shigella spp;*	*Lachnospira;#4* *Bacteroides spp;#7* *Prevotella spp;#8*	150	17	25
4	2020	Tianjin, China	16S RNA	*Bacteroides;#7* *Desulfovibrio;* *Facklamia;* *Ruminiclostridium;#9*	*Helicobacter;* *Lactobacillus;#2* *Parasutterella;#5* *Ruminococcus;#6*	10	12	21
5	2020	Quanzhou, China	16S RNA	*Ruminiclostridium_9;#9* *Fusicatenibacter;* *Adlercreutzia;* *CAG-56;* *Intestinimonas;#10* *Lachnospira;#4* *Bacteroides;#7* *Pseudoflavonifractor*	*Uncultured_bacterium_f_* *Saccharimonadaceae; Prevotella_7;#8* *uncultured_bacterium_f_* *Enterobacteriaceae; Cronobacter;* *Prevotellaceae_NK3B31_group;* *Weissella*	40	40	This study

AS: Ankylosing spondylitis; HC: Healthy controls; # With same number indicated the same or equal genera, which was found in different studies.

gut microbiome were found at the genus level, the identified genera did not match those found in a previous microbial signature discovery study.[21]

We must state that this study has some limitations. Microorganisms were obtained from fecal preparations. Contaminated nonbacterial DNA was inevitably present in the fecal total DNA extractions, even though many of them were removed. To ensure the relative accuracy of the results, we equally extracted DNA from each stool specimen without visible food residues. Nutritional factors such as diet can also have an impact on gut microbiota. We failed to sufficiently control participants' eating behavior in this study. In addition, we had a relatively lower sample size (n=40) in our study. The obtained data are still worthy of peer reference.

These findings show that the fecal microbiota is imperative for further study the development of AS. Future studies should profile the species constituting the AS-related microbiota as well as the clinical indicator-related microbiota and shed light on how the gut microbiome contributes to the inflammatory response. Characterization of the detailed mechanism of the gut microbiome in AS should be a major project for future research.

In conclusion, our study demonstrated that the gut microbiota of patients with AS differs from that of healthy individuals. Some alterations are related to the development of AS, as evidenced by the changes at various taxonomic levels. We inferred that some biomarker microbes are involved in the pathogenesis and development of AS, including the genera *Ruminiclostridium_9, Fusicatenibacter, Adlercreutzia, CAG-56, Intestinimonas, Lachnospira, Bacteroides,* and *Pseudoflavonifractor*, which were increased in abundance in patients with AS, and *uncultured_bacterium*_f_Saccharimonadaceae, *Prevotella_7*, *uncultured_bacterium_f_Enterobacteriaceae, Cronobacter, Prevotellaceae_NK3B31_group,* and *Weissella*, which were decreased in abundance in patients with AS. Our study highlights the signature microbiota involved in the pathogenesis of AS and provides a guidance tool for assessing the morbidity of AS. Furthermore, the discovery of microbes related to AS activities may help develop a new strategy for the treatment of AS.

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Ethics Committee Approval: The study protocol was approved by the Quanzhou Orthopedics-Traumatological Hospital Ethics Committee (no: 2019-11). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patient Consent for Publication: A written informed consent was obtained from each patient.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Contributed equally to this work: W.Z., Y.Y.; Contributed to the conception and design of the study: J.L., Y.Y., W.Z.; Material preparation and analysis were performed: Y.Y., M.C.; Data collection and diagnosis were performed: M.C., Q.G., Y.C., W.Z., J.W.; The first draft of the manuscript was written: Y.Y., W.Z.; All authors commented on the previous versions of the manuscript and read and approved the final manuscript.

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