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Fecal microbiota in patients with ankylosing spondylitis: Correlation with dietary factors and disease activity

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ABSTRACT

Background: We investigated the characterization of the gut microbiome in Chinese patients with ankylosing spondylitis (AS) and healthy controls (HCs) and to explore the association ofbacteria communities with dietary factors and disease activity.

Methods: 16S ribosomal RNA gene sequencing was performed on fecal DNA isolated from stool samples in consecutive cross-sectional cohorts. Alpha and beta diversities were assessed using QIIME, and comparisons were performed using one-way ANOVA, Student's *t*-test, and SKN multiple range comparisons to examine differences between groups and a correlation network analysis was performed.

Results: We investigated 207 samples from 103 AS patients and 104 HCs. Alpha diversity was not significant difference in AS compared with HCs. For the community structure, Bacteroidetes was the most represented class. Megamonas, Dorea, and Blautia were significantly greater in AS than in HCs, whereas the abundance of Lachnospira, Ruminococcus, and Clostridium_XlVb was significantly lower in AS than in HCs. In addition, Specific gut microbiome was significantly correlated with disease activity and dietary factors.

Conclusions: Our results suggest that the human gut microbiome of AS patients was clearly different from that of HCs and bacteria communities are associated with dietary factors and disease activity.

1. Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory autoimmune disease characterized by ankylosis of spine and inflammation in tendons and pelvis. Its prevalence currently ranges from 0.2% to 0.54% in the Chinese Han population [1]. Multiple factors, including family history, repeated trauma to the joint structures, osteoporosis and intestinal inflammation, have been contributing to an increased risk of AS [2–4]. AS is regarded as a genetic disease and is strongly associated with human leukocyte antigen (HLA)-B27 [5]. Furthermore, HLAB27 might predispose to AS by altering the intestinal microbiome [6]. Approximately 50% of AS patients have mucosal chronic inflammation [7], and intestinal inflammation contributes further to the severity of sacroiliac joint inflammation [8].

Gut microbiome has an important role in human body defense system. The imbalance between gut microbiome and immune system may contribute to diseases [9], and intestinal dysbiosiscan drive chronic gut inflammation directly or indirectly through induction of autoimmunity [10]. Several evidence shown that almost 10% of AS patients develop subclinical gut inflammation, and gut microbiome can regulate intestinal function [10–12]. Moreover, AS is a prototype of spondyloarthritis (SpA), and most SpA patients exhibit bowel

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Abbreviations: AS, ankylosing spondylitis; ASDAS, Ankylosing Spondylitis Disease Activity Score; BASDAI, Bath AS Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; CD, Crohn's disease; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HCs, healthy controls; HLA-B27, human leukocyte antigen; IBD, inflammatory bowel disease; OUT, operational taxonomic units; QIIME, Quantitative Insights Into Microbial Ecology; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SpA, spondyloarthritis; TNF, tumor necrosis factor; UC, ulcerative colitis

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inflammation [13]. Recently, a cohort study found that AS patients with chronic intestinal inflammation had a higher level of bone marrow edema in sacroiliac joints compared with patients with normal gut histology [8]. Thus, gut microbiome may be undoubtedly implicated in the pathogenesis of AS, although the study is still ongoing. Therefore, we conducted a study to elucidate the role of gut microbiome in the pathogenesis of AS.

The question is what may alter gut microbiome in patients with AS? The answer may be diet, because diet has a strong influence on gut microbiome [14]. Interestingly, a distinct AS gut microbial signature was reported in a comparison of patients with HCs on the basis of 16S ribosomal RNA gene sequencing [15]. During the last decade, significant differences of microbiomes have been reported for many autoimmune diseases including CD [16], ulcerative colitis(UC) [17,18], rheumatoid arthritis (RA) [19], systemic lupus erythematosus (SLE) [20,21], psoriasis [22] and AS [13,23].

2. Methods

2.1. Patients and controls information

A total of (103 AS patients) were enrolled from 2 hospitals (First Affiliated Hospital of Anhui Medical University and the Second Affiliated Hospital of Anhui Medical University). Patients (85 men, 18 women) aged 16–62 y were diagnosed on the modified New York criteria for AS [24]. Clinical information of the participants (gender, age, body mass index [BMI], clinical manifestation, test for HLA-B27, erythrocyte sedimentation rate [ESR], C-reactive protein [CRP]) was collected, and the Bath Ankylosing Spondylitis Functional Index (BASFI) [25], Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) [26] and Ankylosing Spondylitis Disease Activity Score (ASDAS) were calculated (Shown in Table 1). A total of 104 HCs were collected in the Shunan community and Anhui Medical University. HCs (84 men, 20 women) were aged 15–67 y and were free from any history of inflammatory bowel disease (IBD) or any rheumatic disease. All subjects provided signed informed consent. In addition, individuals with gastrointestinal tract disorders and those who undergoing treatment with antibiotics within one month prior to the stool collection were excluded. Patients with severe systemic diseases or hepatitis were excluded.

Table 1
The basic demographic characteristics, clinical features, laboratory measurements and medication used situation of the enrolled participants.

Variable	AS(n = 103)	Healthy control(n = 105)	χ^2/t	P
Age (y)	33.29 ± 11.66	33.66 ± 12.53	0.221	NS
Male/female	85/18	84/20	0.106	NS
BMI (kg/m^2)	22.85 ± 3.60	22.98 ± 3.38	0.280	NS
ESR (mm/h)	19.0(10.5,37.0)	NA		
CRP (mg/l)	9.2(3.8,24.0)	NA		
WBC	7.04 ± 1.91	NA		
HLA-B27+ (%)	92.6	NA		
Disease characteristics, mean (SD)				
BASFI (cm)	1.20(0.40,4.30)	NA		
BASDAI (cm)	3.06 ± 2.07	NA		
Active AS n (%)	29(28.2)	NA		
Inactive AS n (%)	74(71.8)	NA		
Disease duration (months)	74.5(43.3120.3)	NA		
Medication, n (%)				
NSAIDs	71(68.9)	NA		
biological agents	46(44.7)	NA		
DMARDs	35(34.0)	NA		
other	59(57.3)	NA		

AS, ankylosing spondylitis; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; NA, not applicable; SD, standard deviation; BASFI, Bath Ankylosing Spondylitis Function Index; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; NSAIDs, non-steroidal anti-inflammatory drugs; DMARDs, disease-modifying anti-rheumatic drugs; other included Chinese patent medicine.

A dietary questionnaire that recorded the complete diet information and dietary habits was completed. The questionnaire was used to exclude individuals that had specific dietary habits such as alcohol consumption or a completely vegetable-based diet. Clinical iand dietary information on the participants are shown in Table S1-S2. Clinical diagnosis and blood examination data were obtained from the hospitals.

2.2. AS patient fecal sample collection

Fresh fecal samples from all individuals were frozen immediately at −20 °C, and transported within 6 h from the hospital to the laboratory, where they were stored at −80 °C until analysis.

2.3. Genomic DNA extraction and Illumina sequencing

Bacterial DNA was extracted from the fecal samples (200 mg) using the MiSeq Reagent Kit v3 (Illumina) according to the manufacturer's protocol. The quality of DNA was measured using a NanoDrop 2000 (Thermo Fisher Scientific, used to estimate the DNA concentrations) and agarose gel electrophoresis (used to measure the molecular sizes and genomic DNA integrity). TruSeq DNA HT Sample Prep Kit was used for library construction. The quality of the DNA library was estimated using Invitrogen Qubit 3.0 Spectrophotometer (Thermo Fisher Scientific) to estimate the DNA concentration and Agilent 2100 bioanalyzer (used to measure the insert sizes). The insert sizes of all samples were in the range of 120–200 bp. All the samples were sequenced on Miseq.

The bacterial genomic DNA was used as the template for amplification of the V3-V4 hypervariable region of the 16S rRNA gene with the forward primer 5′-CCTACGGGNGGCWGCAG-3′ and the reverse primer 5′-GACTACHVGGGTATCTAATCC-3′.

2.4. Quality control and host genome filtering

The raw reads from the AS patients and HCs that had 90% low-quality bases (quality ≤ 20) or > 2 ambiguous bases were excluded. Subsequently, reads with low-quality tails (quality ≤ 20) were trimmed, the remaining reads were mapped to the human genome (hg19) by SOAP alignment (v2.21) [27], and the matching reads were removed as being contaminants from the host genome.

2.5. Bioinformatics analysis

The forward primers in front of the reads from the original DNA fragments were trimmed with CutAdapt software (−-discard-untrimmed -Q 20). Since the original DNA fragments were shorter than the length of the reads, we also trimmed the primers at the end of both reads. Then, pairs of reads were merged by using FLASH, i.e., a very fast and accurate software tool that was designed to merge pairs of reads when the original DNA fragments were shorter than twice the length of the reads. Sequencing reads were assigned to each sample according to the unique barcode and were analyzed with the QIIME [28] software package (Quantitative Insights Into Microbial Ecology) and the UPARSE pipeline [29]. A sequencing read was assigned to each sample according to the unique barcode of each sample. Sequences were analyzed with the UPARSE pipeline. First, the reads were filtered by UPARSE quality filters (maxee = 1, min_length = 200) [29]. Then, we used the UPARSE pipeline to select operational taxonomic units (OTUs) to make the OTU table. Sequences were assigned to OTUs at 97% similarity [30]. We picked a representative sequence for each OTU and used the mother to assign taxonomic data to each representative sequence (the OTU and the quality of the raw data sequencing is shown in Additional file 1: Table S3 and Table S4). The subsequent analyses of alpha diversity and beta diversity were all performed based on this output of normalized data.

2.6. Statistical analysis

To compute alpha diversity, we rarified the OTU table and calculated 3 metrics: Chao1, which is an ACE metric that estimates the richness; the observed OTUs metric, which was simply the count of unique OTUs found in the sample; and the Shannon index. Rarefaction curves were generated based on these 3 metrics. One-way ANOVA, Student's *t*-test, and SKN multiple range comparison were performed separately on the soil property datasets using the Statistical Package for the Social Sciences (SPSS ver 16.0). The correlation between community planning (OTU) and environmental factors was calculated using software R.

3. Results

3.1. Study population

We investigated 207 samples from 103 subjects with AS and 104 HCs. All subjects provided a fecal sample. The study population consisted 81.6% males, and had a mean age of 33.48 y (range, 15–67 y) and a mean BMI of 22.91 kg/m^2 (range, 14.69–34.16 kg/m^2).

3.2. Characteristics of the abundance and riches of gut microbiota

We obtained 23,086,590 raw reads and 17,721,650 reads remained after filtering. Sequencing analysis of the 207 samples identified 1277 OTUs. The rarefaction curves had a tendency to approach a saturation plateau, indicating that the number of samples was sufficient. The same tendency was found in the ShannonWiener curves and Rank-Abundance curves, indicating that the number of 16S rRNA gene sequences in the database was very abundant and reflected the vast majority of the microbiota.

The α diversity assessed by the number of the observed genera was no significant difference in AS compared with HCs ($P > .05$, Fig. 1).

For community structure, phylotypes with a median relative abundance larger than 1% and 0.1% at the class and genus levels were assessed. Bacteroidetes (64.2%) was the most represented class in AS samples, followed by Clostridia (23.8%) and Betaproteobacteria (4.3%), while Bacteroidetes (62.8%) was the most represented class in control samples, followed by Clostridia (27.8%) and Betaproteobacteria (4.8%). Bacteroides (35.6%) and Prevotella (19.9%) were enriched in AS at genus level, while Bacteroides (36.1%) and Prevotella (16.6%) were more than half of the proportion in HCs (Figs. 2A,B). These results indicated that Bacteroidetes were the first taxa in both the AS patients and HCs, which in agreement with other studies [31]. Examination at the genus level showed that the relative abundance of Megamonas, Dorea, and Blautia was significantly greater in AS than in controls, whereas abundance of Lachnospira, Ruminococcus, Clostridium_IV, and Clostridium_XlVb was significantly lower in AS than in control samples ($P < .05$) (Fig. 3, Table S5). Furthermore, compared with HCs, Prevotellaceae of the microbial communities in AS was not significantly different. This observation is different from the data of Costello [32]. The study by Lin [33] noted marked effects of HLA (human leukocyte antigen)-B27 on the gut microbiota; and an increase in the Prevotella spp. was observed in HLA-B27 transgenic rats. P. copri may stimulate an immune reaction that then targets joint tissues and this species was strongly correlated with the disease severity in untreated new-onset RA patients [34], whereas P. melaninogenica can interact with human lactoferrin.

The most abundant species in AS and HC groups were primarily from the Bacteroides genus. Of the 3 species for which the abundance was most decreased in the AS group, one was Bacteroidesspp. It has been reported that Bacteroides spp. are reduced in RA [34] and IBD [35]. Noticeably, our study reported that uncultured_Christensenellaceae_bacterium ($p = .003$, Wilcoxon rank-sum test), Alistipes_shahii ($p = .006$), and Bacteroides_cellulosilyticus ($p = .02$) were decreased in the AS group (Fig. 4).

Beta diversity analysis showed that the microbiota composition was significantly different between the two groups (Non-MetricMulti-Dimensional Scaling, NMDS, stress = 0.13; ADONIS Analysis, $p = .026$, Figs. 5A, B). Discriminant analysis using LEfSe identified significant taxa variations distinguishing each sample group from another (Fig. 6). Patients with AS had an increase in Bacteroides plebeius belonging to the Bacteroidaceae family including Megamonas, Sutterella genera, and Actinobacteria from the Coriobacteriaceae family. Increased species included Streptococcus, Parasutterella and Clostridium.

3.3. Correlations of the environmental factors, diet and gut microbiome

The information of environmental factors and diet including smoking, alcohol use, milk, noise, physical activity, damp, etc. were collected. Based on the problems that discovered in the past investigation to the food, with related information and document, we put forward the four groups (A,B,C,D), which in view of the energy and composition of food in individual diet by factor analysis. Distribution of nutrients in dietary intake and the environmental factors were significantly different between patients and controls, as shown in Table 2.

When we analyzed the associations between environmental factors, diet and gut microbiome, the results showed that the alpha diversity was not correlated with environmental factors and diet, as shown for AS and HCs ($P > .05$, Fig. S1, S2).

The structure of the microbiome community was compared between different diet groups in AS and HCs. At the genus level, there were significantly different the abundance of Odoribacter, Alistipes, Megamonas, Bifidobacterium and Blautia ($p < .05$) in different dietary groups in AS. The HCs exhibited a different richness of the gut microbiome in different diet groups, including Roseburia, Leuconostoc, Dysgonomonas, Plesiomonas, Ruminococcus2, Cellulosilyticum, Olsenella, Candidatus_Kuenenia, Acetivibrio, Dysgonomonas and Plesiomonas ($p < .05$, Table S6).

Discriminant analysis using LEfSe identified significant taxa variations distinguishing each sample group from others. Patients with AS had an increase in Anaeroplasmataceae family in group A, Peptococcaceae.1 family in group C and Erysipelotrichaceae family in group D. Increased species included Victivallaceae, Odoribacter, Clostridium, Alistipes and Clostridiumspiroforme. Group A had an increase in Prevotella_histicolaspecies in HCs, and Paraprevotella_xylaniphila species increased in group C, Bifidobacterium genus from Bifidobacteriaceae family increased in group D.

The presence of bacteria in both AS and HCs samples was correlated with environmental factors and diet. However, the taxa of microbiota were very different in these two sample populations, as shown in Additional file 2: Figs. S3, S4. For example, in AS samples, Peptococcus at the genus level was significantly correlated with smoking ($r = 0.3688$, $P < .001$). In HCs samples, Bifidobacteriumand Actinomycesat the genus level was significantly correlated with smoking ($r = -0.3545$, $P < .001$, $r = 0.3292$, $P < .001$, respectively). Firmicutes at the phylum level was inversely and significantly correlated with Nutrient ($r = -0.2298$, $P = .0378$). At the class level, Erysipelotrichia, which belongs to Firmicutes, was inversely and significantly correlated with Nutrient ($r = -0.2943$, $P = .0072$). At the order level, among the class Erysipelotrichia, the order Erysipelotrichales was inversely and significantly correlated with Nutrient ($r = -0.2943$, $P = .0072$), as well as the families Erysipelotrichaceae ($r = -0.2943$, $P = .0072$), Peptoniphilaceae ($r = -0.2879$, $P = .0087$), Bdellovibrionaceae ($r = 0.2907$, $P = .0081$).The genera Hungatella, Eisenbergiella and Vampirovibrio were also signifcantly correlated with Nutrient ($r = -0.3587$, $P = .0009$, $r = -0.3170$, $P = .0037$, $r = 0.2907$, $P = .0081$,respectively) Species Bacteroides_clarus was positively and significantly correlated with Nutrient($r = 0.3503$, $P = .0013$) (Additional file 2: Fig. S5, Fig. S6.)

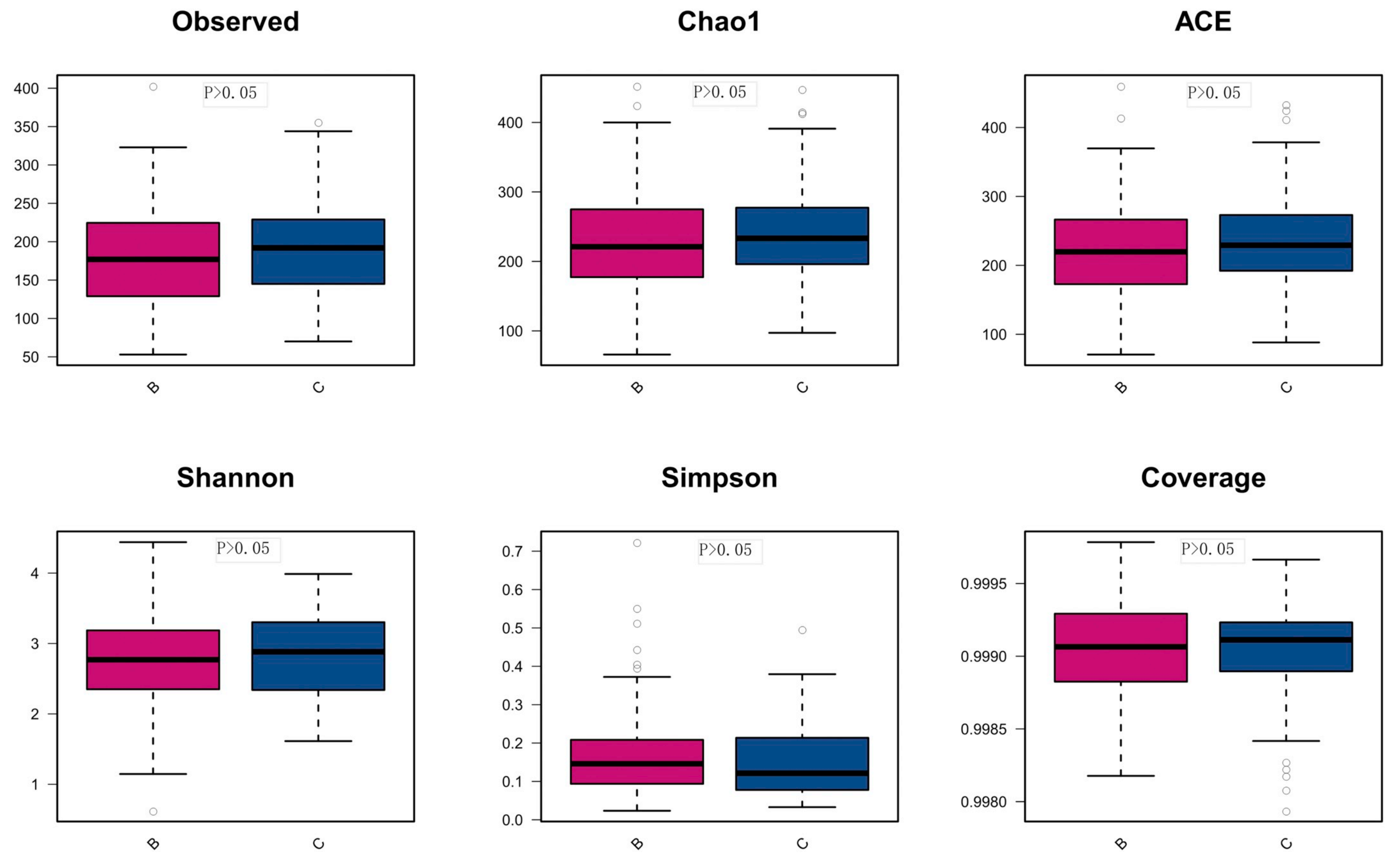

Fig. 1. The α diversity of the gut microbiomes for the AS patients and the healthy controls was similar atthe genus level.

3.4. Correlations between gut microbiota and disease activity

BASDAI, BASFI and ASDAS-CRP were not correlated with bacterium at the phylum level ($P > .05$). At the class level, Bacilli, belongs to Firmicutes, was positively correlated with BASDAI ($r = 0.2705$, $P = .0140$). At the order level among the class Actinomycetes, the order Actinomycetales was positively associated with BASFI and ASDAS-CRP ($r = 0.2601$, $P = .0183$; $r = 0.2424$ $P = .0282$, respectively). At the family level, Micrococcaceae was significantly correlated with BASFI ($r = 0.3277$, $P = .0027$), and Nocardiaceae was positively correlated with BASDAI and ASDAS-CRP ($r = 0.2483$, $P = .0245$; $r = 0.2587$, $P = .0189$, respectively). At the genus level, Erysipelotrichaceae was positively correlated with BASDAI, BASFI and ASDAS-CRP ($r = 0.2670$, $P = .0153$; $r = 0.3098$, $P = .0046$; $r = 0.2721$, $P = .0134$, respectively). While Coprobacter was inversely correlated with BASFI ($r = -0.3496$, $P = .0012$). Lactobacillus_mucosae_LM1 was positively correlated with BASDAI, BASFI and ASDAS-CRP ($r = 0.3395$, $P = .0018$; $r = 0.3565$, $P = .0010$; $r = 0.3632$, $P = .0008$, respectively, Fig. S5).

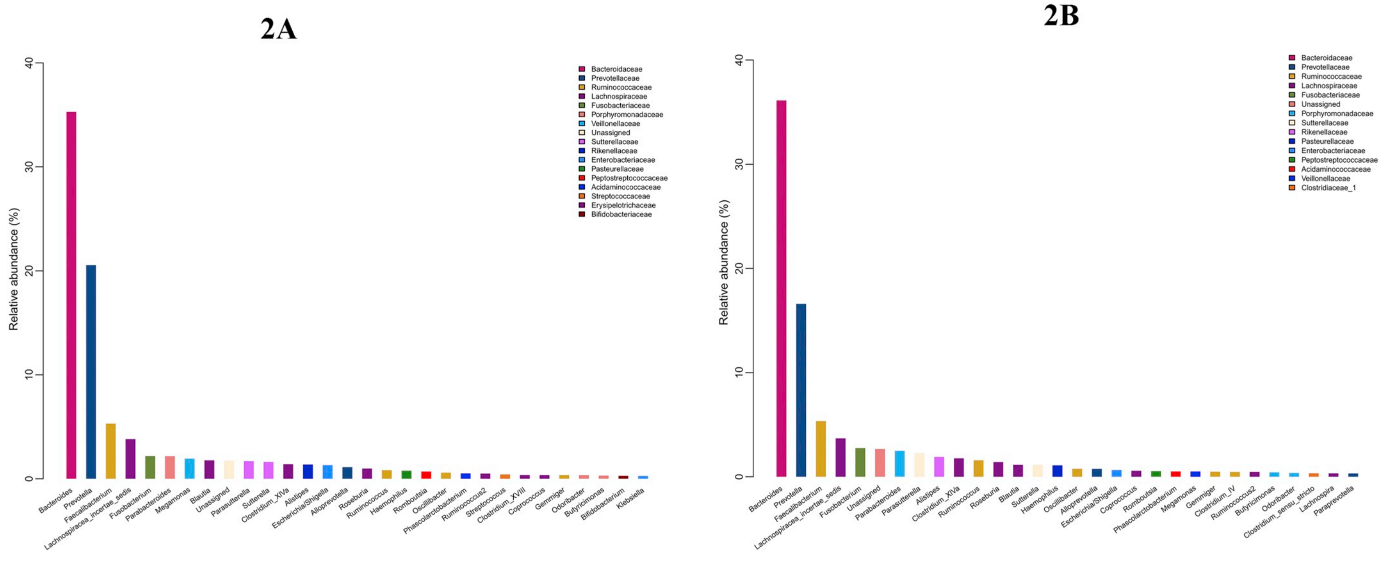

Fig. 2. The abundance of the gut microbiomes for the AS patients (A) and the healthy controls (B) at the genus level.

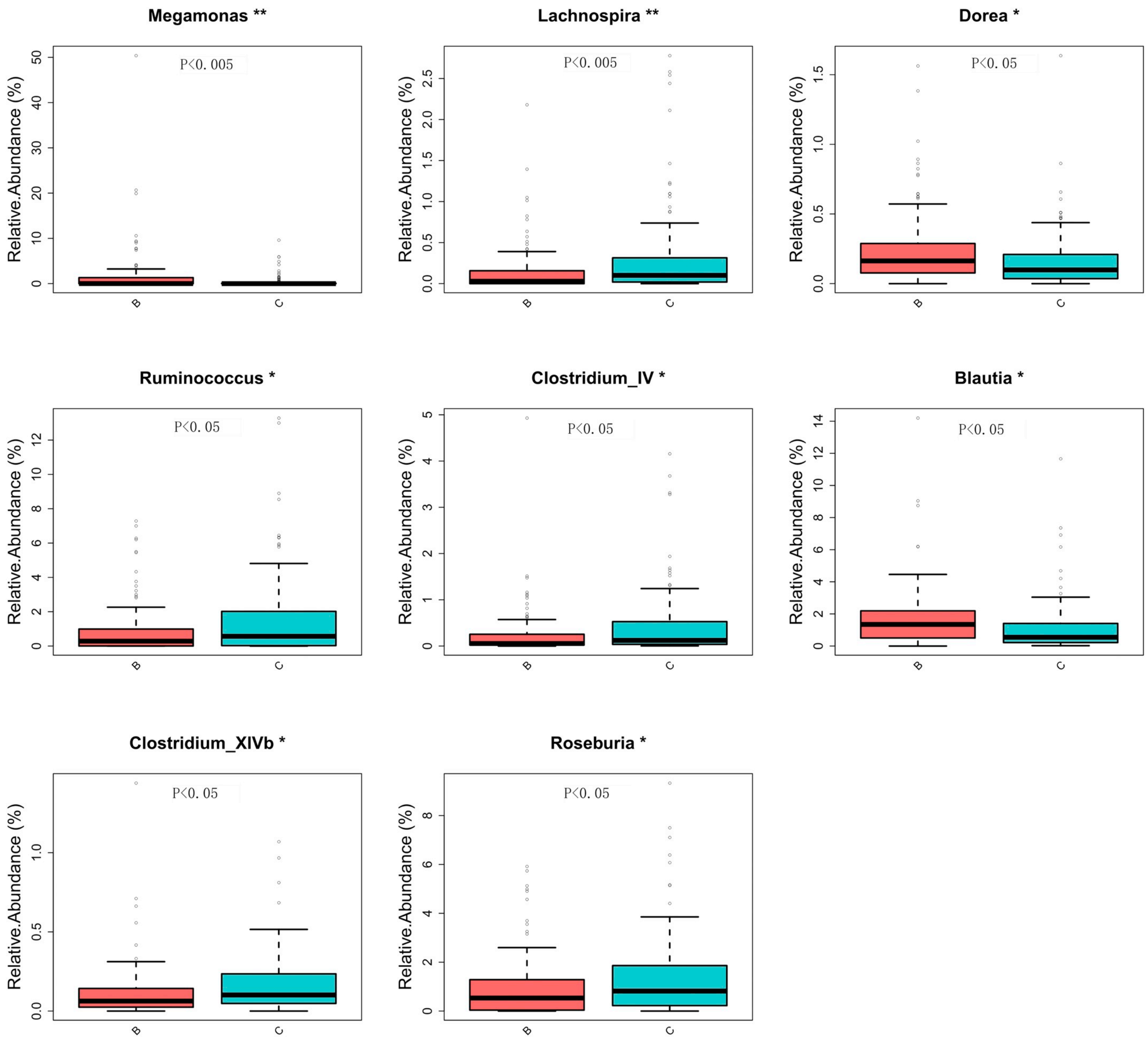

Fig. 3. Differences of phylogenetic abundance between AS patients and healthy controls. The phylotypes that were increased or decreased in the AS patients at the genus levels. Red (B) and blue (C) indicate the AS patients and healthy controls, respectively. Wilcoxon rank-sum tests were applied to identify the differentially abundant genera. Among these, the highest medians of the phylogenetic abundance in the enriched cohort were drawn as boxplots. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

It has recently become clear that the influence of microbiota extends beyond the intestinal tract and affects the systemic immune system. In the current study, we present compelling evidence that the gut microbiome in AS is different from HCs. The alteration might have a role in the pathogenesis of AS by modulating the innate and adaptive immune systems. To better understand gut microbiome variations across population groups and cross-talk between the microbiota, diet and its host, we assessed the richness and abundance of the gut microbiome between AS and HCs and analyzed the correlations between disease activity and gut microbiota, diet.

Our results demonstrating that Actinobacteria was enriched in AS patients, we speculated that Actinobacteria might modulate the ubiquitination of NF-κB. This, in turn, could facilitate the development of AS through the activation of NF-κB signaling and the accumulation of pro-inflammatory factors in AS. A large study showed that proteasomes have an obvious correlation with autoimmune diseases including SLE, RA and scleroderma, and the serum proteasome concentration was significantly increased in many autoimmune diseases [36]. Some bacteria, such as Bacteroides vulgatus, have been reported to play an important role in AS pathogenesis. Evidence from animal models indicated that Bacteroides was associated with the inflammatory development in peripheral joint or intestinal disease. In the present study, AS patients had an increase in Bacteroides compared to HCs. The result was consistent with previous findings [33,37], Bacteroides vulgatus has been shown as increase in the HLA-B27 transgenic rat gut was sufficient to trigger both IBD and arthritis.

The results of correlation analysis between disease activity and bacteria showed that the class Bacilli, the order Actinomycetales, the family Micrococcaceae, Nocardiaceae, the genus Erysipelotrichaceae, Coprobacter, Lactobacillus_mucosae_LM1 were associated with disease

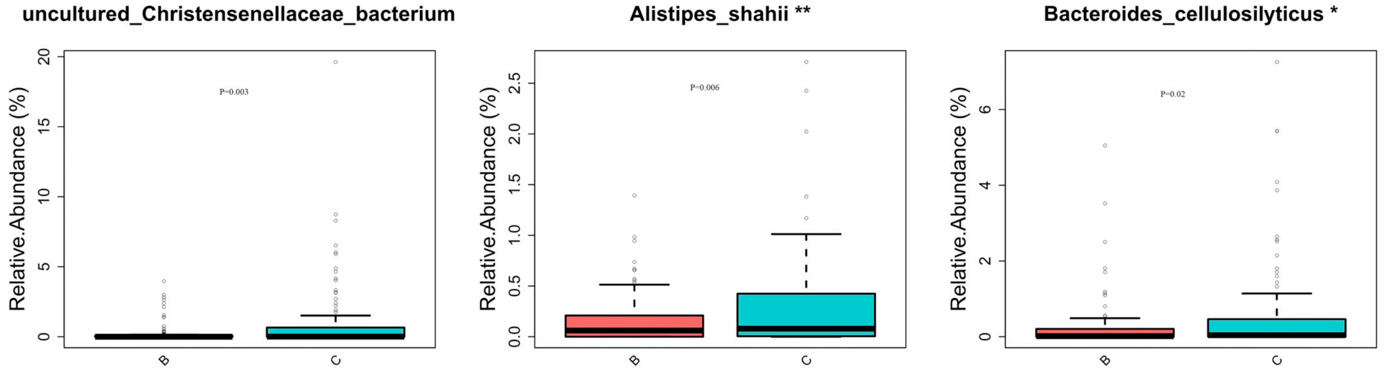

Fig. 4. Differences of phylogenetic abundance between AS patients and healthy controls. The phylotypes that were increased or decreased in the AS patients at the species levels. Red (B) and blue (C) indicate the AS patients and healthy controls, respectively. Wilcoxon rank-sum tests were applied to identify the differentially abundant genera. Among these, the highest medians of the phylogenetic abundance in the enriched cohort were drawn as boxplots. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

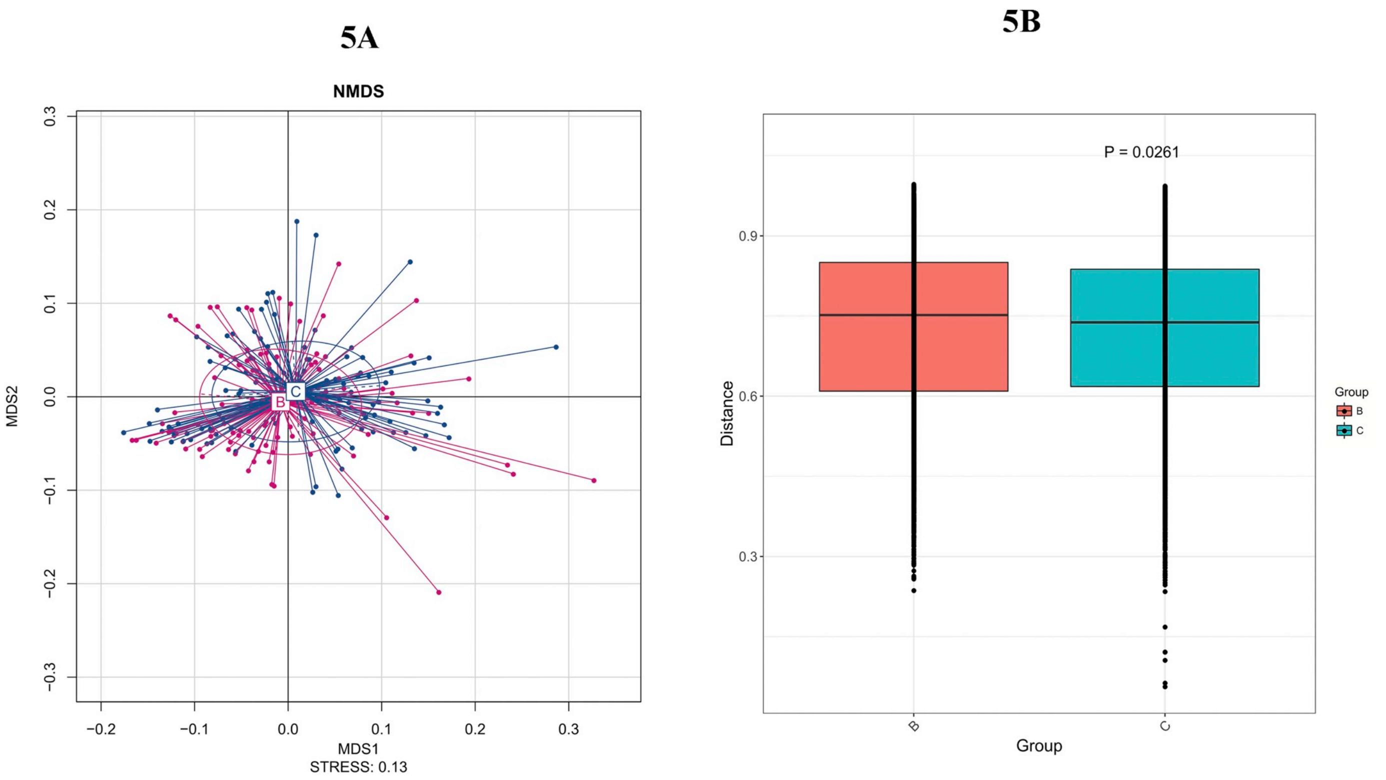

Fig. 5. Beta diversity analysis showed that the microbiota composition was significantly different between the two groups (Non-MetricMulti-Dimensional Scaling, NMDS, stress = 0.13; ADONIS Analysis, $p = .026$, B for AS and C for controls).

activity. The results indicated that intestinal microbiota are critically important in the development of AS.

Taken together, multiple evidence firmly implicated intestinal microbiome as a possible contributor to the pathogenesis of AS. Therefore, characterization of the species composition of microbiota associated with AS and the exact mechanism for the functional role of the intestinal microbiome in disease progression should be the focus of research in the future.

Another goal of our study was to test whether diet could prevent or induce the development of AS. Distribution of nutrients in dietary intake was significantly different between patients and controls. Diet has a strong influence on the intestinal microbiota. In different diet groups, the structure of the microbiome community was different. Therefore, the effect of diet on AS development may involve direct interaction of proteins or lipids with the immune system, or indirect interaction involving the gut flora. In addition, AS development might be helped by proteins or other non-bacterial factors contained in the food. For example, members of the Bacteroides, one of predominant genera in the human gut, are particularly well-adapted to metabolizing complex carbohydrates and have large numbers of genes encoding glycosyl hydrolases and polysaccharide lyases. A correlation analysis was performed between the bacterial populations at the phylum, class, order, family and genus levels and Nutrient. Nutrient associated bacteria were part of the phylum Firmicutes. The taxa that showed a significant correlation was selected and studied from the phylum to family level. The phylum Firmicutes in arthritis-susceptible mice and arthritis-resistant mice was increased. Firmicutes were correlated with serum levels of pro-inflammatory cytokines such as IFN-α. The Erysipelotrichia are a subclass of bacteria of the phylum Firmicutes. Species of this subclass are known to be common in the gut microbiome, increase in the composition of the mouse gut microbiome for mice switched to diets high in fat. The result showed that Erysipelotrichiamay is

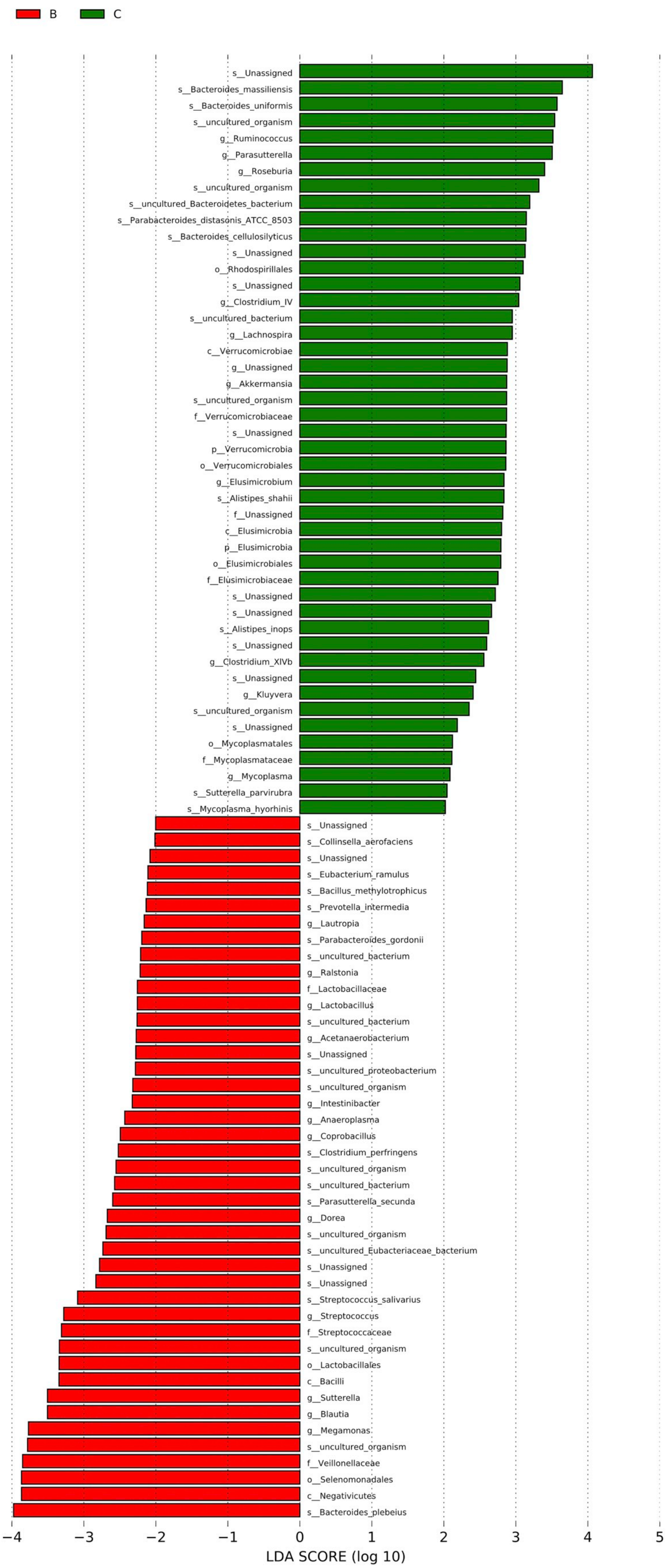

Fig. 6. Bacterial taxa that were differentially represented in AS and HCs with statistical level of significance according to linear discriminant analysis (LDA score > 2). The histogram displays all taxa that were increased in each group, as compared with another, and the corresponding level of significance (LDA score). Taxa were identified at the gender level and the legend is shown on the right-hand side. Red (B) and blue (C) indicate the AS patients and healthy controls. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
The distribution of AS patients and healthy control in different dietary groups.

	AS (n = 103)	Healthy control (n = 104)	χ^2	P
A	32	19	*9.540*	*0.023*
B	30	22		
C	22	30		
D	19	33		

AS, ankylosing spondylitis; Chi square test; A,B,C,D diet grouping based on the results of factor analysis.

associated with fat intake and AS.

In this study, we did not address the mechanism of the observed protection. However, the objective of our future studies will to investigate whether it involves modification of the gut microbiota as observed in spondyloarthropathies or psoriatic arthritis. Due to a few AS participants who did not take any medicine, a major limitation of our study is that we do not know whether medication use is driving the difference seen in AS patients compared with HCs. This will require further studyin the analysis of newly diagnosed AS patients.

5. Conclusions

The human gut microbiome of AS patients was clearly different from that of HCs. Our results demonstrated that some alteration of gut microbiome is associated with developments of AS, evidenced by the changes in various taxonomic levels. According to previous reports, we inferred that some biomarkers participate in the pathogenesis and development process of AS, such as Bifidobacterium, Prevotell. Other markers that were not clear in pathogenesis could provide new information for further research. Lastly, the discovery of the associated microbes of AS in the gut microbiome may help us to seek more treatments for this disease.

Ethics

We stated that our study complies with the Declaration of Helsinki, thatthe Anhui Medical University Ethics Committee has approved the research protocol. And patients and healthy controls data were retrospectively analyzed from themselves. All participants gave informed consent to participate in this study.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2019.07.038.

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