Journal of Inflammation Research
Dovepress
Taylor & Francis Group

Open Access Full Text Article
ORIGINAL RESEARCH

Dysbiosis of Gut Microbiota in Ankylosing Spondylitis Patients

Lianjun Yang[1,*], Kun Wang[1,*], Bin Liu[2,*], Tao Chen[1], Tao Ma[3], Zhifei Cui[1], Dawei Zhang[1], Shiyanjin Zhang[1], Xiang Liu[1], Jun Shen[4,5], Hai Lu[1]

[1]Department of Spine Surgery, The Fifth Affiliated Hospital, Sun Yat-Sen University, Zhuhai, Guangdong, 519000, People's Republic of China; [2]Department of Orthopedics, The First Affiliated Hospital of Jinan University, Guangzhou, Guangdong, 510630, People's Republic of China; [3]Department of Biobank, The Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, Guangdong, 519000, People's Republic of China; [4]Department of Orthopedics, The Seventh Affiliated Hospital, Sun Yat-sen University, Shenzhen, Guangdong, 518107, People's Republic of China; [5]Shenzhen Key Laboratory of Bone Tissue Repair and Translational Research, Shenzhen, Guangdong, 518107, People's Republic of China

*These authors contributed equally to this work

Correspondence: Jun Shen, Department of Orthopedics, The Seventh Affiliated Hospital, Sun Yat-sen University, Shenzhen, Guangdong, 518107, People's Republic of China, Email 13421398163@163.com; Hai Lu, Department of Spine Surgery, The Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, Guangdong, 519000, People's Republic of China, Email lvhai@mail.sysu.edu.cn

Journal of Inflammation Research downloaded from https://www.dovepress.com/
For personal use only.

Purpose: Ankylosing spondylitis (AS) is a chronic inflammatory disease associated with genetic, immune, and microbial factors. The role of gut microbiota in the pathogenesis of AS is increasingly recognized, with studies suggesting that intestinal dysfunction may trigger systemic inflammation. This study aimed to investigate the gut microbiota profiles of AS patients from Southern China and explore the relationship between gut microbiota and the occurrence and development of AS.

Patients and Methods: We enrolled 30 AS patients and 25 healthy controls from the Fifth Affiliated Hospital of Sun Yat-sen University. Fecal samples were collected, and DNA was extracted for 16S rDNA sequencing to analyze the V3-V4 variable regions. Bioinformatic processing and statistical analysis were performed to assess the microbial community structure, diversity, and function.

Results: The study revealed significant differences in gut microbiota composition between AS patients and healthy controls. AS patients exhibited a decrease in beneficial bacteria such as Firmicutes and Actinobacteria and an increase in harmful bacteria like Proteobacteria and Enterobacteriaceae. The functional prediction of gut microbiota indicated significant metabolic pathway alterations, particularly in energy metabolism, degradation metabolism, and nucleotide metabolism, which may be linked to the pathophysiology of AS.

Conclusion: This study demonstrates that the gut microbiota of Han Chinese AS patients in Guangdong Province is characterized by a decrease in beneficial bacterial communities and an increase in harmful ones, potentially contributing to AS progression through intestinal barrier disruption and intensified inflammatory responses. These findings provide a theoretical basis for developing new intervention strategies targeting the gut microbiota.

Keywords: inflammation, 16S rDNA sequencing, abundance, metabolic pathways

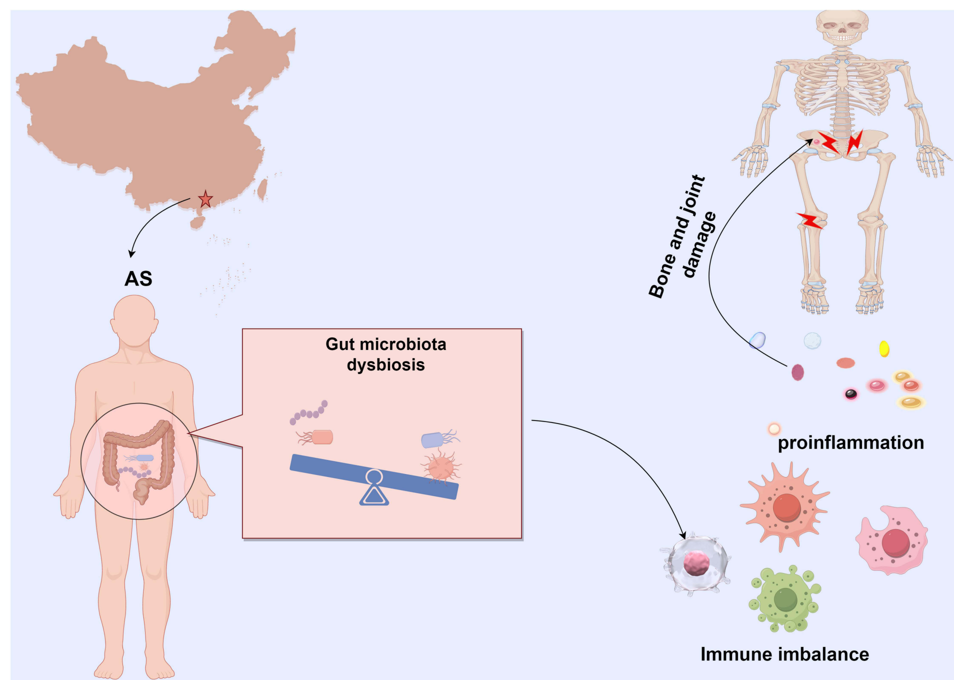
Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disease characterized by chronic lower back pain and stiffness, with morning stiffness in the lower back that improves with activity but is not relieved by rest.[1] The average prevalence of AS per 10, 000 individuals was found to be 23.8 in Europe, 16.7 in Asia, 31.9 in North America, 10.2 in Latin America, and 7.4 in Africa.[2] The etiology of AS may involve multiple factors, including genetic background, immune function, pathogen infection, and endocrine abnormalities. The human leukocyte antigen (HLA)-B27 gene is closely associated with the development of AS, although the exact pathogenic mechanisms are not yet fully understood.[3] HLA-B27 may be involved in pathogenesis of AS through mechanisms such as molecular mimicry, misfolding, and peptide presentation.[3,4] In addition, variations in ERAP1, IL-23R, and several other genes, such as IL6R, TYK2, and STAT3, collectively contribute to the pathogenesis of AS, indicating that the genetic susceptibility to AS is a complex

Received: 6 February 2025
Accepted: 16 May 2025
Published: 15 June 2025

© 2025 Yang et al. This work is published and licensed by Dove Medical Press Limited. The full terms of this license are available at https://www.dovepress.com/terms.php and incorporate the Creative Commons Attribution – Non Commercial (unported, v4.0) License (http://creativecommons.org/licenses/by-nc/4.0/). By accessing the work you hereby accept the Terms. Non-commercial uses of the work are permitted without any further permission from Dove Medical Press Limited, provided the work is properly attributed. For permission for commercial use of this work, please see paragraphs 4.2 and 5 of our Terms (https://www.dovepress.com/terms.php).

Graphical Abstract



multifactorial process.[5] Pathogen infection may promote the onset of AS by activating the IL-23/IL-17 axis, Genetic studies have indicated that the increased expression of IL-23 and IL-17 promotes inflammatory processes and affects the activation and function of various immune cells, including Th17 cells, γδT cells, MAIT cells, and ILC3 cells, which contribute to the inflammation and pathophysiology of bone in AS through the production of IL-17.[6,7] Furthermore, endocrine factors are involved in the pathogenesis of AS through various mechanisms, including the association with Metabolic Syndrome (MetS), inflammatory factors, the regulation by microRNAs, changes in adipokines, and the impact of oxidative stress.[8] This prompts us to delve into the pathophysiological mechanisms of AS and to explore new therapeutic strategies.

In recent years, more and more evidence has shown that the inflammatory activation of AS is related to intestinal dysfunction. Impaired intestinal barrier function leads to the translocation of bacteria and their products, thereby activating a systemic inflammatory response, particularly through the inflammasome pathway, which promotes the secretion of IL-1β and IL-18, and subsequently affects the IL-23/IL-17 axis. This not only triggers inflammation locally in the gut but is also associated with the systemic inflammatory state in patients with AS.[9] Changes in the composition of the gut microbiota may exacerbate joint inflammation by affecting immune interactions between the gut and joints, as well as the migration of immune cells.[10] Studies have shown that in patients with AS who have a history of inflammatory bowel disease (IBD), the abundance of Ruminococcus gnavus is positively correlated with the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI). This suggests that specific gut microbiota may be involved in the inflammatory process of AS and may be related to disease activity.[11]

Previous studies have shown that the clinical pattern of AS varies considerably according to the subject's race and geographic location.[12] AS is not rare in mainland China. China comprises 56 ethnic groups, including the Han majority and 55 minority nationalities. Research on HLA antigens shows that the Han Chinese can be divided into Southern and Northern Han populations based on significant differences in HLA antigen frequencies.[13] One report indicates that patients with ankylosing spondylitis in Southern and Northern China exhibit distinct clinical characteristics. Meanwhile, significant diagnostic delay and higher prevalence of HLA-B27 were found in southern AS patients.[14] The connection

between gut microbiota and its role in the pathogenesis of AS is increasingly recognized. Differences in microbiota profiles related to geography and lifestyle have been observed in various populations.[15] Southern China has distinct dietary habits (fresh, lightly seasoned ingredients, with an emphasis on seafood, vegetables, and soups),[16] environmental factors, and genetic backgrounds, which can significantly influence gut microbiota composition. Investigating microbiotas in Southern China AS patients may reveal unique microbial taxa or metabolites that influence disease-specific immune pathways. Such insights could clarify the pathophysiological mechanisms by which gut dysbiosis contributes to AS progression. However, there are very few studies on Southern China AS patients. Our present study aimed to investigate the gut microbiota profiles of AS patients from Southern China (Guangdong province) and to provide a potential offering of new targets for microbial or dietary interventions.

Materials and Methods

Subjects

This study enrolled 30 patients diagnosed with AS at the Fifth Affiliated Hospital of Sun Yat-sen University. Additionally, 25 healthy volunteers who underwent health checkups at our outpatient clinic were selected as controls. Inclusion criteria for AS patients were as follows: aged 18 to 45 years, with no gender restrictions; diagnosed with AS based on the modified New York criteria for AS 1984;[17] not involved in other clinical trials, and had not consumed antibiotics, probiotics, or prebiotics for treatment within the past 3 months; exclude patients with cachexia such as cancer, lymphoma, leukemia or lymphoproliferative diseases, active tuberculosis, HIV, etc. The criteria for healthy volunteers included: being aged 18–45 years, gender unrestricted, and in good health; no history of allergies to drugs, food, or other substances; no past infectious diseases or significant illnesses; not being involved in other clinical trials and had not taken antibiotics, probiotics, or prebiotics for treatment within the last 3 months. The clinical characteristics (ESR, CRP, HLA-B27, BASDAI) of patients with AS and Healthy control subjects are shown in Supplementary Table 1 (This study focused on biomarker profiles within the patient population. Future investigations incorporating healthy control groups may further elucidate these parameters' diagnostic and prognostic roles). This study was approved by the Ethics Committee of the Fifth Affiliated Hospital of Sun Yat-sen University (project No. ZDWY.JZWK.015).

Fecal Sample Collection and DNA Extraction

The 16S rDNA sequencing technology offers significant advantages in gut microbiota research, including high sensitivity and specificity, broad species coverage, provision of ecological and evolutionary information, monitoring of microbial community changes, and aiding in disease diagnosis and biomarker discovery.[11] These features make us choose 16S rDNA sequencing as an important tool to reveal gut microbial diversity and function, and to explore the relationship between gut microbiota and the occurrence and development of AS. Collected fresh fecal samples from participants using a sterile spoon, with each sample weighing approximately 1 g. Placed the collected samples into sterile tubes and stored them at −80 °C until DNA extraction can be performed. The DNA was extracted from 200 mg fecal samples in each group using the E.Z.N.A.® Stool DNA Kit (D4015, Omega, inc., USA) according to the manufacturer's instructions. Nuclear-free water was served as the blank. The total DNA was eluted with 50 μL of Elution buffer and stored at −80 °C until it was measured by PCR.

Library Construction and Sequencing

The primers used were 341F forward primer (5′-CCTACGGGNGGCWGCAG-3′) and 805R reverse primer (5′-GACTACHVGGGTATCTAATCC-3′) for PCR amplification of the V3-V4 variable regions of 16S rDNA gene. PCR amplification was performed in a total volume of 25 μL reaction mixture containing 25 ng template DNA, 12.5 μL PCR Premix, 2.5 μL of each primer, and PCR-grade water. The PCR products were confirmed using 2% agarose gel electrophoresis. The PCR products were purified by AMPure XT beads (Beckman Coulter Genomics, Danvers, MA, USA). The amplicon pools were prepared for sequencing, with the size and quantity of the amplicon library being evaluated using the Agilent 2100 Bioanalyzer (Agilent, USA) and the Library Quantification Kit for Illumina (Kapa

Biosciences, Woburn, MA, USA), respectively. The libraries were sequenced on a NovaSeq PE250 platform (Illumina, CA, USA) by LC-Bio Technology (Hangzhou) Co., Ltd, China.

Bioinformatic Processing and Statistical Analysis

Filtered sequences were clustered by 97% identity into operational taxonomic units (OTUs, Sequence clusters representing microbial taxa) using UPARSE.[18] Alpha diversity was utilized to assess the complexity of species diversity within samples, employing five indices: Chao1 (Estimates species richness in a community, accounting for rare taxa), Observed species, Goods coverage, Shannon, and Simpson (Measure alpha diversity, combining species richness and evenness, Shannon emphasizes rare species, Simpson focuses on dominant species), which were calculated with QIIME2. To elucidate patterns of beta diversity, a multivariate analysis of community diversity employing principal coordinate analysis (PCoA, specifically refers to the unweighted analysis of microbial composition differences based on a distance matrix) was performed, A Bray-Curtis distance matrix was used as the dissimilarity index. Linear discriminant analysis effect size (LEfSe) analysis was performed to detect differential abundance between each group, only the taxonomic groups with p values < 0.05 and threshold logarithmic Linear discriminant analysis (LDA) sores > 3 were considered. Kruskal–Wallis tests followed by Dunn's multiple-comparison test were used to determine whether there are significant differences between groups. Based on the Kyoto Encyclopedia of Gene and Genomes (KEGG), the metagenome function was predicted using a Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt), A bioinformatics tool to predict microbial functional potential based on 16S rRNA gene data by mapping taxa to reference genomes. Statistical analyses were performed using R software version 3.5.2, and * $p < 0.05$ was considered statistically significant. We also performed Benjamini-Hochberg FDR correction ($p_{FDR} < 0.05$) for 16S rRNA taxonomic comparisons. The adjusted results are provided in Supplementary Table 2 for further reference by the readers. Sequencing data in this study are available via National Genomics Data Center, China National Center for Bioinformation Genome Sequencing Achieve under Project PRJCA038041.

Results

The Gut Microbial Community Structure and Abundance from the AS and Healthy Groups

The number of shared OTUs in AS and Healthy groups was 1084, the number of unique OTUs in healthy group was 2529, while the number of unique OTUs in AS group decreased to 2472, and the difference was statistically significant ($p < 0.05$, Figure 1a). The abundance and evenness of the species in different samples were evaluated by the rank-abundance curve (Figure 1b), we observed significant differences in species abundance and evenness between healthy

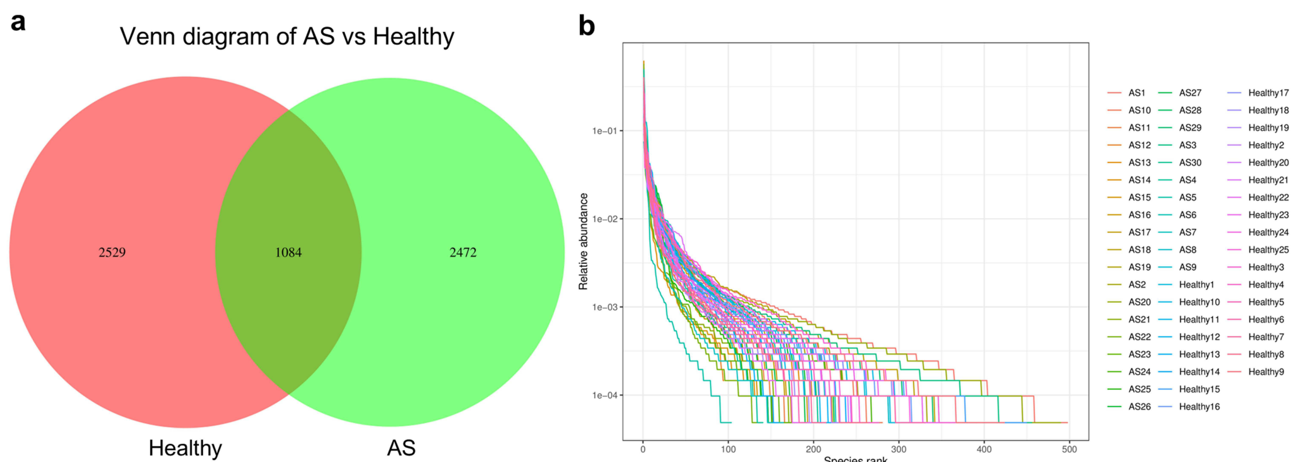


Figure 1 Microbial community OUT analysis. (**a**) Venn diagram of shared and unique OTUs among different groups. (**b**) Rank-abundance curve diagram of AS and Healthy groups, the y-axis indicates the abundance of OTUs, while the x-axis represents the sequence number of corresponding abundance of OTUs.

and AS samples, specifically, the rank-abundance curve of AS samples rapidly decreased at low species levels, indicating that a few species occupy higher abundances in these samples, while the abundance of most species is lower, demonstrating lower species evenness. On the contrary, the curve of healthy samples decreases relatively smoothly, especially at high species levels, indicating the presence of more moderately abundant species in the healthy samples and a relatively more uniform distribution of species.

Diversity of Gut Microbiota in the AS and Healthy Groups

The richness and uniformity of samples in each group were evaluated using α-diversity analysis. We used the Chao1 index to evaluate the differences in species richness between AS and Healthy groups, the Wilcoxon signed rank test showed that the data of Healthy group had higher species richness ($p = 0.019$, Figure 2a); the Observed OTUs index showed that the number of microbial communities in AS group is significantly lower than Healthy group ($p = 0.027$, Figure 2b); the Goods coverage of AS group is lower than that of the Healthy group ($p = 0.0044$, Figure 2c); indicating that the species richness of AS group was significantly lower than that of Healthy group. Although the Shannon index value of the Healthy group is higher than that of the AS group ($p = 0.48$, Figure 2d); a higher Simpson index value in the AS group may suggest the presence of one or several dominant species in the community, but this trend is not statistically significant ($p = 0.79$, Figure 2e); this indicates that there is no significant difference in species diversity between AS and Healthy group.

To evaluate the β-diversity of microbial communities between samples, the Principal Component Analysis (PCA, the method used to reduce dimensionality and visualize structural differences based on variance) was used to reveal structural differences in AS and Healthy groups. Each sample was projected into a two-dimensional space based on

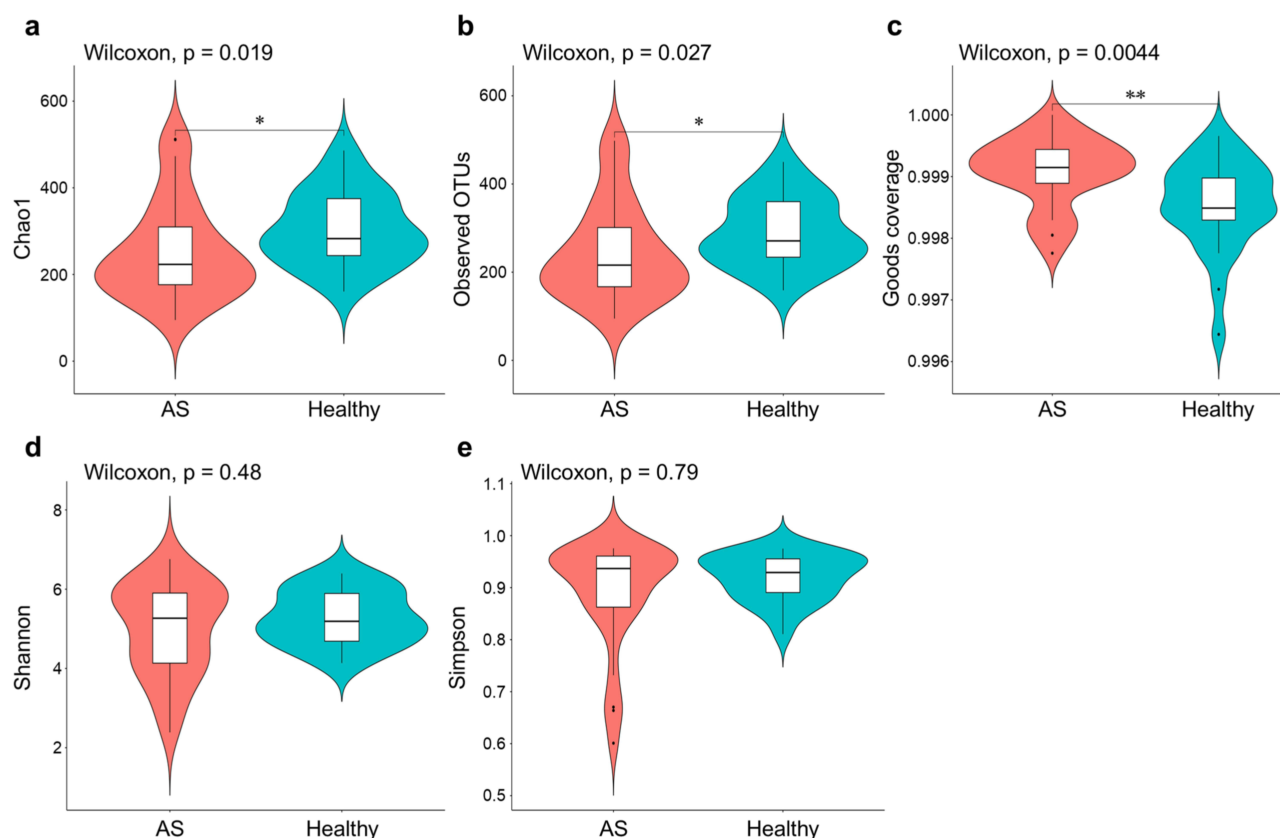


Figure 2 α-diversity of the gut microbial community between AS and Healthy groups. (**a**) Comparison of the richness of microbiota in fecal samples between AS and Healthy groups based on Chao1 index. (**b**) Comparison of the richness between two groups based on the Observed OTUs index. (**c**) Comparison of the OTU coverage between two groups based on the Goods coverage index. (**d**) Diversity of the microbiota between two groups based on the Shannon index. (**e**) Diversity of the microbiota between two groups based on the Simpson index. Statistical analysis was performed with Wilcoxon signed rank test, and the *p* values are shown, * $p < 0.05$, ** $p < 0.01$.

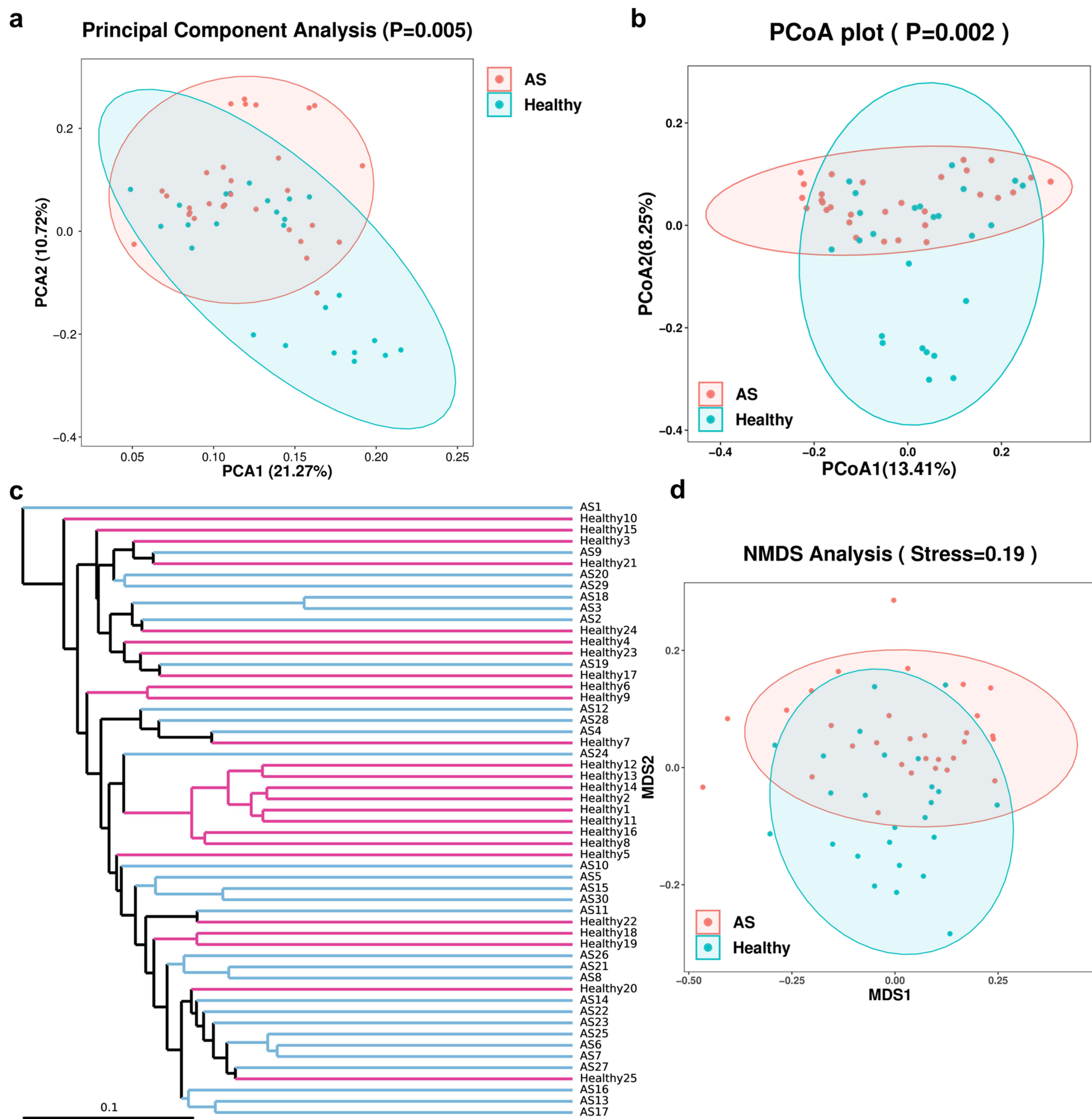


Figure 3 β-diversity of the gut microbial community between AS and Healthy groups. (**a**) Comparison of the structural differences in AS and Healthy groups based on PCA. (**b**) Unweighted Principal Coordinate Analysis (PCoA) plot. (**c**) Unweighted Pair Group Method with Arithmetic Mean (UPGMA). (**d**) Unweighted Non-metric Multidimensional Scaling (NMDS) analysis.

microbial composition, and the PCA results emphasized the significant differences in microbial community structure between AS and Healthy groups (p = 0.005, Figure 3a); the unweighted Principal Coordinate Analysis (PCoA) showed the differences in microbial composition among different samples (p = 0.002, Figure 3b); we applied the Unweighted Pair Group Method with Arithmetic Mean (UPGMA, A hierarchical clustering algorithm for constructing phylogenetic trees or grouping samples based on similarity) to construct clustering relationships between samples, the tree diagram revealed a clear separation of microbial community structures between AS and Healthy states, with branch lengths generally longer between AS and Healthy groups, emphasized the significant differences in microbial communities between the two groups of samples (Figure 3c); the unweighted Non-metric Multidimensional Scaling (NMDS) analysis

provided an intuitive way to display the compositional differences of microbial communities between different samples, it can be observed that there is a significant separation between the AS and Healthy groups on the MDS1 axis, a Stress value of 0.19 indicated that the two-dimensional configuration of NMDS is still an acceptable model fit compared to the distance matrix of original data (Figure 3d).

Taxonomic Distribution of Gut Microbial Communities at the Phylum to Genus Levels

In this study, we conducted a detailed taxonomic assignment of microbial communities in the samples, revealing the microbial composition at the phylum to genus levels for both groups. At the phylum level, we found that Firmicutes and Actinobacteria were the dominant microbial phyla in both groups, but their relative abundance decreased in the AS group. However, the proportion of Proteobacteria is higher in the AS group (13.46%) than in the Healthy group (3.96%) ($p < 0.05$, Figures 4a and S1a). The main microbial class in the two groups was Clostridia. Similarly, the relative abundance of Actinobacteria in the Healthy group was 10.37% but decreased to 8.30% in the AS group. Conversely, the

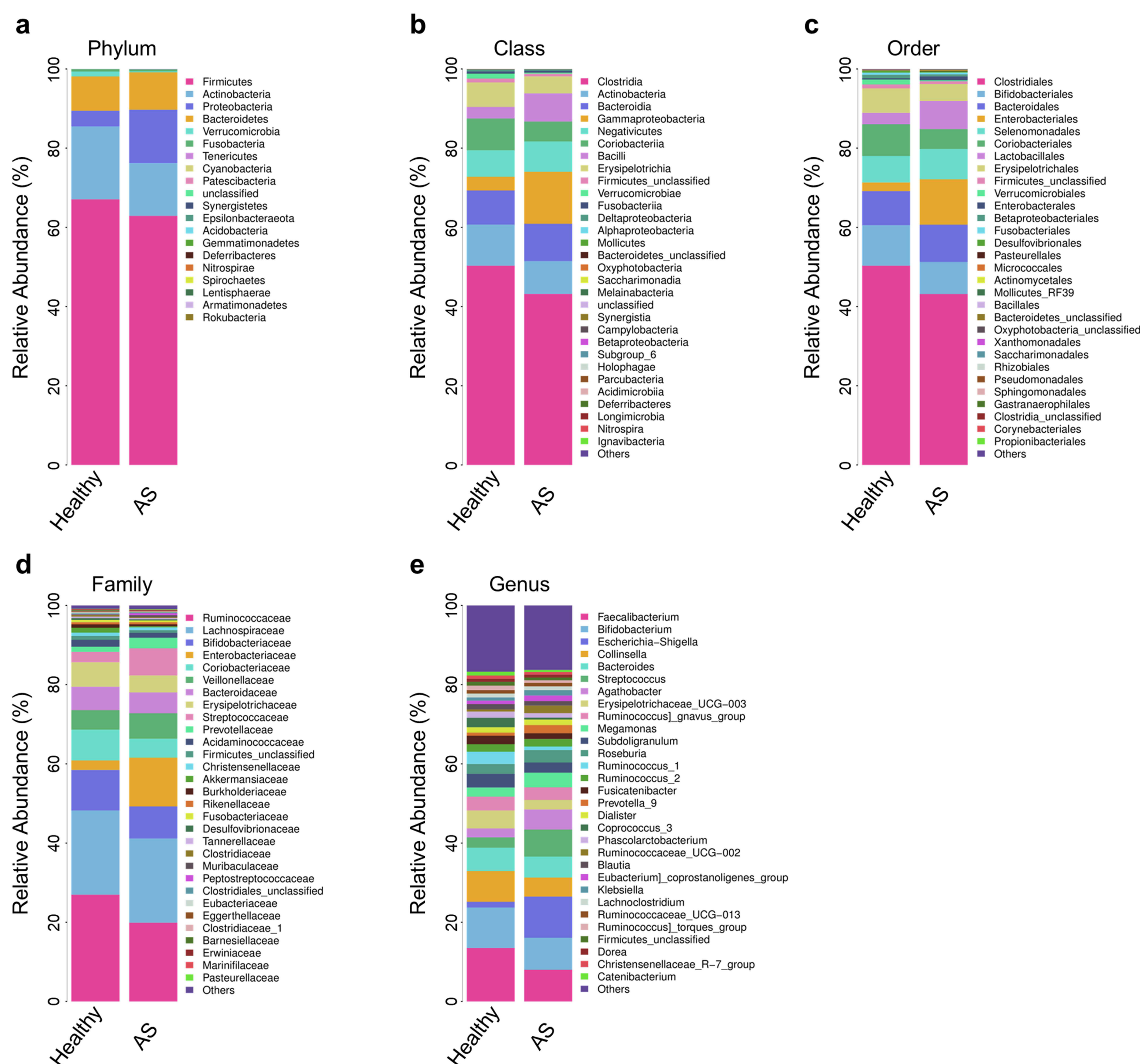


Figure 4 Taxonomic assignments of the gut microbial community of participants. Presented at the (**a**) Phylum, (**b**) Class, (**c**) Order, (**d**) Family, and (**e**) Genus levels, respectively.

relative abundance of Gammaproteobacteria in the AS group was significantly higher than in the Healthy group, increasing from 3.46% to 13.13% ($p < 0.05$, Figures 4b and S1b). At the Order level, the relative abundance of Clostridiales and Bifidobacteria decreased in the AS group, while the relative abundance of Enterobacteria was significantly higher in the AS group than in the Healthy group, increasing from 2.17% to 11.47% ($p < 0.05$, Figures 4c and S1c). At the family level, compared to the healthy group, the relative abundance of Peptostreptococcaceae and Enterobacteriaceae was significantly higher in the AS group ($p < 0.05$, Figures 4d and S1d). At the genus level, compared to the healthy group, the relative abundance of Faecalibacterium, Lachnospiraceae_unclassified, Xanthomonadaceae_unclassified, Bilophila, Carnobacterium, Alicycliphilus, and Bifidobacterium was decreased in the AS group, while the relative abundance of Prevotella, Escherichia-Shigella, Tyzzerella_4, and Romboutsia was higher in the AS group ($p < 0.01$, Figures 4e and S1e).

Gut Microbiota Taxonomic Signature Differences Between Groups

We further performed a taxonomic profile analysis of each sample, LEfSe demonstrated significant differences ($p < 0.05$) in gut microbiota composition between the two groups with a threshold score of LDA > 3. In the cladogram diagram, the size of nodes is proportional to the relative abundance of the taxonomic group, the lines connecting nodes represent evolutionary relationships, it showed the differences in microbial communities between the two groups at different levels of phylogenetic development (Figure 5a). A bar plot further illustrated the significance between two groups, with positive values indicating enrichment in the Healthy group and negative values indicating enrichment in the AS group (Figure 5b). Compared with the Healthy group, the AS group showed a significant decrease in f_Ruminococcaceae and s_Faecalibacterium, while the c_Gammaproteobacteria, p_Proteobacteria, f_Enterobacteriaceae, o_Enterobacteriales, and s_Escherichia-Shigella increased (Figure 5).

Function Prediction for Gut Microbiota in the AS and Healthy Groups

The potential influence of gut microbiota on metabolic function in AS and Healthy group participants was further predicted using PICRUSt analysis. The KEGG pathway analysis results showed significant differences between the AS and Healthy group in multiple metabolic pathways (Figure 6), especially in the AS group, which was significantly upregulated in the following energy metabolism-related pathways: hexitol fermentation to lactate, ethanol and acetate ($p = 0.0002$); superpathway of glycolysis, pyruvate dehydrogenase, tricarboxylic acid cycle (TCA), and glyoxylate bypass ($p = 0.0018$); TCA cycle IV (2-oxoglutarate decarboxylase) ($p = 0.0014$); superpathway of glyoxylate bypass and TCA ($p = 0.0018$). We also found that pathways related to degradation metabolism were slightly downregulated in the AS group, such as catechol degradation II (meta-cleavage pathway) ($p = 0.0003$); L-tryptophan degradation IV ($p = 0.0003$); 2-nitrobenzoate degradation I ($p = 0.0003$); while the superpathway of hexitol degradation (bacteria) was upregulated ($p = 0.0009$). In addition, the AS group showed significantly higher levels of bacterial iron metabolism pathway – enterobactin biosynthesis – than the Healthy group ($p = 0.0007$). However, in terms of nucleotide metabolism-related pathways, the AS group showed significant downregulation compared to the Healthy group, such as the superpathway of adenosine nucleotides de novo biosynthesis II ($p = 0.0013$); guanosine deoxyribonucleotides de novo biosynthesis II ($p = 0.0018$); adenosine deoxyribonucleotides de novo biosynthesis II ($p = 0.0018$). These differences may be related to the pathophysiological processes of AS, providing important clues for further research on the metabolic mechanisms of AS.

Discussion

Geographical and lifestyle disparities profoundly influenced the human gut microbiome.[19] These variations are shaped not merely by dietary practices – where Western diets, in contrast to Asian diets, are linked to reduced microbial diversity[20] – but also by a spectrum of elements including environmental interactions, standards of hygiene, and lifestyle choices.[21] Moreover, the gut microbiota's contribution to the etiology of AS is an area of growing interest, with its metabolic activities being implicated in the inflammatory processes characteristic of AS.[11] In our research, we conducted 16S rDNA sequencing analysis on fecal samples from individuals with AS and Healthy controls in Southern China (Guangdong Province). Our analysis revealed notable disparities in the gut microbiota composition between the AS patients and the Healthy cohort, particularly emphasizing the diversity of microbial species and the relative abundance of

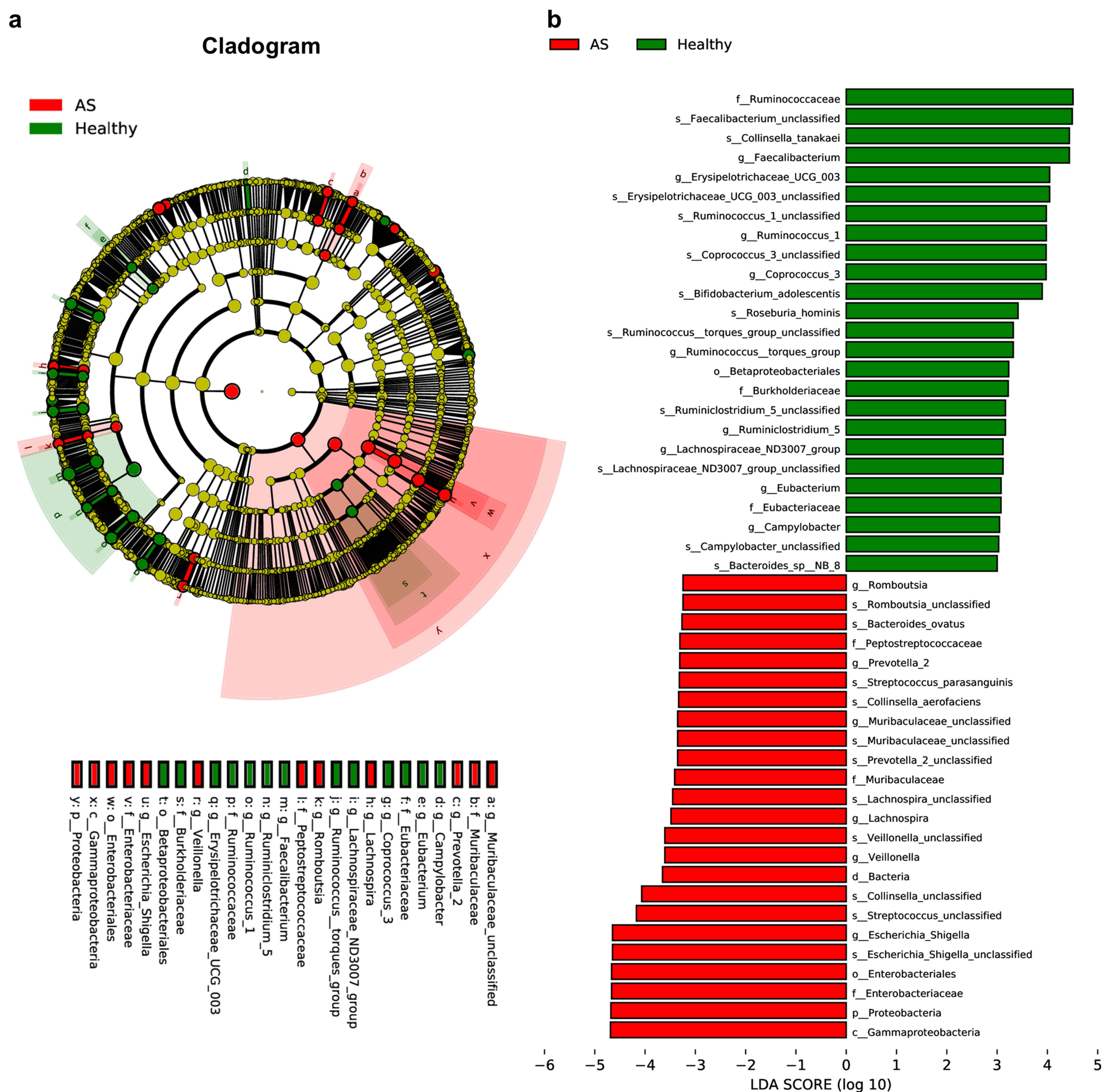


Figure 5 Linear discriminant analysis effect size (LEfSe) analysis of gut microbiota. (**a**) LEfSe analysis of evolutionary clades of the gut microbiota in the AS and Healthy groups. (**b**) LEfSe identified the taxa with the greatest differences in abundance between the AS and Healthy groups.

certain bacterial genera. Furthermore, we performed function predictions of the gut microbiota, which could offer vital insights into understanding the pathophysiological mechanisms underlying AS. Our study provides compelling evidence that the gut microbiota composition is significantly altered in individuals with AS compared to Healthy controls (Figures 1–3). Specifically, the depletion of butyrate-producing taxa (eg, Faecalibacterium) observed in our cohort mirrors trends reported in recent meta-analyses,[22] suggesting a potential loss of microbial-derived immunomodulatory metabolites such as butyrate. Butyrate deficiency may exacerbate intestinal barrier dysfunction and systemic inflammation, as demonstrated in experimental AS models where butyrate supplementation ameliorated axial inflammation.[23] Notably, dietary patterns in Guangdong – rich in fiber from vegetables and legumes – theoretically favor butyrogenesis, yet AS patients still exhibited reduced butyrate producers, implying disease-specific overrides of dietary influences. Furthermore, dysbiosis in AS may be compounded by host genetic factors (eg, HLA-B27) and disease activity.[24,25] While

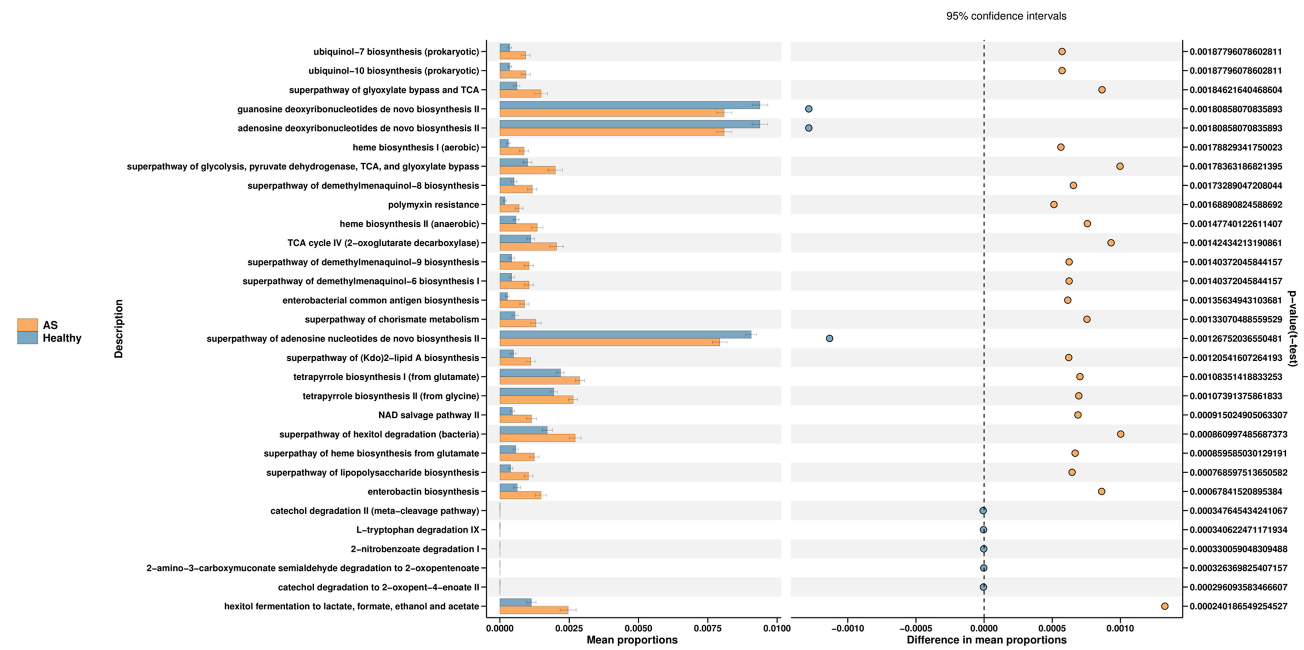


Figure 6 KEGG pathway analysis of the AS and Healthy groups. Mean proportions, difference in mean proportions of the AS and Healthy groups, and p values are shown.

our study did not assess disease activity scores or HLA-B27 status, these factors warrant investigation in future cohorts to clarify their interplay with regional dietary and environmental exposures.

The reduction of beneficial microbiota, especially Firmicutes and Actinobacteria, may have adverse effects on the intestinal barrier function and immune regulation of AS patients.[26] Faecalibacterium possesses the ability to generate short-chain fatty acids, which play a crucial role in preserving intestinal barrier integrity, these acids enhance epithelial tight junctions, act as a source of energy for colonocyte ATP synthesis and modulate the immune system.[27] In our previous animal experiments on AS, we observed that AS model mice exhibited gut microbiota dysbiosis and intestinal mucosal barrier impairment, accompanied by increased inflammatory cells such as Th17 cells and the release of pro-inflammatory cytokines.[28] When AS mice were treated with agents known to modulate gut dysbiosis, such as indole-3-Acetic Acid,[28] FITC,[29] or rifaximin,[30] we found that intestinal barrier function improved, and inflammatory responses were suppressed. An increase in Proteobacteria, especially Enterobacteriaceae may be associated with metabolic disorders and IBD, elevated nitrate and oxygen concentrations in the inflamed intestinal tract, as well as with dysregulation of bile acid metabolism.[31,32] Research indicates the enrichment of Gammaproteobacteria may correlate with chronic inflammation, such as Crohn's disease.[33] Additionally, the increase of Escherichia-Shigella is suspected to be associated with an amplified inflammatory response, impaired intestinal mucosal barrier, and increased intestinal permeability.[34] The decrease of beneficial bacteria and the increase of harmful bacteria in AS patients may collectively advance the disease by disrupting the intestinal barrier, intensifying inflammatory responses, and promoting pathological immune reactions (Figures 4, 5 and S1). This highlights the potential significance of modulating the gut microbiota in disease management and provides new insights for future therapeutic strategies.

Consistent with previous studies,[35] we found an increase in pro-inflammatory bacteria (such as Prevotella and Escherichia-Shigella) and a decrease in anti-inflammatory bacteria (such as Faecalibacterium) in the AS group (Figures 4, S1, and Supplementary Table 2). However, while some earlier studies reported an upregulation of Lachnospira in AS patients,[36,37] our study found a decrease in Lachnospiraceae. Additionally, compared to previous studies, we identified some novel findings: f_Peptostreptococcaceae, g_Tyzzerella_4, and g_Romboutsia were elevated in the gut microbiota of the AS group, while g_Xanthomonadaceae_unclassified, g_Bilophila, g_Carnobacterium, and g_Alicycliphilus were reduced. Peptostreptococcaceae is a family of anaerobic, Gram-positive bacteria classified under the phylum Firmicutes. Members of this family are often found in diverse environments, including the human oral cavity

and gastrointestinal tract, where they contribute to the normal microbial flora. Notably, Peptostreptococcus is commonly associated with various clinical conditions, including periodontitis and other infections. Studies have focused on the role of different Peptostreptococcus species in health and disease. For example, Peptostreptococcus asaccharolyticus has been implicated in rare cases of septic arthritis, often arising from hematogenous dissemination.[38] The literature describing Tyzzerella 4 is very limited, but previous reports have found this genus to be increased in patients with inflammatory bowel disease.[39] Romboutsia is a recently described genus of bacteria that has garnered attention in the scientific community due to its significant association with the health of the gastrointestinal tract. Previous study found that genus Romboutsia was significantly increased in the obese group.[40] It was reported that Xanthomonadaceae was related to formation of biofilms[41] and family Xanthomonadaceae was decreased in patients with systemic lupus erythematosus.[42] The function of Xanthomonadaceae may need further research in AS. Meanwhile, we found that Carnobacterium decreased in the AS group. Carnobacterium is a genus of lactic acid bacteria (LAB) that has gained attention for its role as a probiotic, particularly in food products, and its potential health benefits in humans and animals.[43] The differences in gut microbiota of AS patients in Southern China compared to previous studies may be due to variations in diet, genetics, and geographic factors.

By employing PICRUSt, we predicted the metabolic functional spectrum of the gut microbiota, thereby revealing potential metabolic differences between AS patients and Healthy individuals (Figure 6). In the AS group, pathways related to energy metabolism – hexitol fermentation to lactate, ethanol and acetate – were significantly upregulated, and these energy metabolism disorders affected the metabolic function of the gut microbiota, which may in turn affect the host's metabolic status and inflammatory response.[44] The upregulation of the glycolysis pathway enables cells involved in pro-inflammatory responses – inflammatory M1 macrophages, Th1 and Th17 lymphocytes, or activated microglia – to rapidly provide energy through glycolysis to promote inflammation.[45] Glycolysis also plays a crucial role in the energy supply, biosynthesis, redox balance, and regulation of skeletal cell fate, and its metabolic disorders will promote the development of osteoarthritis.[46] TCA intermediates, especially α-ketoglutarate, participate in inflammatory responses by regulating the NF-κB signaling pathway and epigenetic modifications, affecting the expression of inflammation-related genes.[47] The TCA metabolic disorder may affect the inflammatory process of AS. In addition, the degradation metabolism pathways were slightly downregulated in the AS group, such as catechol degradation II, which is supposed to alleviate cellular oxidative stress caused by long-term hypoxia effectively, facilitate the reconstruction of an immune-friendly microenvironment, and ultimately enhance cartilage regeneration,[48] its downregulation may affect intracellular redox balance and inflammatory response, leading to the occurrence of AS. Dysregulation of L-tryptophan degradation IV reduces the ability of bacteria to metabolize tryptophan into regulatory metabolites, thereby enhancing tryptophan metabolism in human cells, which may lead to fatigue and contribute to spondyloarthritis.[49] Downregulation of the 2-nitrobenzoate degradation I pathway may be related to the accumulation of inflammatory mediators and the enhancement of cellular stress response in AS patients, thereby affecting cellular function and promoting inflammatory response. Besides, the high expression of the enterobactin biosynthesis pathway results in an increase of its metabolic product, the enterobactin siderophore, which in turn induces the activation of NF-κB, thereby exacerbating the inflammatory response.[50] Furthermore, in nucleotide metabolism pathway, the AS group exhibited a notable decrease compared to the Healthy group. Metabolites of nucleotide metabolism, such as cGAMP, can activate the innate immune response by stimulating the STING pathway, indicating that nucleotide metabolism plays a role not only in intracellular metabolism but also potentially in immune signaling transduction. Alterations in nucleotide metabolism may impact the function of regulatory T cells (Tregs), which are crucial in maintaining immune tolerance. Abnormalities in nucleotide metabolism could lead to the disruption of immune tolerance, thereby promoting the development of autoimmune diseases and inflammation.[51] The alterations in these metabolic pathways of the gut microbiota in the AS group may be related to the pathophysiological processes of the disease, providing significant clues for further investigation into the metabolic mechanisms of AS.

This study still has limitations – it has a relatively small sample size and is limited to the Southern Han Chinese population in Guangdong Province, China – which may not fully represent the AS patients from other regions or ethnic groups. Our conscious focus on Southern Han Chinese participants aimed to minimize genetic heterogeneity for initial biomarker discovery (particularly given HLA-B27 subtype variations across ethnicities). However, we revealed significant

differences in the gut microbiota composition between AS patients and Healthy individuals, particularly in alterations related to metabolism pathways. In the future, research can be considered in a wider geographical area and different ethnic groups to enhance the universality and applicability of research findings. Recruitment was challenging given the stringent inclusion criteria and the relatively low regional prevalence of AS. Similar exploratory studies (eg, Costello et al recruit 10 AS patients and 9 healthy controls;[52] Chen et al recruit 20 AS patients and 19 healthy controls[53]) have employed comparable cohort sizes for hypothesis generation. And we are currently expanding this cohort through multi-center collaboration and will incorporate power calculations in subsequent validation phases; We acknowledge that 16S rDNA sequencing has limitations in species-level resolution and functional predictions. However, this method remains widely used for microbiota profiling due to its efficiency and cost-effectiveness.[52,54] To strengthen our analysis, we validated our findings by comparing them with existing literatures. While PICRUSt-based functional predictions are indeed inferential, similar approaches have provided valuable insights into microbiome studies.[55,56] In future research, we plan to incorporate metabolomics and transcriptomics to enhance the functional interpretation of microbial changes; It would be worthwhile to delve into the molecular mechanisms of the metabolic changes in the gut microbiota of AS patients, including how the gut microbiota affects the host's immune response and inflammatory pathways; Exploring the impact of modulating the gut microbiota on the progression of AS, including assessing the long-term effects of different interventions such as probiotics, prebiotics, and specific dietary changes on the composition of gut microbiota and AS disease activity.

Conclusion

This study indicates that in the gut microbiota of Han Chinese AS patients in Guangdong Province, the relative abundance of beneficial bacterial communities decreases, and harmful bacterial communities increase. This may promote the progression of AS by disrupting the intestinal barrier, intensifying inflammatory responses, and pathological immune reactions. It provides a solid theoretical foundation for developing new AS intervention strategies, including microbiota-targeted drugs or personalized dietary plans.

Abbreviations

AS, Ankylosing spondylitis; HLA, Human leukocyte antigen; MetS, Metabolic syndrome; IBD, Inflammatory bowel disease; OUT, Operational taxonomic units; PCA, Principal component analysis; PCoA, Principal coordinate analysis; LEfSe, Linear discriminant analysis effect size; LDA, Linear discriminant analysis; KEGG, Kyoto Encyclopedia of Gene and Genomes; PICRUSt, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States; UPGMA, Unweighted Pair Group Method with Arithmetic Mean; NMDS, Non-metric Multidimensional Scaling; TCA, Tricarboxylic acid cycle.

Ethics Approval

The Ethics Committee of the Fifth Affiliated Hospital of Sun Yat-sen University approved this study (project No. ZDWY. JZWK.015). Prior to the commencement of the study, informed consent was obtained from all participants. This study adheres to the ethical standards outlined in the Helsinki Declaration and its subsequent amendments.

Acknowledgments

The Graphical Abstract was drawn by Figdraw (https://www.figdraw.com), with authorization ID: OSUWOd6d1d.

Funding

This study was supported by the National Natural Science Foundation of China (No. 82302035, L. Yang), the Basic and Applied Basic Research Foundation of Guangdong Province (No. 2023A1515111101, K. Wang), the Project of Administration of Traditional Chinese Medicine of Guangdong Province of China (No. 20241046, L. Yang), R&D project of Pazhou Lab (Huangpu) (No. 2023K0604, H. Lu), the Joint Funding Scheme 2022 for Scientific Research Projects (FDCT-GDST Projects) by the Science and Technology Development Fund of Macau, the Department of Science and Technology of Guangdong Province (No. 2022A0505020019 & 0056/2021/AGJ, H. Lu), Shenzhen Key Laboratory of Bone Tissue Repair and Translational Research (No. ZDSYS20230626091402006, J.Shen).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Sieper J, Poddubnyy D. Axial spondyloarthritis. *Lancet*. 2017;390(10089):73–84. doi:10.1016/s0140-6736(16)31591-4
2. Dean LE, Jones GT, MacDonald AG, et al. Global prevalence of ankylosing spondylitis. *Rheumatology*. 2014;53(4):650–657. doi:10.1093/rheumatology/ket387
3. Zhu W, He X, Cheng K, et al. Ankylosing spondylitis: etiology, pathogenesis, and treatments. *Bone Res*. 2019;7(1). doi:10.1038/s41413-019-0057-8
4. Chen B, Li J, He C, et al. Role of HLA-B27 in the pathogenesis of ankylosing spondylitis. *Mol Medi Rep*. 2017;15(4):1943–1951. doi:10.3892/mmr.2017.6248
5. Nagit R-E, Rezus E, Cianga P. Exploring the pathogenesis of spondylarthritis beyond HLA-B27: a descriptive review. *Int J Mol Sci*. 2024;25(11):6081. doi:10.3390/ijms25116081
6. Tsukazaki H, Kaito T. The role of the IL-23/IL-17 pathway in the pathogenesis of spondyloarthritis. *Int J Mol Sci*. 2020;21(17):6401. doi:10.3390/ijms21176401
7. Jostins L, Ripke S, Weersma RK, et al. Host–microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature*. 2012;491(7422):119–124. doi:10.1038/nature11582
8. Pishgahi A, Abolhasan R, Danaii S, et al. Immunological and oxidative stress biomarkers in Ankylosing Spondylitis patients with or without metabolic syndrome. *Cytokine*. 2020:128. doi:10.1016/j.cyto.2020.155002
9. Guggino G, Mauro D, Rizzo A, et al. Inflammasome activation in ankylosing spondylitis is associated with gut dysbiosis. *Arthritis Rheumatol*. 2021;73(7):1189–1199. doi:10.1002/art.41644
10. Asquith M, Elewaut D, Lin P, et al. The role of the gut and microbes in the pathogenesis of spondyloarthritis. *Best Pract Res*. 2014;28(5):687–702. doi:10.1016/j.berh.2014.10.018
11. Breban M, Tap J, Leboime A, et al. Faecal microbiota study reveals specific dysbiosis in spondyloarthritis. *Ann Rheumatic Dis*. 2017;76(9):1614–1622. doi:10.1136/annrheumdis-2016-211064
12. Wei J, Zhu X, Wang J, et al. Mendelian randomization studies in ankylosing spondylitis: a systematic review. *Int J Rheum Dis*. 2024;27(11):e15408. doi:10.1111/1756-185x.15408
13. Lee TD, Zhao TM, Mickey R, et al. The polymorphism of HLA antigens in the Chinese. *Tissue Antigens*. 1988;32(4):188–208. doi:10.1111/j.1399-0039.1988.tb01656.x
14. Ma HJ, Yin QF, Hu FP, et al. Different clinical features in patients with ankylosing spondylitis from Southern and Northern China. *Int J Rheum Dis*. 2012;15(2):154–162. doi:10.1111/j.1756-185X.2011.01697.x
15. Lin D, Wang R, Luo J, et al. The core and distinction of the gut microbiota in Chinese populations across geography and ethnicity. *Microorganisms*. 2020;8(10):1579. doi:10.3390/microorganisms8101579
16. Hou Y, Wang SF, Zhou K, et al. Comparison and recommendation of dietary patterns based on nutrients for eastern and western patients with inflammatory bowel disease. *Front Nutr*. 2022;9:1066252. doi:10.3389/fnut.2022.1066252
17. Van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. *Arthritis Rheum*. 1984;27(4):361–368. doi:10.1002/art.1780270401
18. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods*. 2013;10(10):996–998. doi:10.1038/nmeth.2604
19. Yatsunenko T, Rey FE, Manary MJ, et al. Human gut microbiome viewed across age and geography. *Nature*. 2012;486(7402):222–227. doi:10.1038/nature11053
20. David LA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature*. 2014;505(7484):559–563. doi:10.1038/nature12820
21. Van Hul M, Cani PD, Petitfils C, et al. What defines a healthy gut microbiome? *Gut*. 2024;73(11):1893–1908. doi:10.1136/gutjnl-2024-333378
22. Su QY, Zhang Y, Qiao D, et al. Gut microbiota dysbiosis in ankylosing spondylitis: a systematic review and meta-analysis. *Front Cell Infect Microbiol*. 2024;14:1376525. doi:10.3389/fcimb.2024.1376525
23. Min HK, Na HS, Jhun J, et al. Identification of gut dysbiosis in axial spondyloarthritis patients and improvement of experimental ankylosing spondyloarthritis by microbiome-derived butyrate with immune-modulating function. *Front Immunol*. 2023;14:1096565. doi:10.3389/fimmu.2023.1096565
24. Berland M, Meslier V, Berreira Ibraim S, et al. Both disease activity and HLA-B27 status are associated with gut microbiome dysbiosis in spondyloarthritis patients. *Arthritis Rheumatol*. 2023;75(1):41–52. doi:10.1002/art.42289
25. Păsăran ED, Diaconu AE, Oancea C, et al. An actual insight into the pathogenic pathways of ankylosing spondylitis. *Curr Issues Mol Biol*. 2024;46(11):12800–12812. doi:10.3390/cimb46110762
26. Mushraf S, Chawla K, Fayaz SMA, et al. Exploring the effects of probiotics on olanzapine-induced metabolic syndrome through the gut microbiota. *Gut Pathog*. 2024;16(1):77. doi:10.1186/s13099-024-00664-2
27. Hays KE, Pfaffinger JM, Ryznar R. The interplay between gut microbiota, short-chain fatty acids, and implications for host health and disease. *Gut Microbes*. 2024;16(1):2393270. doi:10.1080/19490976.2024.2393270
28. Shen J, Yang L, You K, et al. Indole-3-acetic acid alters intestinal microbiota and alleviates ankylosing spondylitis in mice. *Front Immunol*. 2022;13:762580. doi:10.3389/fimmu.2022.762580
29. Liu B, Wang K, Yang L, et al. 6-formylindolo[3, 2-b]carbazole alters gut microbiota and prevents the progression of ankylosing spondylitis in mice. *Int Immunopharmacol*. 2024;128:111562. doi:10.1016/j.intimp.2024.111562
30. Yang L, Liu B, Zheng J, et al. Rifaximin alters intestinal microbiota and prevents progression of ankylosing spondylitis in mice. *Front Cell Infect Microbiol*. 2019;9:44. doi:10.3389/fcimb.2019.00044
31. Rizzatti G, Lopetuso LR, Gibiino G, et al. Proteobacteria: a common factor in human diseases. *Biomed Res Int*. 2017;2017:9351507. doi:10.1155/2017/9351507

32. Baldelli V, Scaldaferri F, Putignani L, et al. The role of Enterobacteriaceae in gut microbiota dysbiosis in inflammatory bowel diseases. *Microorganisms*. 2021;9(4):697. doi:10.3390/microorganisms9040697
33. Sekido Y, Nishimura J, Nakano K, et al. Some gammaproteobacteria are enriched within CD14(+) macrophages from intestinal lamina propria of Crohn's disease patients versus mucus. *Sci Rep*. 2020;10(1):2988. doi:10.1038/s41598-020-59937-w
34. Yan J, Pan Y, He J, et al. Toxic vascular effects of polystyrene microplastic exposure. *Sci Total Environ*. 2023;905:167215. doi:10.1016/j.scitotenv.2023.167215
35. Song ZY, Yuan D, Zhang SX. Role of the microbiome and its metabolites in ankylosing spondylitis. *Front Immunol*. 2022;13:1010572. doi:10.3389/fimmu.2022.1010572
36. Tito RY, Cypers H, Joossens M, et al. Brief report: dialister as a microbial marker of disease activity in spondyloarthritis. *Arthritis Rheumatol*. 2017;69(1):114–121. doi:10.1002/art.39802
37. Zhang F, Ma C, Zhang B, et al. Dynamic changes in gut microbiota under the influence of smoking and TNF-α-blocker in patients with ankylosing spondylitis. *Clin Rheumatol*. 2020;39(9):2653–2661. doi:10.1007/s10067-020-05032-4
38. Costa C, Santiago M, Ferreira J, et al. Septic arthritis caused by Peptostreptococcus asaccharolyticus. *Acta Reumatol Port*. 2016;41(3):271–272.
39. Olaisen M, Flatberg A, Granlund AVB, et al. Bacterial mucosa-associated microbiome in inflamed and proximal noninflamed ileum of patients with Crohn's disease. *Inflamm Bowel Dis*. 2021;27(1):12–24. doi:10.1093/ibd/izaa107
40. Therdtatha P, Song Y, Tanaka M, et al. Gut microbiome of Indonesian adults associated with obesity and type 2 diabetes: a cross-sectional study in an Asian City, Yogyakarta. *Microorganisms*. 2021;9(5):897. doi:10.3390/microorganisms9050897
41. Mhedbi-Hajri N, Jacques MA, Koebnik R. Adhesion mechanisms of plant-pathogenic Xanthomonadaceae. *Adv Exp Med Biol*. 2011;715:71–89. doi:10.1007/978-94-007-0940-9_5
42. Li BZ, Zhou HY, Guo B, et al. Dysbiosis of oral microbiota is associated with systemic lupus erythematosus. *Arch Oral Biol*. 2020;113:104708. doi:10.1016/j.archoralbio.2020.104708
43. Evangelista AG, Danielski GM, Corrêa JAF, et al. Carnobacterium as a bioprotective and potential probiotic culture to improve food quality, food safety, and human health - a scoping review. *Crit Rev Food Sci Nutr*. 2023;63(24):6946–6959. doi:10.1080/10408398.2022.2038079
44. Heiss CN, Olofsson LE. Gut microbiota-dependent modulation of energy metabolism. *J Innate Immun*. 2018;10(3):163–171. doi:10.1159/000481519
45. Soto-Heredero G, Gómez de Las Heras MM, Gabandé-Rodríguez E, et al. Glycolysis - a key player in the inflammatory response. *Febs J*. 2020;287(16):3350–3369. doi:10.1111/febs.15327
46. Stegen S, Carmeliet G. Metabolic regulation of skeletal cell fate and function. *Nat Rev Endocrinol*. 2024;20(7):399–413. doi:10.1038/s41574-024-00969-x
47. Choi I, Son H, Baek JH. Tricarboxylic acid (TCA) cycle intermediates: regulators of immune responses. *Life*. 2021;11(1). doi:10.3390/life11010069
48. Zhao F, Jia Z, Zhang L, et al. A MnO(2) nanosheets doping double crosslinked hydrogel for cartilage defect repair through alleviating inflammation and guiding chondrogenic differentiation. *Biomaterials*. 2025;314:122875. doi:10.1016/j.biomaterials.2024.122875
49. Berthelot JM, Darrieutort-Laffite C, Trang C, et al. Contribution of mycobiota to the pathogenesis of spondyloarthritis. *Joint Bone Spine*. 2021;88(6):105245. doi:10.1016/j.jbspin.2021.105245
50. Tomás A, Lery L, Regueiro V, et al. Functional genomic screen identifies Klebsiella pneumoniae factors implicated in blocking nuclear factor κB (NF-κB) signaling. *J Biol Chem*. 2015;290(27):16678–16697. doi:10.1074/jbc.M114.621292
51. Ali ES, Ben-Sahra I. Regulation of nucleotide metabolism in cancers and immune disorders. *Trends Cell Biol*. 2023;33(11):950–966. doi:10.1016/j.tcb.2023.03.003
52. Costello ME, Ciccia F, Willner D, et al. Brief report: intestinal dysbiosis in ankylosing spondylitis. *Arthritis Rheumatol*. 2015;67(3):686–691. doi:10.1002/art.38967
53. Chen Z, Qi J, Wei Q, et al. Variations in gut microbial profiles in ankylosing spondylitis: disease phenotype-related dysbiosis. *Ann Transl Med*. 2019;7(20):571. doi:10.21037/atm.2019.09.41
54. Zhang L, Han R, Zhang X, et al. Fecal microbiota in patients with ankylosing spondylitis: correlation with dietary factors and disease activity. *Clin Chim Acta*. 2019;497:189–196. doi:10.1016/j.cca.2019.07.038
55. Langille MG, Zaneveld J, Caporaso JG, et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat Biotechnol*. 2013;31(9):814–821. doi:10.1038/nbt.2676
56. Douglas GM, Beiko RG, Langille MGI. Predicting the functional potential of the microbiome from marker genes using PICRUSt. *Methods Mol Biol*. 2018;1849:169–177. doi:10.1007/978-1-4939-8728-3_11

Journal of Inflammation Research

Dovepress
Taylor & Francis Group

Publish your work in this journal

The Journal of Inflammation Research is an international, peer-reviewed open-access journal that welcomes laboratory and clinical findings on the molecular basis, cell biology and pharmacology of inflammation including original research, reviews, symposium reports, hypothesis formation and commentaries on: acute/chronic inflammation; mediators of inflammation; cellular processes; molecular mechanisms; pharmacology and novel anti-inflammatory drugs; clinical conditions involving inflammation. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit http://www.dovepress.com/testimonials.php to read real quotes from published authors.

Submit your manuscript here: https://www.dovepress.com/journal-of-inflammation-research-journal