Gut microbiota differences between psoriatic arthritis and undifferentiated arthritis patients

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Research article

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Abstract

Introduction Psoriatic arthritis (PSA) is a form of immune-mediated inflammatory arthritis. Studying the gut microbiota of PSA patients may offer new insights into the pathophysiology of this inflammatory joint disease. We designed a prospective study to examine gut microbiome from patients with PSA, primarily with enthesitis and dactylitis, and compared the data with undifferentiated arthritis patients (NO PSA), without enthesitis or dactylitis.

Methods We enrolled nine PSA patients and 10 NO PSA patients in this study. The fecal samples were investigated by using 16S rRNA amplicon sequencing, followed by bioinformatics and statistical analyses. Results None of the available objective clinical laboratory data could differentiate PSA from the NO PSA subgroup. The microbiota result shows that Family: XIII_AD3011 is significantly higher in NO PSA patients than PSA patients' stool samples (p=0.039). Megasphaera elsdenii in the PSA was 10000 times higher than in the NO PSA group.

Conclusion Our results demonstrated high intra-group homogeneous and high inter-group heterogeneous microbiota. The clinical symptoms of either enthesitis or dactylitis link to the specific microbiota in the current study. In the future, a larger cohort and thorough biochemical study is needed for confirmation.

Introduction

Psoriatic arthritis (PSA) is an immune-mediated inflammatory arthritis that primarily occurs in middle-aged patients with a several-year history of psoriasis. The prevalence of PSA worldwide is 133 cases per 100,000 people (1), which can cause joint deformities and considerable comorbidity, even leading to an inability to work and a reduced lifespan (2, 3). The potential pathophysiology of PSA is multidimensional and has been recently understood in the fields of genetics and molecular biology as involving innate and adaptive immune systems (4). Based on this knowledge, the commercialization of tumor necrosis factor inhibitors and interleukin (IL)-12/23 and IL-17 inhibitors represents a breakthrough in PSA treatment (5).

Gut microbiota has been a very hot topic in recent years. Compared to other parts of the human body, the intestinal flora has both the most bacteria and the most species. The relationship between certain gut flora and humans is not only a commensal but also a mutualistic relationship. Human gut microbiota is determined by genetic, epigenetic, and dietary factors (6). When diets and overall health change, the composition of human intestinal microbiota also changes over time (7, 8). Interestingly, intestinal flora disorders may be associated with various forms of inflammation and autoimmune diseases.

In recent years, convincing evidence has revealed multi-component bidirectional signaling pathways between the gut and joints (9–11). The "skin-joint-gut axis" concept refers to the relationship between gut microbiota and skin and joint function and has been applied to PSA (12). Therefore, studying the gut microbiota of PSA patients may provide new insights into the pathophysiology of this inflammatory joint disease (13, 14). Previous evidence has suggested that host-microbe interactions play a key role in arthritis development (9, 11, 15). DMARDs are known to correct gut dysbiosis in rheumatoid arthritis

patients, as well as further modify the host immune system (15). In psoriatic arthritis patients, a lower relative abundance of multi-intestinal bacteria and dysbiosis have also been noted (16, 17), although whether probiotics can correct them remains unknown.

Currently, few reports have been made on microbiota in Asian PSA patients. Furthermore, few studies have compared the microbiota data between PSA and other arthritis categories, even though the clinical differential diagnosis is clearly different. Since gut microbiota is related to dietary habits, we are not certain whether the previous results of microbiome analysis of PSA patients in Asia are eligible for post-hoc analysis. Therefore, we designed a prospective study to examine the gut microbiome from PSA patients with mainly enthesitis and dactylitis and compare the data with other arthritis patients, especially those undifferentiated forms of arthritis, without enthesitis or dactylitis.

Methods

Study participants

This study's criteria for PSA arthritis patients (n = 9) consisted of the following: a clinical diagnosis of psoriasis by a senior dermatologist based on his/her clinical judgments should be obtained before rheumatologists confirm the PSA diagnosis based on CASPAR criteria (18). All patients in the PSA subgroup have a history of either dactylitis or enthesitis, as confirmed by rheumatologists. We excluded the following patients from our study: those with a history of neuropsychiatric diseases or major physical illnesses (such as intellectual disabilities, psychotic disorders), diabetes mellitus, neuropathy patients, and patients currently taking probiotics or antibiotics.

Regarding the arthritis control subgroup (NO PSA group), we enrolled arthritis with definite synovitis, confirmed by rheumatologists, with the aid of sonography in every case (n = 10). All patients in this subgroup have undifferentiated arthritis, without a definite clinical associated autoimmune disease while enrolled in the study.

This study consisted of eligible patients with arthritis treated in outpatient rheumatology clinics, with minimum oral medication treatment, at Kaohsiung Chang Gung Memorial Hospital in Taiwan. Most patients do not take any medication or only take non-steroid anti-inflammatory drugs to treat pain. We obtained the written informed consent from the parents or guardians of the participants as required by the IRB prior to the start of this study. Our research protocol was approved by the Institutional Review Board (IRB) at Chang Gung Memorial Hospital in Taiwan (IRB: 201700509B0).

Sample collection

Fecal samples were obtained from either PSA patients (PSA group, n = 9) or arthritis controls (NO PSA group, n = 10) using the standard method of scooping a piece of feces, placing it in a 50 mL Falcon tube, and storing it at 4 °C immediately after collection and then at − 80 °C within 24 h. Total DNA extraction of the fecal was carried out using a QIAamp ® DNA Stool Mini Kit (QIAGEN, Tokyo, Japan) in accordance with the manufacturers' instructions. To increase the recovery of bacterial DNA, samples were pretreated

with lytic enzymes prior to extraction using the stool kit. Briefly, 100 mg of fecal matter was suspended in 10 mL of Tris–EDTA buffer (pH around 7.5), and 50 µL of 100 mg/mL lysozyme type VI from chicken egg white (MPBIO, Derby, UK) and 50 µL of 1 mg/mL purified achromopeptidase (Wako, Osaka, Japan) were mixed. The solution was incubated at 37 °C for 1 hour. Next, 100 µL of 20 mg/mL proteinase K (Wako) was added, followed by incubation at 55 °C for 1 hour. The cell lysate was then subjected to ethanol precipitation. The precipitant was dissolved in 1 mL of ASL buffer from the kit and subsequently purified using the QIAamp ® DNA Stool Mini Kit (QIAGEN).

16S rRNA amplicon sequencing

The DNA samples were put into the first-run PCR reaction, where the specified forward primer (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTCGGGNGGCWGCAG) and reverse primer (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC) were designed to amplify the V3–V4 genomics region of bacterial 16S rRNA genes. Using approximately 550 base pair PCR products confirmed with gel electrophoresis, the products were then set to library preparation for 16S rRNA sequencing. A DNA library developed according to the 16S rRNA Sequencing Library Preparation instructions (Illumina, California, USA) was made available to us by the manufacturing company. The prepared amplicons were sequenced on the MiSeq sequencer (Illumina, California, USA) using the 600-cycle sequencing reagent and specifying the pair-end mode.

Clinical measurements

Clinical data, including age, gender, leukocyte counts, differential counts, absolute eosinophil count, platelets, erythrocyte sedimentation rate (mm/hour), C-reactive protein (mg/L), rheumatoid factor (IU/mL), AST (mg/dL), ALT (mg/dL), creatinine (mg/dL), uric acid (mg/dL), total IgE, and total cholesterol were all collected by chart review.

Statistical and bioinformatics analysis

We analyzed data with the statistical software package SPSS, version 16 (SPSS Inc., Chicago, IL, USA). Variables were presented as either the mean (standard deviation) or frequency. Two-tailed p-values < 0.05 were considered statistically significant. We applied the independent t-test or Mann–Whitney U test to determine potential differences in age and clinical assessments between the PSA patients and no PSA arthritis controls. 16S rRNA gene amplicon sequences were analyzed using Mothur v1.39 in accordance with the MiSeq SOP. The 16S rRNA V3–V4 sequencing reads were initially de-multiplexed using MiSeq Reporter v2.6. The de-multiplexed paired reads were assembled into a single contig with the following parameters: minimum length of 405 bp, maximum length of 428 bp, and no ambiguity. The single contig consisted of the effective reads from all samples clustered into OTUs based on a 97% sequence similarity according to Illumina MiSeq SOP and pursuant to the steps described as follows: sequencing filtering and trimming to de-replicate the working sequence set and align it with the SILVA bacteria reference 16S alignment (release 132) distributed with Mothur (v1.38). Sequencing error reduction was performed with PCR chimera removal after screening with UCHIME (v4.2).

Taxonomy assignment of sequences and Clustering into OTU

We performed the Mothur implementation of the Naïve Bayesian Classifier against the homemade RDP rRNA training set (v9) to create a taxonomic assignment for every sequence with a minimum bootstrap confidence score of 80%. The clustering of sequences was carried out at a threshold identity of 0.03% using the average neighbor algorithm. We performed alpha faction analysis, including observed OTUs, Chao1, and PCoA plots, which were generated using R scripts. Finally, we used linear discriminant analysis effect size (LEfSe) to determine the taxa that could most likely explain the differences between the experimental samples and the control samples.

Results

Demographic data of enrolled patients

All the clinical data, including age, gender, leukocyte counts, differential counts, absolute eosinophil count, platelets, erythrocyte sedimentation rate (mm/hour), C-reactive protein (mg/L), rheumatoid factor (IU/mL), AST (mg/dL), ALT (mg/dL), creatinine (mg/dL), uric acid (mg/dL), total IgE, and total cholesterol collected by chart review did not differ significantly between the PSA and NO PSA subgroups (all $p>0.05$) (Table 1).

Alpha diversity and beta diversity

Microbial diversity was assessed either within a subgroup (alpha diversity) or between the sample subgroups (beta diversity). We calculated several different values to evaluate alpha diversity: “Rarefaction Curve” to calculate species richness for a given number of individual samples (Figure 1), “Chao Index” to estimate species abundance (Figure 2a), and “observed species” to estimate the amount of unique OTUs found in each sample (Figure 2b). Gut microbiota communities from the PSA patients demonstrated a Chao index ($p = 0.014$) and observed species ($p=0.048$) that were significantly increased compared to the NO PSA arthritis controls (Figure 2a, b). The Rarefaction Curve demonstrates that both the PSA and the NO PSA subgroups were adequately sampled and the species richness of the microbial DNA reach plateau (Figure 1). Beta diversity was assessed using a partial least squares discriminated analysis (PLS-DA) plot to compare between NO PSA arthritis controls and PSA patients. The results demonstrate that the microbial diversity differed significantly between the PSA and NO PSA groups (Figure 3a). The unweighted unifrac and weighted unifrac PCoA plots indicated that the gut microbiomes were different between the NO PSA arthritis controls and the PSA patients (Figure 3b). Overall, these results indicated that the gut microbiota communities differed between NO PSA arthritis controls and PSA patients.

Gut microbiome profiling

The gut microbiome dataset from NO PSA arthritis controls and PSA patients revealed a total of ten phyla, four of which accounted for more than 99% of all the bacteria. The NO PSA arthritis controls

demonstrated a dominance of Bacteroidetes (75.95%), followed by Firmicutes (14.13%), Proteobacteria (9.24%), and Fusobacteria (0.34%). The bacteria composition at the phylum level of the PSA group closely resembled that of the NO PSA arthritis controls. The bacteria composition at the phylum level of the PSA group consisted of Bacteroidetes (68.06%), followed by Firmicutes (24.11%), Proteobacteria (6.1%), and Actinobacteria (1.10%). The Actinobacteria was significantly higher in the PSA patients than in the NO PSA arthritis controls ($p<0.01$, see following Figure 4b).

Comparison microbiology differences between PSA and NO PSA subgroups with Welch's t-test, metagenome sequencing, and LEfSe analysis

We used the Welch's t-test (Figure 4a), Metagenome Sequencing (Figure 4b), and LEfSe analysis (Figure 4c) to identify the specific bacteria phylotypes that were differentially altered between the NO PSA arthritis controls and the PSA patients. The Welch's test, named for creator Bernard Lewis Welch, is an adaptation of the Student's t-test (19) for analysis of significantly different families of species between NO PSA arthritis controls and PSA patients. The result shows that Family: XIII_AD3011 is significantly higher in the stool samples of NO PSA patients than those of PSA patients ($p=0.039$, Figure 4a). Metagenome Sequencing is used to demonstrate the significant difference of kingdom, phylum, class, order, and family between the NO PSA arthritis controls and the PSA patients. In Figure 4b, only those that differed significantly between NO PSA arthritis controls and PSA patients are shown (Figure 4b, all $p<0.05$). These differences include Actinobacteria Phylum, Erysipelotrichia Class, Erysipelotrichales Order, Fusobacteriales Order, Acidaminococcaceae Family, Erysipelotrichaceae Family, Peptostreptococcaceae Family, Clostridium innocuum Genus, Catenibacterium Genus, Family XIII_AD3011 Genus, Intestinimonas Genus, Morganella Genus, Turicibacter Genus, and Tyzzerella Genus (Figure 4b, all $p<0.05$).

The LEfSe plot as a result of comparison Genus, and Species between NO PSA arthritis controls and PSA patients is shown in Figure 4c. The relative abundance of Megasphaera elsdenii in the PSA group was significantly higher than 10000 times in the NO PSA arthritis controls group.

Discussion

Psoriatic arthritis is a member of the seronegative spondylopathy family and may be defined as an inflammatory arthropathy associated with psoriasis and is usually negative for rheumatoid factor. Until the 1950s, inflammatory arthritis that occurred in the presence of psoriasis was thought to represent rheumatoid arthritis (RA) that occurred coincidentally with psoriasis. PSA synovium shows infiltration with lymphocytes, macrophages, and NK receptor–expressing cells, with upregulation of leukocyte homing receptors. Clonally expanded T cells are frequent in PSA, which is similar to RA. There is also an abundant synovial overexpression of proinflammatory cytokines. IL2, TNF-α, and IL-1β, -6, -8, and − 15 are found in PSA synovium or synovial fluid. TH17 derived cytokines are found in PSA, given their prominence in PSO and other SPA, the genetic association with genes in the IL-12/IL-23 axis, and the therapeutic response. All of the aforementioned inflammatory pathways were partially demonstrated in

other inflammatory arthritis, such as RA, lupus, vasculitis, and so on. Therefore, differentiating PSA from other types of arthritis in an early stage, i.e., undifferentiated arthritis in the current study, is difficult.

Several articular features distinguish PSA from other joint disorders. Dactylitis occurs in > 30%; enthesitis and tenosynovitis are also common and are likely present in most patients, although they may go unnoticed in physical examinations. Shortening of digits due to underlying osteolysis is particularly characteristic of PSA, and there is a much greater tendency for both fibrous and bony ankylosis of small joints than in RA. In this study, we defined our target PSA patients by clinical manifestation of dactylitis and/or enthesitis, which are unique symptoms in PSA.

The microbiota differ significantly between PSA and NO PSA subgroup patients. These differences are Actinobacteria Phylum, Erysipelotrichia Class, Erysipelotrichales Order, Fusobacteriales Order, Acidaminococcaceae Family, Erysipelotrichaceae Family, Peptostreptococcaceae Family, Clostridium innocuum Genus, Catenibacterium Genus, Family XIII_AD3011 Genus, Intestinimonas Genus, Morganella Genus, Turicibacter Genus, and Tyzzerella Genus (Fig. 4b, all $p < 0.05$). Among these Metagenome Sequencing differences, species abundance is found to be significantly higher in PSA patients than in NO PSA patients in most comparisons, except for the Acidaminococcaceae Family, Clostridium innocuum Genus, Family XIII_AD3011 Genus, and Tyzzerella Genus (higher in the NO PSA subgroup, $p < 0.05$). In the last, most important of all, the Megasphaera elsdenii species are 10000 times higher in PSA patients than in NO PSA patients.

Megasphaera elsdenii species, previously called Peptostreptococcus elsdenii, is one of the gram-negative cocci that can use both carbohydrates and organic acids and is considered one of the main organisms involved in lactic acid catabolism (20) and amino acid deamination (21). Its role in lactic acid metabolism and its links to PSA have yet to be clarified, but its role in PSA patients, compared to undifferentiated arthritis patients, has been demonstrated in our study for the first time. In the future, additional study concerning the immune-modulation, glycobiology (22), and inflammation effect (23) of Megasphaera elsdenii is required to establish the relationship between this organism and PSA disease.

In the current study, we compared microbiota in PSA patients with undifferentiated arthritis, and the result demonstrated high intra-group homogeneous and high inter-group heterogeneous microbiota. According to this, we linked the clinical symptoms of either enthesitis or dactylitis to the specific microbiota and dominate Megasphaera elsdenii species in the current study. A larger cohort and a thorough biochemical study are needed for confirmation.

Conclusion

1. There are substantial differences in the microbiota between those patients with dactylitis and/or enthesitis and those without, which implies that the axis of gut-joint exist.
2. The clinical available serum markers may not be enough to reflect the details of patients with different patterns of joint and soft tissue rheumatism, and further biomarkers from gut micro-

organism could be future research directions.

3. Megasphaera elsdenii species could be a link between gut flora and enthesitis and/or dactylitis clinically.

Abbreviations

ALT	Alanine transaminase
AST	Aspartate transaminase
CASPAR criteria	The Classification Criteria for Psoriatic Arthritis
DMARDs	Disease-modifying anti-rheumatic drugs
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
IgE	Immunoglobulin E
IL	interlukine
IRB	Institutional Review Board
LEfSe	linear discriminant analysis effect size
NO PSA	undifferentiated arthritis patients
OTUs	operational taxonomic units
PCoA	Principal Coordinates Analysis
PLS-DA	partial least squares discriminated analysis
PSA	Psoriatic arthritis
rRNA	Ribosomal ribonucleic acid
SPSS	Statistical Package for the Social Sciences Software
USA	the United States of America

Declarations

- **Ethics approval and consent to participate**

Our research protocol was approved by the Institutional Review Board (IRB) at Chang Gung Memorial Hospital in Taiwan (IRB: 201700509B0). All the participates have provided and signed inform consents.

- **Consent for publication**

No personal individual data was included in any form in this article, and all participates were adults and were provided with inform consents.

- **Availability of data and material**

All the data are available upon requested.

- **Competing interests**

The authors declare to have no conflicts of interest with regard to this article.

- **Funding**

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- **Authors' contributions**

CYH drafted the manuscript and clinical evaluation of patients, HCK participated in the design of the study, YJS conducted the sequence alignment and clinical evaluation of patients and participated in its design and coordination

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- **Authors' information (optional)**

NONE.

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Tables

Table 1. Demographic data of enrolled patients, in either the psoriatic arthritis (PSA) or the undifferentiated arthritis (NO PSA) subgroup.

	PSA	NO PSA	p-value
Case Number	9	10	
Gender	6M/3F	10M/0F	0.087
Age	42.44±2.43	34.86±3.63	0.109
Leukocytes (/uL)	9.14±1.42	7.32±0.63	0.274
Neutrophils (%)	68.93±4.57	60.78±2.77	0.131
Lymphocytes (%)	26.2±3.41	30.39±2.41	0.322
Monocytes (%)	4.44±0.71	5.26±0.61	0.292
Absolute eosinophil count	277.67±95.51	236.44±42.85	0.661
Platelets (1000/uL)	299.0±15.08	262.1±17.60	0.142
Hemoglobin (g/dL)	14.03±0.51	12.69±0.71	0.164
Hematocrit (%)	41.8±1.40	38.26±2.08	0.2
Erythrocyte sedimentation rate (mm/hour)	14 (5,16)	8 (5,10)	0.29
C-reactive protein (mg/L)	5.88 (1.7,7.27)	5 (3.5,10)	0.68
Rheumatoid factor (IU/mL)	3.5 (0.52,5.42)	7.5(5,21)	0.30
Aspartate transaminase (mg/dL)	21 (16,26)	ND	0.14
Alanine transaminase (mg/dL)	22 (17,26)	15 (8.5,36.5)	0.46
Creatinine (mg/dL)	0.75 (0.53,0.97)	0.81 (0.65,2.19)	0.39
Uric acid (mg/dL)	5.2 (3.6,4.36)	3.4 (2.17,3.14)	0.12
Immunoglobulin E	62.9 (8.04,204.)	208 (98,765)	0.42
Total cholesterol	196 (144,153.)	207. (147.,189.)	0.44

Figures

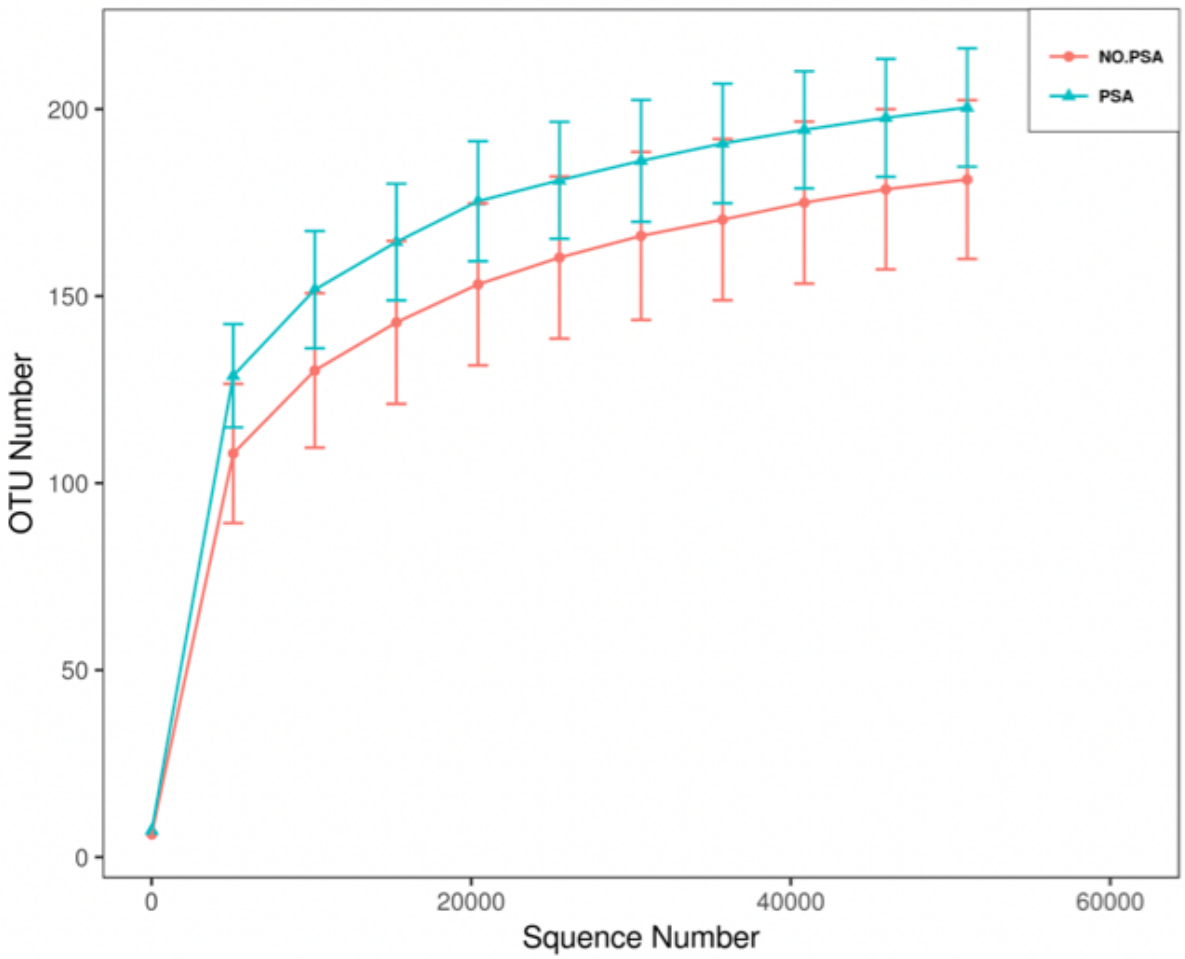


Figure 1

Alpha Diversity-Rarefaction Curve in either the psoriatic arthritis (PSA) or the undifferentiated arthritis (NO PSA) subgroup. The Rarefaction Curve demonstrates that both the PSA and the NO PSA subgroups are adequately sampled, as well as the species richness of the microbial DNA reach plateau.

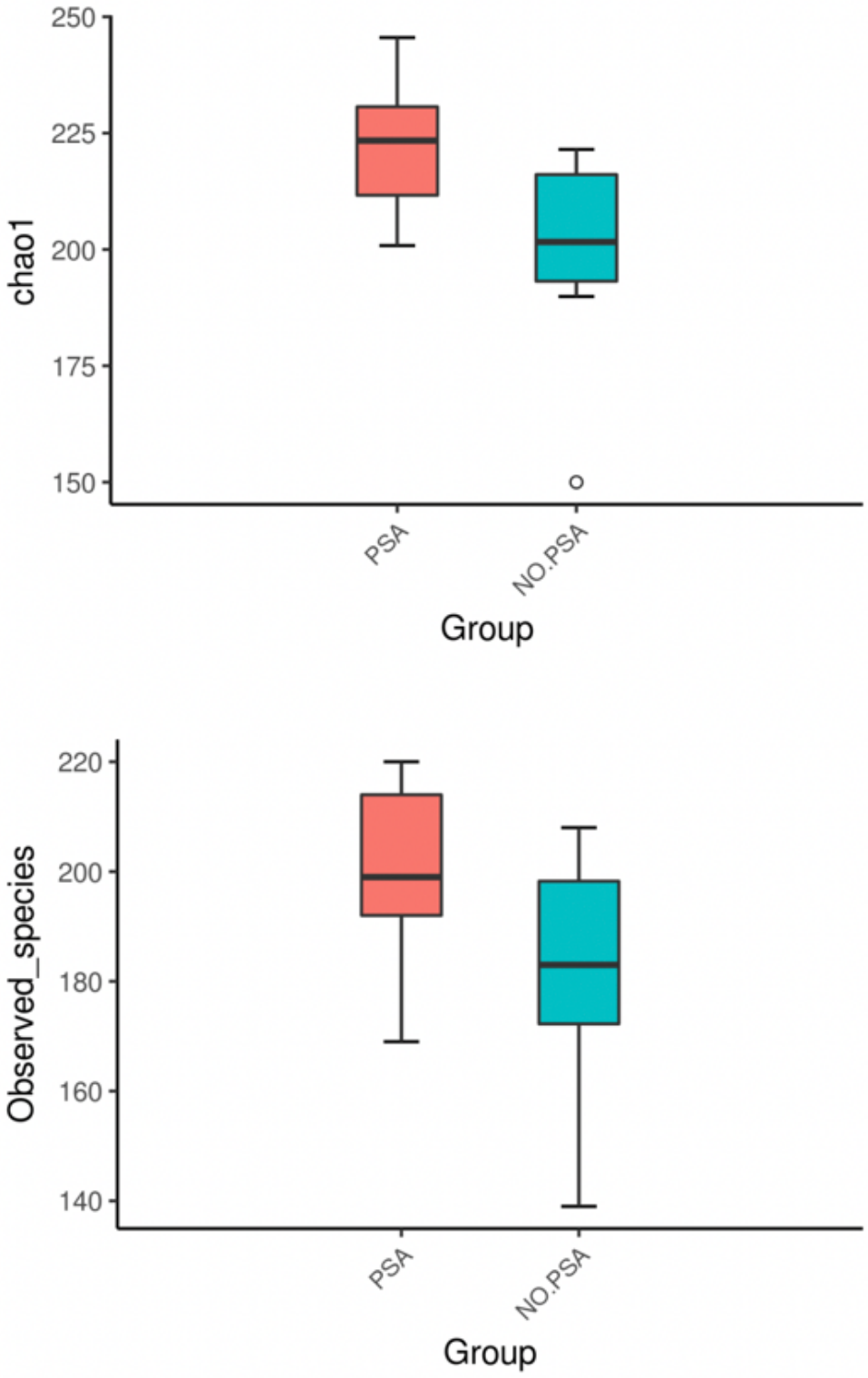
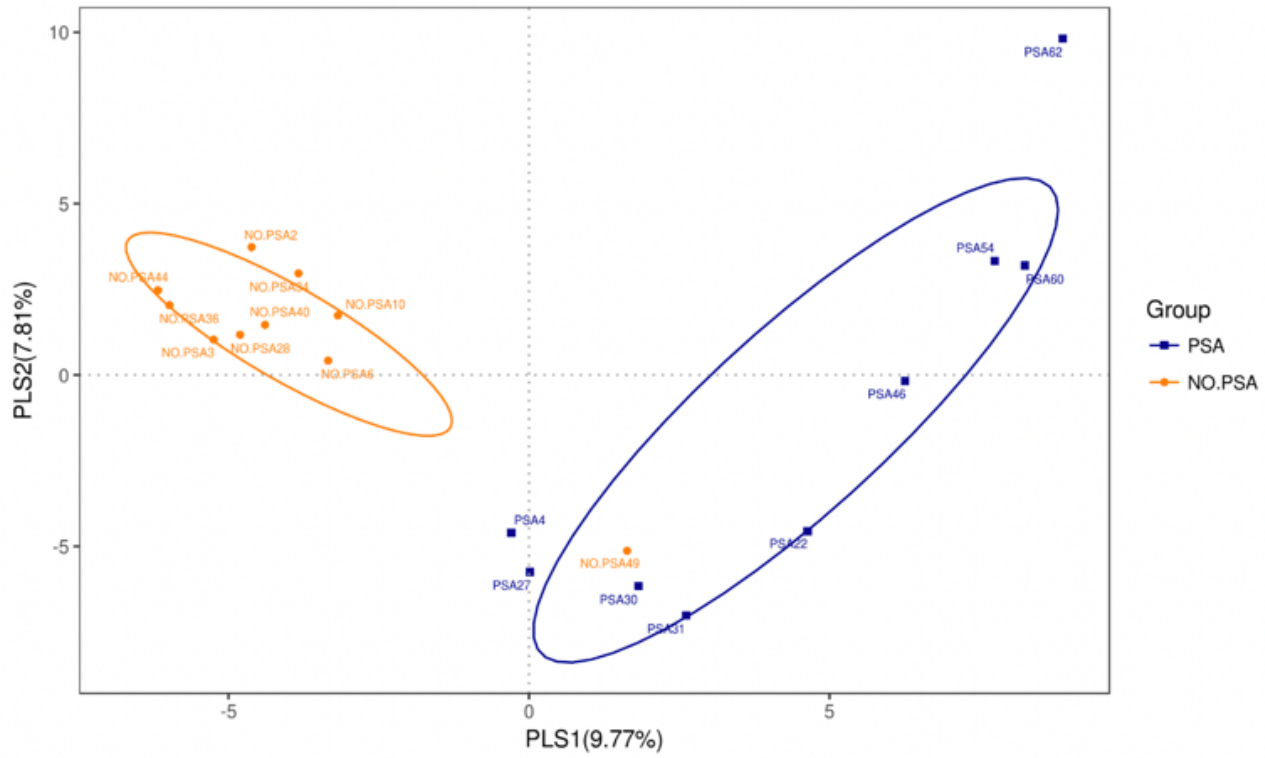


Figure 2

Alpha Diversity in either the psoriatic arthritis (PSA) or the undifferentiated arthritis (NO PSA) subgroup, demonstrated with Chao index and observed species index, both $p<0.05$. Gut microbiota communities from the PSA patients demonstrated Chao index ($p = 0.014$) and observed species ($p=0.048$) that were significantly increased compared to the NO PSA arthritis controls.

3a.



3b.

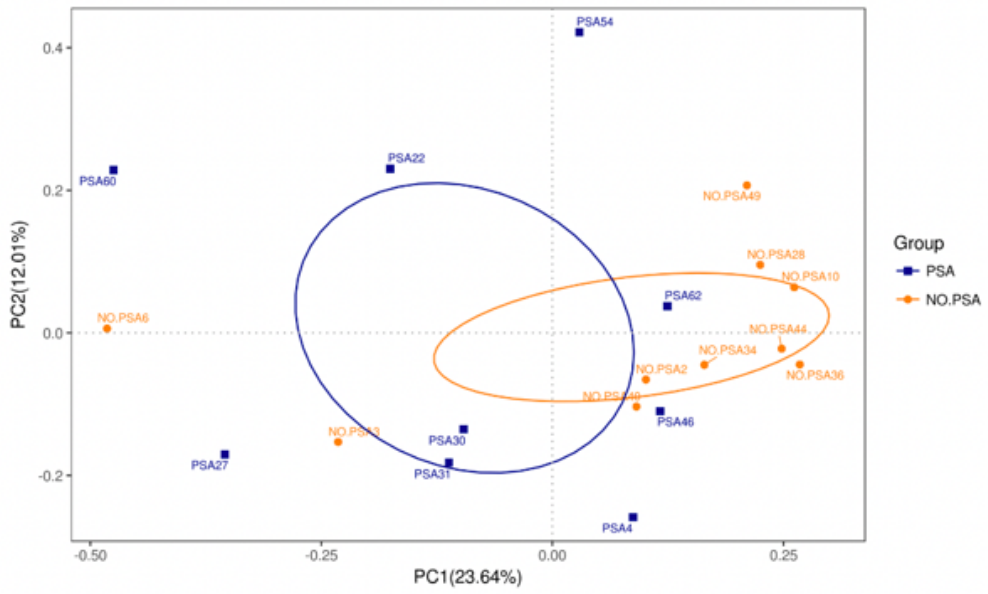


Figure 3

Beta Diversity in either the psoriatic arthritis (PSA) or the undifferentiated arthritis (NO PSA) subgroup. The results demonstrated that the microbial diversity differed significantly between the PSA and NO PSA groups.

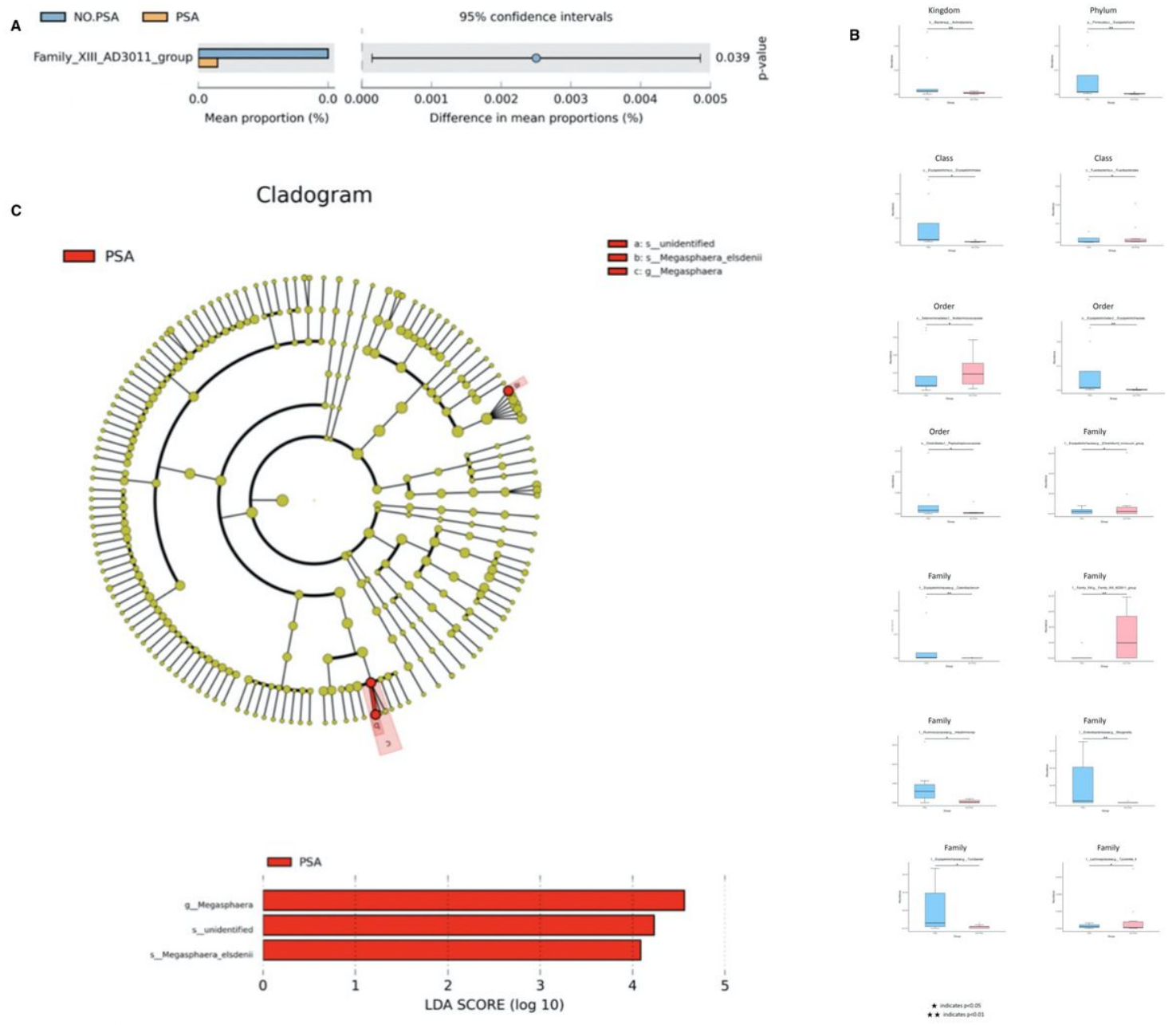


Figure 4

A) Inter-group differences analysis in either the psoriatic arthritis (PSA) or the undifferentiated arthritis (NO PSA) subgroup. Welch's t-test. B) Metagenome Sequencing in either the psoriatic arthritis (PSA) or the undifferentiated arthritis (NO PSA) subgroup. C) Linear discriminant analysis effect size (LEfSe) in the psoriatic arthritis (PSA) subgroup. a. The results show that Family: XIII_AD3011 is significantly higher in NO PSA patients than PSA patients' stool samples (p=0.039). b. Actinobacteria Phylum, Erysipelotrichia Class, Erysipelotrichales Order, Fusobacteriales Order, Acidaminococcaceae Family, Erysipelotrichaceae Family, Peptostreptococcaceae Family, Clostridium innocuum Genus, Catenibacterium Genus, Family XIII_AD3011 Genus, Intestinimonas Genus, Morganella Genus, Turicibacter Genus, and Tyzzerella Genus differ significantly between the PSA and NO PSA subgroups, all $p<0.05$. c. Megasphaera elsdenii in the PSA was 10000 times higher than in the NO PSA group.