Gut microbiome-immune interactions and their role in rheumatoid arthritis development

Rassul Meiram, Zharkyn Jarmukhanov, Nurislam Mukhanbetzhanov and Almagul Kushugulova

Laboratory of Microbiome, Center for Life Sciences, National Laboratory Astana, Nazarbayev University Astana, Kazakhstan

Entire research is based on the previous one: Nurgaziyev, M., Issilbayeva, A., Bersimbaev, R., Ilderbayev, O., Vinogradova, E., Jarmukhanov, Z., Nurgozhina, A., Sergazy, S., Kozhabergen, N., Akhmetova, Z., Meiramova, A., Chulenbayeva, L., Ibrayeva, A., Mukhanbetzhanov, N., Mukhanbetzhanova, Z., Kozhakhmetov, S., Ainabekova, B., Kushugulova, A. (2024). Gut microbiome-immune interactions and their role in rheumatoid arthritis development. PeerJ, 12, e17477. https://doi.org/10.7717/peerj.17477

1. INTRODUCTION

1.1 Background

Millions of people worldwide suffer from **rheumatoid arthritis (RA)**, a chronic autoimmune illness that is characterised by extra-articular involvement and inflammatory arthritis *(Brent LH, et al.*). Rheumatoid arthritis (RA) prevalence in Kazakhstan was expected to be between 0.36% and 0.38% between 2017 and 2019 (*Issilbayeva, A., et al*). Even if the precise origins of RA are still unknown, new study indicates that the gut microbiota is important for the disease's progression. Maintaining general health depends on the **gut microbiome**, a diverse population of bacteria in the human gastrointestinal system. Changes in host gene expression associated with modifications in the gut microbiota may cause aberrant systemic immunity, which in turn may result in autoimmune disorders such as RA *(Zhang, C., et al)*. Some strains can stimulate immune responses, beneficial for individuals with weakened immune systems, while others can suppress the immune response, particularly in RA patients.

1.2 Objectives

The complex connections between the gut microbiota and RA metabolic pathways are examined in this paper. Although our understanding of the relationship between the gut microbiota and RA has advanced, many important mechanisms—particularly the way in which distinct gut microbial communities affect the immune system in RA—remain unknown. By comparing the gut microbiome makeup and variety of Kazakhstani female RA patients to that of healthy persons, this study attempts to close these disparities. We also look into relationships between the metabolic pathways linked to different microbiome profiles. We intend to learn more about these connections in order to develop fresh approaches to treating RA and other autoimmune conditions.

2. METHODS

2.1 Sample collection

Recruitment took place at City Polyclinics No. 10 and No. 12, as well as the Treatment and Diagnostic Center located within the City Multidisciplinary Hospital No. 2 in Astana, Kazakhstan. All RA patients were evaluated by a rheumatologist. The control group included healthy female individuals, matched with the RA patients in terms of age (over 18 years) and ethnicity, and who had no family history of RA or other autoimmune disorders. These healthy controls underwent the same laboratory tests as the RA patients.

2.2 DNA Extraction, and Sequencing

Samples were collected from all study participants, including 25 RA patients and 25 control subjects, following infectious safety protocols. Fecal samples were obtained using the DNA/RNA Shield Fecal Collection Tube (Zymo Research, R1101; Tustin, CA, USA). All biomaterials were labeled according to a coding system.

The study adhered to good laboratory practice (GLP) guidelines for the investigation of the gut microbiome. Genomic DNA extraction from fecal samples was conducted using the ZymoBIOMICS DNA Miniprep Kit (Zymo Research, D4300; Tustin, CA, USA), following the manufacturer's protocol. The quality of the isolated DNA was verified through electrophoresis in a 1% agarose gel.

Finally, the extracted DNA was sequenced to analyze its composition.

2.3 Analyzing Sequencing Data

The 16S amplicon sequencing data, starting from raw reads, were processed into taxon density tables using the LotuS2 pipeline (Less OTU

Scripts 2). This process involved several key steps, including demultiplexing, quality filtering, and dereplication of reads.

2.4 Analysis of Metabolic Pathways

The analysis of metabolic pathways was performed using PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) version 2.5.0, adhering to its default settings. This tool was employed to predict functional metagenomic profiles based on the 16S rRNA sequencing data.

2.5 Statistical Analysis

All statistical analyses were conducted using Python 3.9.16. Operational taxonomic unit (OTU) tables were rarefied. For comparing the control and RA groups, appropriate tests such as the independent T-test, Welch's T-test.

Alpha diversity metrics, including Shannon and Simpson's indices, were calculated, and beta diversity was assessed using Bray-Curtis distance through ANOSIM and PERMANOVA with 9,999 permutations, performed with "scikit-bio 0.5.6." Principal coordinate analysis (PCoA) was used for ordination of between-sample compositions. Differential analysis of taxonomic features was carried out using "LEfSe 1.0.8," with significance determined by an LDA score greater than 2 and p-value 0.05, following the methodology described by Issilbayeva et al. (2024).

3. RESULTS

3.1 The results of gut microbiome sequencing

The results indicated that there were no significant differences in both alpha and beta diversity between the control and RA groups. Specifically, no significant difference was observed in the Shannon index ($p = 0.77$) and the Simpson index ($p = 0.54$) between the two groups (Fig. 1A).

A histogram illustrating the structure of the gut microbiota is presented in Fig. 1B. In the RA group, the most abundant taxa at the genus level were *Ruminococcus*, *Blautia*, and *Bacteroides*. In contrast, the control group was dominated by *Asteroleplasma, Phyllobacterium, and Sphingomonas.*

4. DISCUSSION

The results of the taxonomic analysis showed that the gut microbiota of the healthy control group and RA group slightly differed. The cer-

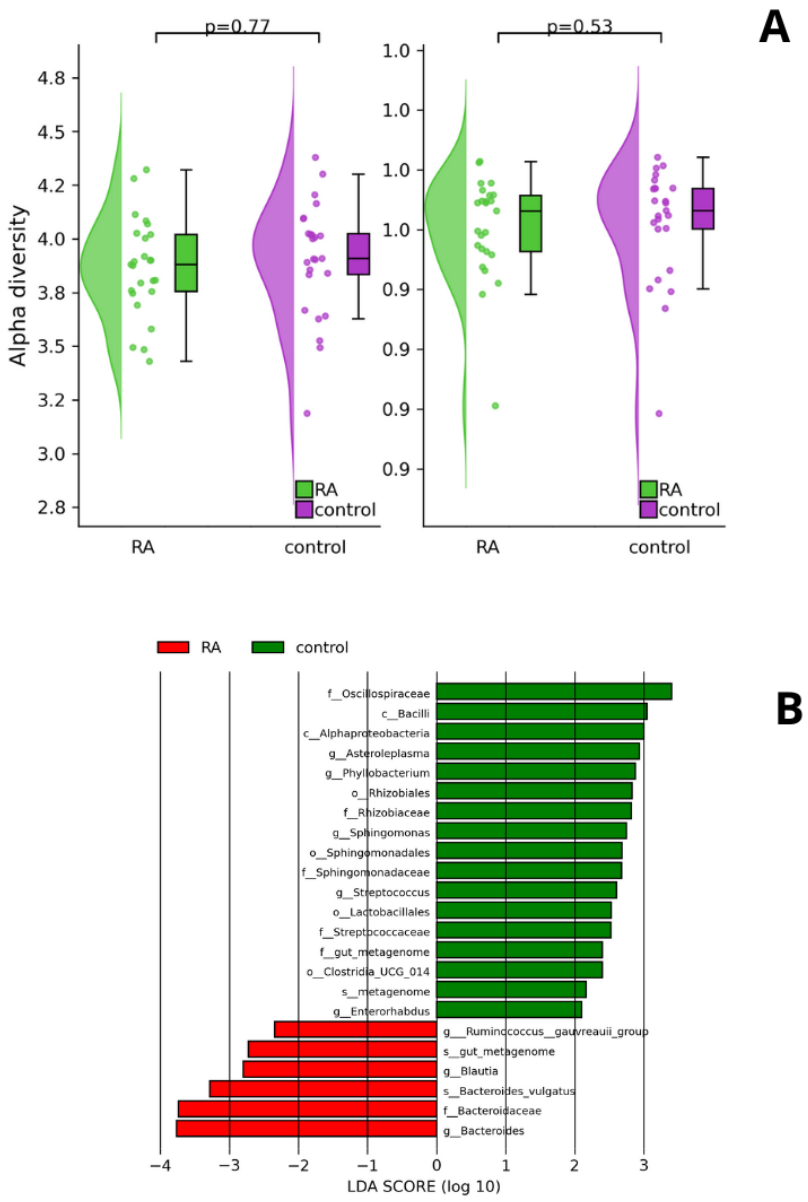


Figure 1. Variation in microbial diversity within the gut microbiome and relative abundance of both study groups. A) Comparing the distribution of alpha diversity in individuals with incident RA and healthy controls. B) ariations in the taxonomic profile among individuals with RA are depicted. A bar plot displaying LDA scores, with each score indicating the magnitude and order of each taxon that exhibits differential abundance

.

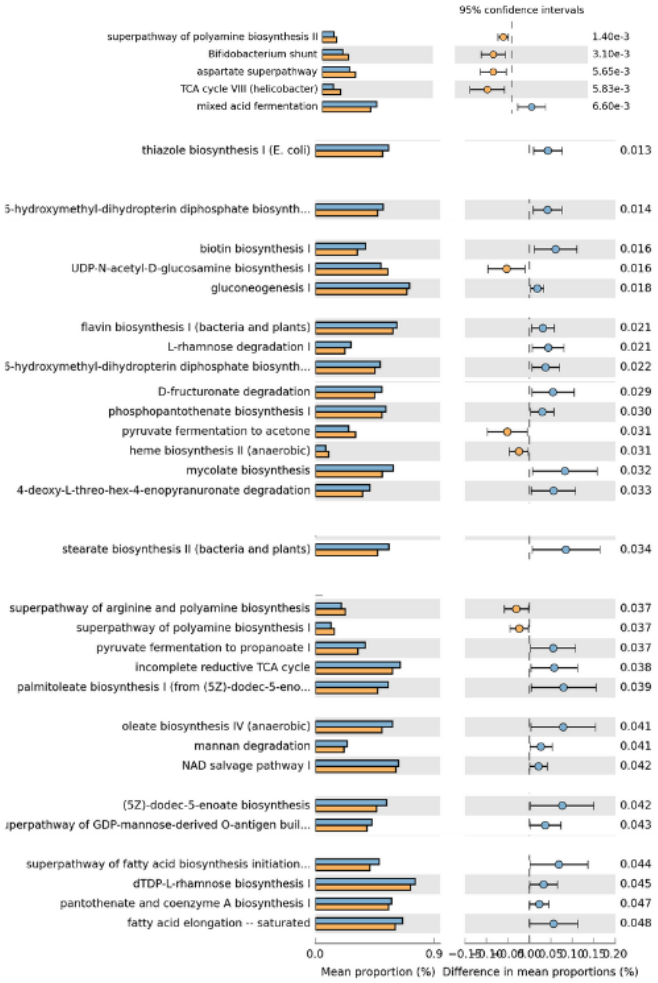


Figure 2. Metabolic pathways.

tain correlations between metabolic pathways were determined. Our findings identified that contrary to Chen et al. (2016) and Chiang et al. (2019b) we did not reveal any significance in a-diversity in subgroups status. Generally, data on taxa relative abundance differ from previous study on the oral microbiome, where a decrease in butyrate and propionate-producing bacteria was observed*(Kozhakhmetov et al., 2023)*.

Metabolic pathways related to Bacteroidaceae are known to influence homeostasis and are linked to proinflammatory cytokines (Vacca et al., 2020). Our findings align with global research, particularly a recent animal study by Liu et al. (2023), which showed an increase in Bacteroidaceae, and Shi et al. (2019), who connected this taxon to atherosclerosis in RA.

According to recent research, Bacteroidaceae may have a negative correlation with lung cancer *(Zhou et al., 2020)*, obesity (Konikoff Gophna, 2016), and inflammatory diseases like RA .According to Yang et al. (2021), Bacteroidaceae may be a potential candidate for probiotics in the future because it produces short-chain fatty acids (SCFAs) like butyrate.

The most predictive pathway was the fermentation pathway: mixed fermentation acid fermentation. It also showed a positive correlation with the most abundant taxon in the RA group (figure 2), Bacteroidaceae, and a negative correlation with the most abundant taxon in the Control group, Bacteroidaceae. This pathway is crucial for the regulation of lactic acid, which has been extensively researched from the standpoint of autoimmunity, especially rheumatoid arthritis.

Our findings on microbiome abnormalities and metabolic pathways may offer a new direction for treating and potentially preventing RA, opening up possibilities for personalized medical approaches. By modifying the gut microbiota through dietary changes or fecal microbiota transplantation, we could influence RA's progression and treatment response. Understanding the role of these microbes in RA could also reveal new therapeutic targets, providing alternative options for patients who do not respond to current treatments.

5. CONCLUSION

Our research has revealed differences in the gut microbiome between RA patients and healthy individuals, with each group displaying distinct microbial patterns. Notably, RA patients had a high abundance of Bacteroidaceae, while the healthy control group showed a prevalence of Oscillospiraceae, each linked to opposing metabolic pathways. These findings open up new avenues for research and the development of precision treatment approaches for RA.

5.1 REFERENCES

Entire research is based on the previous one: Nurgaziyev, M., Issilbayeva, A., Bersimbaev, R., Ilderbayev, O., Vinogradova, E., Jarmukhanov, Z., Nurgozhina, A., Sergazy, S., Kozhabergen, N., Akhmetova, Z., Meiramova, A., Chulenbayeva, L., Ibrayeva, A., Mukhanbetzhanov, N., Mukhanbetzhanova, Z., Kozhakhmetov, S., Ainabekova, B., Kushugulova, A. (2024). Gut microbiome-immune interactions and their role in rheumatoid arthritis development. PeerJ, 12, e17477. https://doi.org/10.7717/peerj.17477

Chen YL, Lin JZ, Mo YQ, Liang JJ, Li QH, Zhou CJ, Wei XN, Ma JD, Yang ZH, Zheng DH, Dai L. 2018. Joint damage is amplified in rheumatoid arthritis patients with positive thyroid autoantibodies. PeerJ 6:e4216 DOI 10.7717/peerj.4216.

Chen J, Wright K, Davis JM, Jeraldo P, Marietta EV, Murray J, Nelson H, Matteson EL, Taneja V. 2016. An expansion of rare lineage intestinal microbes characterizes rheumatoid arthritis. Genome Medicine 8(1):431 DOI 10.1186/s13073-016-0299-7.

Chen X, Zhang D, Sun H, Jiang F, Shen Y, Wei P, Shen X. 2021. Characterization of the gut microbiota in Chinese children with overweight and obesity using 16S rRNA gene sequencing. PeerJ 9(6275):e11439 DOI 10.7717/peerj.11439.

Chiang H, Li J, Liu C, Liu P, Chen H. 2019a. An association of gut microbiota with different phenotypes in Chinese patients with rheumatoid arthritis. Journal of Clinical Medicine 8:1770 DOI 10.3390/jcm8111770.

Chiang HI, Li JR, Liu CC, Liu PY, Chen HH, Chen YM, Lan JL, Chen DY. 2019b. An association of gut microbiota with different phenotypes in Chinese patients with rheumatoid arthritis. Journal of Clinical Medicine 8(11):1770 DOI 10.3390/jcm8111770.

Chen X, Zhang D, Sun H, Jiang F, Shen Y, Wei P, Shen X. 2021. Characterization of the gut microbiota in Chinese children with overweight and obesity using 16S rRNA gene sequencing. PeerJ 9(6275):e11439 DOI 10.7717/peerj.11439.

Chen X, Zhang D, Sun H, Jiang F, Shen Y, Wei P, Shen X. 2021. Characterization of the gut microbiota in Chinese children with overweight and obesity using 16S rRNA gene sequencing. PeerJ 9(6275):e11439 DOI 10.7717/peerj.11439.

Kozhakhmetov S, Babenko D, Issilbayeva A, Nurgaziyev M, Kozhakhmetova S, Meiramova A, Akhmetova Z, Kunz J, Ainabekova B, Marotta F, Kushugulova A. 2023. Oral microbial signature of rheumatoid arthritis in female patients. Journal of Clinical Medicine 12(11):3694 DOI 10.3390/jcm12113694.

Chiang H, Li J, Liu C, Liu P, Chen H. 2019a. An association of gut microbiota with different phenotypes in Chinese patients with rheumatoid arthritis. Journal of Clinical Medicine 8:1770 DOI 10.3390/jcm8111770.

Chiang HI, Li JR, Liu CC, Liu PY, Chen HH, Chen YM, Lan JL, Chen DY. 2019b. An association of gut microbiota with different phenotypes in Chinese patients with rheumatoid arthritis. Journal of Clinical Medicine 8(11):1770 DOI 10.3390/jcm8111770.

Konikoff T, Gophna U. 2016. Oscillospira: a central, enigmatic component of the human gut microbiota. Trends in Microbiology 24(7):523–524 DOI 10.1016/j.tim.2016.02.015.