LETTER TO EDITOR

Alteration of circulating microbiome and its associated regulation role in rheumatoid arthritis: Evidence from integration of multiomics data

To the Editor:

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterized by symmetric polyarthritis and the presence of autoantibodies. Recent studies have shown that blood is not as sterile as previously supposed, and blood microbial dysbiosis is implicated in the pathogenesis of some diseases, but not reported in RA.

Our previously identified RA-associated interferon-inducible gene network has strongly implicated that the circulation microbial dysbiosis probably is served as an important RA-associated environmental factor. To identify the alterations of circulating microbiome associated with RA and its regulation role, we generated and integrated three omics datasets (microbiome, methylome, and transcriptome) from the same subjects, described the composition and richness of blood bacteria between RA cases and controls, identified significant RA-associated taxa, and performed in-depth correlation analysis and causal inference test to evaluate regulation role of the detected bacteria community on the pathogenesis of RA.

A total of 28 female patients and 15 age- and sex-matched controls (Table S1) were enrolled according to several steps detailed in the Supporting Information. We determined blood microbiome by bacterial 16S ribosomal DNA sequencing. The 1352 OTUs detected could be classified into 12 phyla, 26 classes, 54 orders, 115 families, and 216 genera (Table S2). Among the 10 known phyla, Proteobacteria, Actinobacteria, Bacteroidetes, Candidatus Saccharibacteria, and Firmicutes have average relative abundances of 77.56% (predominated), 13.23%, 3.98%, 3.05%, and 1.28%, respectively (Table S3). This result was consistent with previous findings from healthy individuals.

No significant α-diversity was detected between the two groups (Figure 1A), but significant major separation in β-diversity was observed between RA patients and the controls (Figure 1B). Compared to the controls, the microbiome of RA blood showed significantly higher mean distances in PCoA1 axis, but no significant difference in PCoA2 axis (Figure 1C).

Linear discriminant analysis effect size (LEfSe) algorithm was used to compare bacterial abundances at different taxonomic levels (five phyla, 14 classes, 37 orders, 97 families, and 196 genera) (Figure 1D). Some bacterial taxa (two phyla, three classes, six orders, 10 families, and 13 genera) have significant differences in abundance between the RA cases and controls (Table S4), for example, Bacteroidetes (0.0126 ± 0.0200 vs 0.0669 ± 0.0341, FDR q-value = 3.51 × 10^{-7}) and Candidatus Saccharibacteria (0.0605 ± 0.1137 vs 0.0006 ± 0.0023, FDR q-value = 1.08 × 10^{-4}) (Table S3). When compared our results with the findings from other studies focused on gut and synovial fluid microbiome, both consistent and inconsistent results exist. For example, the lower abundance of Bacteroidetes in the blood was consistent with that in the synovial fluid, but inconsistent with that in the gut of RA patients. Proteobacteria are one of the most abundant phyla in human microbiota. The proportion of Proteobacteria in the synovial fluid of RA patients was higher than that of controls. Our results consistently suggested that the prevalence of genera Pelagibacterium, Halomonas, Aureimonas, Chelativorans, and others that belong to the Proteobacteria phylum was higher in RA than the controls.

These results taken together showed that alterations of circulating microbiome were associated with RA. In addition, the differences in microbiomes of RA patients and controls suggested some bacteria-derived functions, including cellular processes, environmental information processing, genetic information processing (transcription and translation), and metabolism (Table S5).

Peripheral blood mononuclear cells (PBMCs) containing monocytes and lymphoid cells represent circulating mixture cells directly involved in autoimmunity and chronic inflammation. It is intuitively inferred that PBMCs...
and circulating microbiota live in the same environment of peripheral blood, and they can directly contact and have potential effective interactions.9,10 Therefore, we generated the mRNA expressions and DNA methylation data in PBMCs from the same sample set by using the Human Gene Expression Microarray V.4.0 (CaptiveBio, Beijing, China) and Illumina 450 K Infinium Methylation BeadChip (Illumina, Inc., USA), respectively.

To detect the potential regulation roles of the identified microbial dysbiosis, we integratively analyzed the three omics data (microbiome, methylome, and transcriptome). We first performed differential expression analyses, and a total of 47 DNA methylations (\(|\Delta \beta| > 0.05\) and \(P < 5.0 \times 10^{-4}\)) and 749 mRNAs (fold change > 2, \(P < 5.0 \times 10^{-4}\)) were selected. As expected, we detected significant correlations between seven taxa within Proteobacteria phylum and DNA methylation (Table S6), and the DNA methylation site cg00959259 in PARP9 was highlighted as its consistent associations with three taxa of order Rhizobiales, family Hyphomicrobiaceae, and genus Pelagibacterium, with spearman correlation coefficients of \(-0.6070 (P = 1.66 \times 10^{-3})\), \(-0.5539 (P = 4.98 \times 10^{-3})\), and \(-0.5626 (P = 4.21 \times 10^{-3})\), respectively (Figure 2).

For mRNA expression, the microbial abundances in order Rhizobiales, family Hyphomicrobiaceae, and genus Pelagibacterium were significantly associated with PARP9 mRNA level, with spearman correlation coefficients of \(0.6040 (P = 1.77 \times 10^{-3})\), \(0.6632 (P = 4.12 \times 10^{-4})\), and \(0.6549 (P = 5.15 \times 10^{-4})\), respectively (Figure 2; Table S7). Through causal inference test, we detected causal effects of order Rhizobiales, family Hyphomicrobiaceae, and genus Pelagibacterium, and the mediation effect of PARP9 mRNA expression on RA risk (Table S8). Previously, we had shown that lower cg00959259 methylation level and higher

---

**FIGURE 1** Comparison of the α-diversity and β-diversity of the blood microbiome in the rheumatoid arthritis (RA) cases and controls. A, We measured the α-diversity of bacterial community in each group using three indices (e.g., Chao1, Shannon, and Simpson), which considered both community richness and evenness. B, ANOSIM test (\(P = 0.001\)) for the within-group and between-group variance. C, Principal coordinates analysis (PCoA) directly and completely present bacterial flora distribution between communities. PCoA plot generated based on Bray-Curtis distances. The x- and y-axes indicate the first and second coordinates, respectively, and the values in parentheses show the percentages of the community variation explained. D, The inner circle represents the classification level from phylum to genus. Each small circle at a different classification level represents a classification at that level, and the diameter of the small circle represents the relative abundance. The significantly different species are colored according to the groups. The red nodes represent the microbial groups enriched in the RA group and the green nodes represent the microbial groups enriched in the control group. The middle layer is the heatmap of the mean abundance. The darker the color, the higher it is; the outermost layer is the species annotation.
Figure 2 Associations between blood bacteria and methylation and mRNA levels of PARP9 in peripheral blood mononuclear cells (PBMCs). A, Heatmap of Spearman correlations between blood bacteria and methylation and mRNA levels of PARP9 in PBMCs. Numbers in the grids are Spearman correlation coefficients (* P < .05, ** P < .01, *** P < .001). B, Scatter plots show the relationships of order Rhizobiales, family Hyphomicrobiaceae, and genus Pelagibacterium with DNA methylation of CpG site cg00959259 and PARP9 mRNA levels in PBMCs. These three taxa belong to the Proteobacteria phylum, family Hyphomicrobiaceae belongs to order Rhizobiales, and genus Pelagibacterium belongs to family Hyphomicrobiaceae.

PARP9 mRNA expression level in PBMCs were associated with higher RA risk. Therefore, by combining the current results with our previously reported results, it seems that blood bacteria within the Proteobacteria phylum may increase RA risk through demethylation of PARP9, which may lead to higher expression of the PARP9 gene in PBMCs. These observations suggested that the identified microbiome alterations may have regulatory effects on gene expressions.

In summary, this was the first study identifying significant alterations in blood microbiome in RA, detecting significant associations between blood bacteria and gene expressions in PBMCs, and highlighting the regulatory effects of circulating bacterial community on RA risk through the mediation of the expression of PARP9. These findings enhanced our understanding of the roles of circulating microbiome in the pathology of RA. These results suggested that the detection of imbalances in microbial composition could facilitate early diagnosis of RA, and bacterial stimuli or control could be developed as useful microbiome-based strategies for RA prevention and treatment.

Acknowledgments
The study was supported by Natural Science Foundation of China (81872681, 81473046, and 31401079), Science and Technology Project of Suzhou: SS202050, Natural Science Fund for Colleges and Universities in Jiangsu Province (18KJB330005), and a Project of the Priority Academic Program Development of Jiangsu Higher Education Institutions.

Conflict of Interest
The authors declare that there is no conflict of interest.

Ethics Statement
The study protocol was approved by ethical committees of Soochow University. All study participants provided their written consent for participation in the study.

Author Contributions
Xing-Bo Mo, Chen-Yue Dong, Pei He, Long-Fei Wu, and Xin Lu recruited the patients and conducted the experiments. Xing-Bo Mo, Chen-Yue Dong, and Yong-Hong Zhang drafted and revised the manuscript. Xing-Bo Mo...
and Chen-Yue Dong analyzed the data. Hong-Wen Deng, Fei-Yan Deng, and Shu-Feng Lei designed and supervised the study, and revised the manuscript. All the authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Xing-Bo Mo1,2  
Chen-Yue Dong1,2  
Pei He1,2  
Long-Fei Wu1,2  
Xin Lu1,2  
Yong-Hong Zhang2,3  
Hong-Wen Deng4  
Fei-Yan Deng1,2  
Shu-Feng Lei1,2

1 Center for Genetic Epidemiology and Genomics, School of Public Health, Medical College of Soochow University, Suzhou, Jiangsu, P. R. China  
2 Jiangsu Key Laboratory of Preventive and Translational Medicine for Geriatric Diseases, Soochow University, Suzhou, Jiangsu, P. R. China  
3 Department of Epidemiology, School of Public Health, Medical College of Soochow University, Suzhou, Jiangsu, P. R. China  
4 Center of Bioinformatics and Genomics, Department of Global Biostatistics and Data Science, Tulane University, New Orleans, Louisiana

Correspondence
Shu-Feng Lei and Fei-Yan Deng, Center for Genetic Epidemiology and Genomics, School of Public Health, Medical College of Soochow University, Suzhou, Jiangsu 215123, P. R. China.
Email: leisf@suda.edu.cn; fdeng@suda.edu.cn

Xing-Bo Mo and Chen-Yue Dong contributed equally to this work.

ORCID
Xing-Bo Mo https://orcid.org/0000-0001-8956-4918

REFERENCES
1. Paisse S, Valle C, Servant F, et al. Comprehensive description of blood microbiome from healthy donors assessed by 16S targeted metagenomic sequencing. Transfusion. 2016;56:1138-1147.
2. Potgieter M, Bester J, Kell DB, Pretorius E. The dormant blood microbiome in chronic, inflammatory diseases. FEMS Microbiol Rev. 2015;39:567-591.
3. Shah NB, Allegretti AS, Nigwekar SU, et al. Blood microbiome profile in CKD: a pilot study. Clin J Am Soc Nephrol. 2019;14:692-701.
4. Zhu H, Wu LF, Mo XB, et al. Rheumatoid arthritis-associated DNA methylation sites in peripheral blood mononuclear cells. Ann Rheum Dis. 2019;78:36-42.
5. Hughes JB, Hellmann JJ, Ricketts TH, Bohannan BJ. Counting the uncountable: statistical approaches to estimating microbial diversity. Appl Environ Microbiol. 2001;67:4399-4406.
6. Segata N, Izard J, Waldron L, et al. Metagenomic biomarker discovery and explanation. Genome Biol. 2011;12:R60.
7. Hammad DBM, Liyanapathirana V, Tonge DP. Molecular characterisation of the synovial fluid microbiome in rheumatoid arthritis patients and healthy control subjects. PLoS One. 2019;14:e0225110.
8. Sun Y, Chen Q, Lin P, et al. Characteristics of gut microbiota in patients with rheumatoid arthritis in Shanghai, China. Front Cell Infect Microbiol. 2019;9:369.
9. Tammen SA, Friso S, Choi SW. Epigenetics: the link between nature and nurture. Mol Aspects Med. 2013;34:753-764.
10. Takahashi K. Influence of bacteria on epigenetic gene control. Cell Mol Life Sci. 2014;71:1045-1054.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of the article.