Causal relationship between gut microbiota and rheumatoid arthritis: a two-sample Mendelian randomisation study

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Abstract

Objective

To assess whether there is a bidirectional causal relationship between the composition of gut microbiota and rheumatoid arthritis (RA), and to identify specific pathogenic bacterial taxa via the Mendelian randomisation (MR) analysis.

Methods

We acquired single nucleotide polymorphisms (SNPs) associated with the composition of gut microbiota (n=18,340) and with RA (n=331,313) from publicly available genome-wide association studies (GWAS). The genome-wide threshold was 1×10^{-5} in the forward MR analysis and was 5×10^{-8} in the reverse MR analysis. Inverse variance weighted (IVW) was the main method to analyse causality, and MR results were verified by several sensitivity analyses including weighted median, MR Egger, and MR Pleiotropy Residual Sum and Outlier (PRESSO).

Results

The IVW method suggested that eight taxa were positively correlated with RA, including: MollicutesRF9 (p_{IVW} <0.01), Alphaproteobacteria (p_{IVW} <0.01), Betaproteobacteria (p_{IVW} =0.04), Bacteroidaceae (p_{IVW} <0.01), Adlercreutzia (p_{IVW} <0.01), Bacteroides (p_{IVW} <0.01), Butyricimonas (p_{IVW} =0.03) and Holdemanella (p_{IVW} =0.03). Six bacterial taxa were negatively correlated with RA, including Desulfovibrionales (p_{IVW} = 0.01), Methanobacteriales (p_{IVW} <0.01), Methanobacteria (P_{IVW} <0.01), Desulfovibrionaceae (p_{IVW} <0.01), Methanobacteriaceae (p_{IVW} <0.01) and Butyrivibrio (p_{IVW} =0.02). Heterogeneity (p>0.05) and pleiotropy (p>0.05) analysis confirmed the robustness of the MR results.

Conclusion

We identified some specific bacterial taxa that were causally associated with the risk of RA, providing new insights into prevention and diagnosis of RA.

Key words

rheumatoid arthritis, gut microbiota, Mendelian randomisation analysis, causality

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Received on May 5, 2023; accepted in revised form on July 24, 2023.

Funding: this work was supported by the National Natural Scientific Foundation of China (81922059, 82273753); the Natural Science Basic Research Plan in Shaanxi Province of China (2021JCW-08).

Competing interests: none declared.

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disorder, characterised by persistent synovitis and joint damage (1). According to a systematic analysis of the Global Burden of Disease (GBD) study 2017, the age-standardised point prevalence and annual incidence rate of RA were 246.6 per 100,000 and 14.9 per 100,000, which respectively increased by 7.4% and 8.2% from 1990, higher in females and increased with age (2). Currently, about 18 million individuals live with RA, contributing to nearly 2.4 million years lived with disability (YLDs), according to a recent analysis of GBD 2019 data (3). RA patients have a higher risk of osteoporosis and cardiovascular disease when compared to non-RA individuals, severely impairing their physical function and quality of life (4). Although methotrexate (MTX) is the cornerstone in the treatment of RA, it has side effects such as hematologic toxicity (5). Therefore, it is essential to explore the potential causal risk factors of RA to promote the current therapies.

The pathogenesis of RA involves different components of the immune system. The close interaction between cells and mediators of the immune system leads to the development of the inflammation (6). The aetiopathogenesis of RA is multifactorial and complex, involving genetic and environmental factors, such as the major histocompatibility complex (MHC) genes, smoking, and infection (7). In particular, microbial infection has been identified as a crucial inducer of RA (8). Experiments conducted on animal models showed that the gut microbiota affects both local and systemic immunity (9). Complex interactions between the host immune system and microbiota are necessary to maintain intestinal homeostasis (10). However, alterations in bacterial function and diversity can disrupt the mutualistic relationship between the host and the microbiota, leading to dysbiosis (11).

Recently, the link between the gut microbiota composition and RA risk has attracted global attention. It was reported that alterations in the gut microbiota affects the development of RA, and dysbiosis presents in all stages of RA (8). Individuals with high RA risk have a distinct gut microbiota environment, including *Bifidobacteriaceae*, *Lachnospiraceae*, *Helicobacteraceae*, and so on (12). Moreover, abundance of *Prevotella copri* was higher in untreated new-onset RA patients, which may induce inflammation in genetically susceptible host (13). Similarly, a previous study indicated that compared with controls, *Bacteroides* and *Escherichia-Shigella* were more abundant in RA patients (14). Nevertheless, the causality between gut microbiota and RA remains to be elucidated.

Establishing causality in observational studies is difficult due to the challenges in detecting, measuring, and adjusting for confounding factors (15). Although randomised controlled trials (RCTs) are the gold standard for demonstrating causality, ethical considerations often make it difficult to conduct (16). Mendelian randomisation (MR) offers a solution by utilising genetic variants as instrumental variables (IVs) to statistically evaluate causality between exposures and outcomes (17). Because Mendel's second law ensures a random combination of genetic variants during meiosis production, the genetic variants selected are typically not associated with confounders (18). With the adding number of genome-wide association studies (GWASs) of gut microbiota and RA, large-scale summary statistics become more widely available (19, 20). MR is becoming an increasingly powerful method for solving problems in epidemiology and human biology.

In this study, we estimated genetic correlations between gut microbiota and RA, and conducted bidirectional two-sample MR analysis to investigate causal relationships between gut microbiota and RA.

Materials and methods

Ethics statement

Summary statistics of GWASs which regarding gut microbiota and RA were obtained from previous studies, and all studies received approval from their respective institutional review boards (IRBs). Figure 1 presents a flowchart of the study procedure.

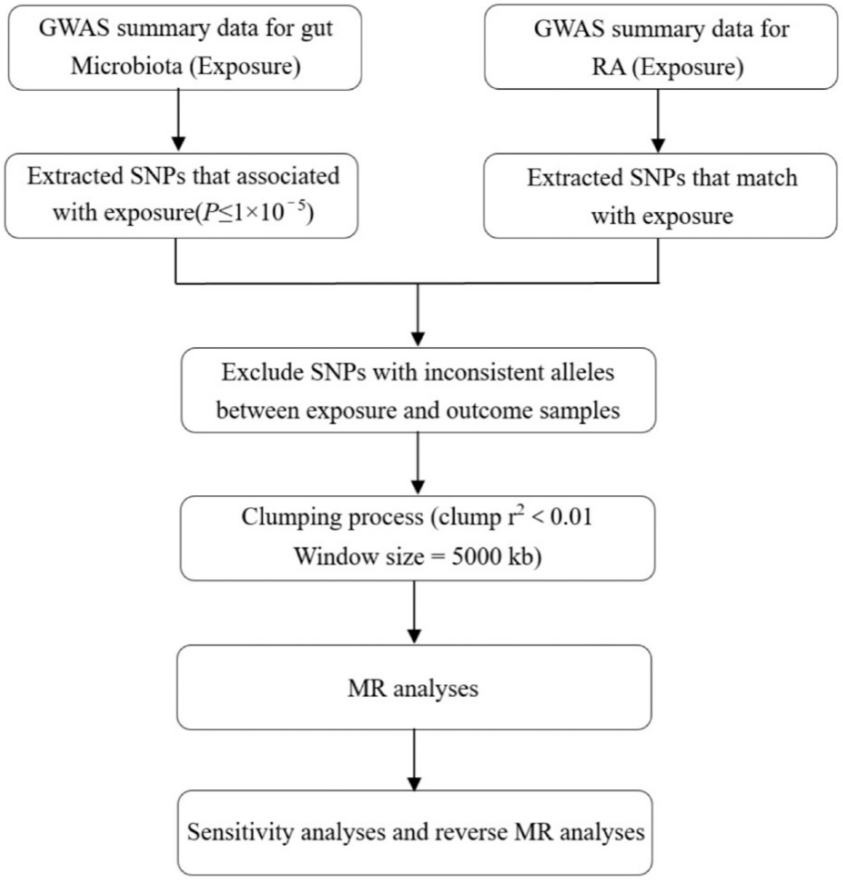

Fig. 1. The flowchart of the study.
GWAS: genome-wide association study; RA: rheumatoid arthritis; SNPs: single nucleotide polymorphisms; MR: Mendelian randomisation.

GWAS summary data of gut microbiota

Genetic instruments of gut microbiota were obtained from a large-scale GWAS meta-analysis involving 18,340 participants from 24 cohorts, which were conducted in various countries including the United States, Canada, Israel, South Korea, Germany, Denmark, the Netherlands, Belgium, Sweden, Finland, and the United Kingdom (20). The datasets were rarefied to 10,000 reads per sample to account for differences in sequencing depth, and taxonomic classification was performed via direct taxonomic. The microbiome quantitative trait loci (mbQTL) mapping analysis was performed for each cohort, which included only the taxa present in more than 10% of the samples. The results showed that the mbQTL analysis contained 211 taxa, including 131 genera, 35 families, 20 orders, 16 classes, and 9 phyla. After adjusting for age, sex, technical covariates and genetic principal components, Spearman's correlation analysis was performed to determine the genetic loci affecting the abundance of bacterial taxa. More detailed information about sample collection, sequencing, microbiome trait preparation, and genotyping analysis was described elsewhere (20).

GWAS summary data of rheumatoid arthritis

The summary-level statistics for RA were obtained from the largest GWAS to date on RA, including 331,313 RA patients and approximately 1 million controls which were from several cohorts including Sweden, Denmark, Iceland, Norway, UK Biobank, and FinnGen (21). Genotyping of all cohorts except UK Biobank and FinnGen was performed by Illumina technology at deCODE genetics, and the sequence variants for imputation were identified through whole-genome sequencing of 67,645 individuals. Logistic regression analysis was conducted to test the association between 64 million sequence variants and RA (22). The sequence variants were classified into five groups based on their genome annotation (23). The summary-level statistics of the study were openly available at: www.decode.com/summarydata/

Selection of instrumental variables

211 bacterial groups were examined at six taxonomic levels to accurately identify pathogenic bacterial taxa. Single nucleotide polymorphisms (SNPs) that were associated with gut bacterial taxa but not with RA and confounders were selected as IVs in forward MR analysis, using a genome-wide significance threshold of 1×10^{-5}. For reverse MR analysis, a threshold of 5×10^{-8} was used. To ensure the quality of IVs, two steps were implemented. First, SNPs with incongruent alleles between exposure and outcome samples were excluded. Second, the existence of strongly linked disequilibrium (LD) may lead to biased results (24), so the independent genetic variables with no LD to other genetic variables were selected. SNPs in each bacterial taxon was conducted by the clumping process to retain independent SNPs. LD was estimated using the European 1000 Genomes Project reference panel, and the LD threshold for clumping was set to $r^2 < 0.01$, with a clumping window size of 5000 kb.

Bidirectional MR analysis

MR analysis is a method that uses genetic factors to evaluate whether a trait will increase the risk of disease, while avoiding the influence of reverse causality and confounding. After qualifying SNPs were selected, we conducted a MR analysis to estimate the causal effect of gut microbiota on RA. The inverse variance weighting (IVW) test is a meta-analysis method that uses a weighted regression of SNP-outcome effects on SNP-exposure effects to obtain an overall estimate of the impact of the gut microbiota on RA risk, where the intercept limit is zero (25). When there is no horizontal pleiotropy, IVW can avoid the influence of confounding factors and obtain an unbiased estimate (24). For bacterial genera with multiple SNPs, we used IVW to provide a more

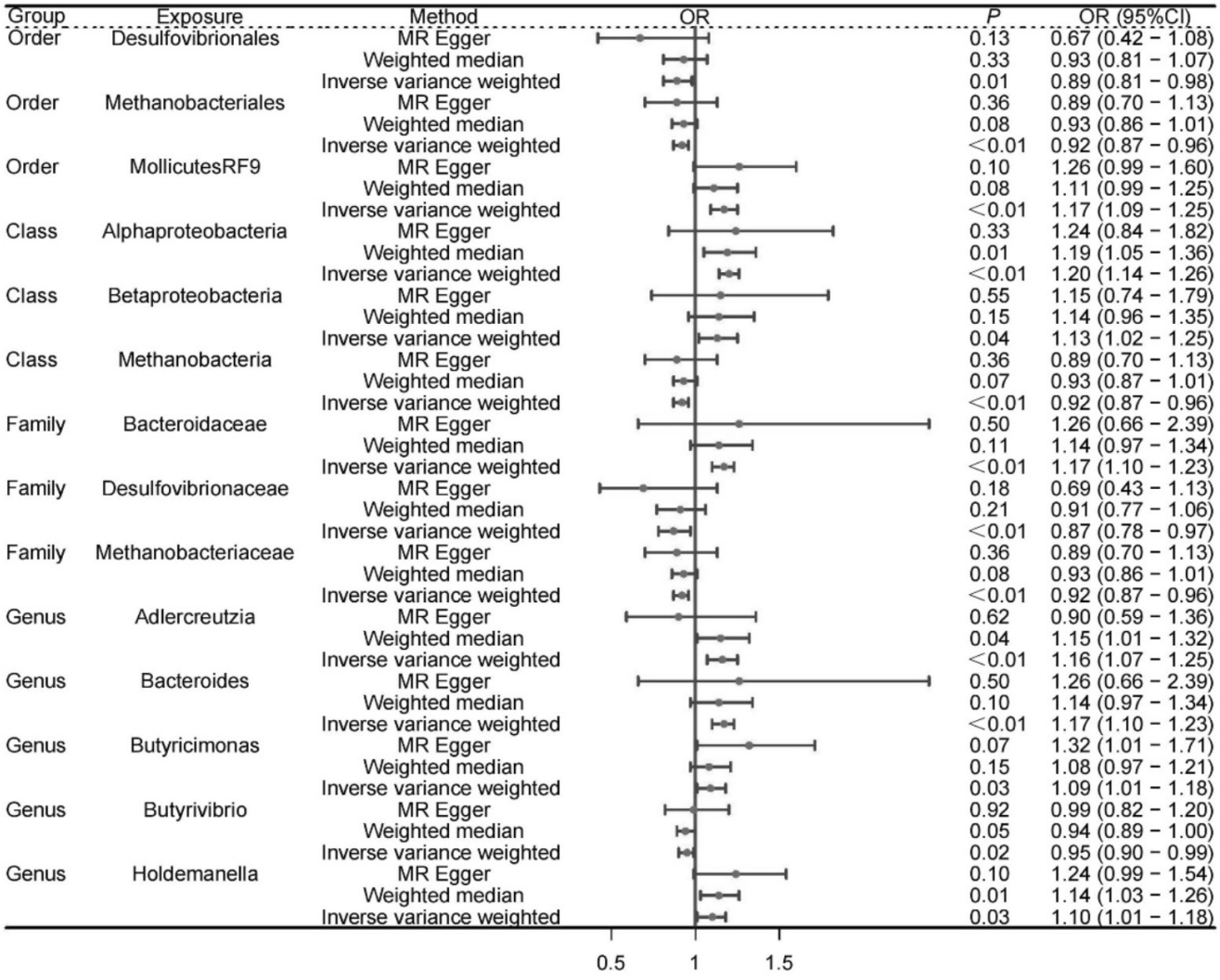

Fig. 2. Forest plot for causal association between bacterial taxa and RA.
OR: odds ratio; CI: confidence interval; $p<0.05$ is the nominal significance.

conservative but robust estimate (26). Although known confounding SNPs were excluded, there were still many unknown confounding factors which lead to pleiotropy and biased effect size estimates. Therefore, two alternative tests were used to cross-validate the reliability and stability of the IVW results, namely MR Egger regression (27) and weighted median estimation (WME) (28).

To ensure the reliability and stability of the significant results, we performed a series of sensitivity analyses. First, MR Egger regression and MR pleiotropy residual sum and outlier (MR-PRESSO) test (29) were used for detecting potential horizontal pleiotropy. The presence of pleiotropy was measured by the *p*-value of the pleiotropy test. If *p*-value >0.05, it is considered that the possibility of pleiotropy in the causal effect is weak, and its influence could be ignored. The MR-PRESSO test evaluated overall horizontal pleiotropy by comparing the observed distance of all SNPs to the regression line (sum of residual squares) with the expected distance under the null hypothesis without horizontal pleiotropy (29). Second, WME was implemented for individual genetic variants, which was more robust with strong outlying causal estimates (15). Finally, we analyzed the potential heterogeneity by means of MR-Egger regression and Q test in IVW test. *P* value >0.05 indicated that there was no heterogeneity in the causal effect.

To explore whether RA has any causal impact on identified significant bacterial taxa, we performed a reverse MR analysis with using RA-related SNPs as IVs. All statistical analyses were performed using R software (v. 4.2.1). The Two Sample MR package was used for IVW, WME and MR-Egger regression methods, while the MR-PRESSO package was used for MR-PRESSO test.

Results

Selection of instrumental variables

Data of gut microbiota consisted of 211 bacterial traits, which were classified into five biological categories: phylum, class, order, family, and genus. After excluding SNPs that had LD effects and were independent from RA, a MR analysis of IVs revealed that 135 independent SNPs in specific bacterial taxa were associated with RA. The main information of SNPs including beta, SE, OR and *p*-value were collected systematically for further analysis.

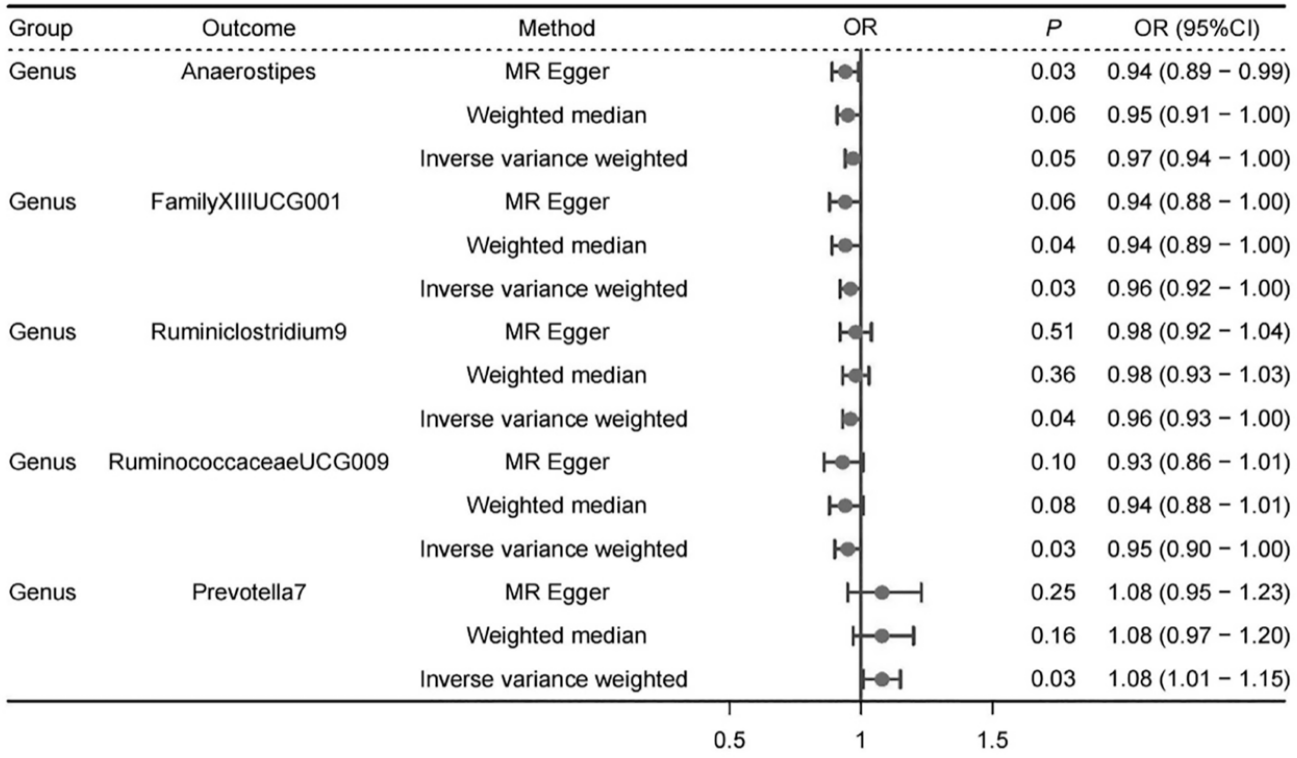

Fig. 3. Forest plot for reverse-direction Mendelian Randomisation analysis
OR: odds ratio; CI: confidence interval; $p<0.05$ is the nominal significance.

Causal effects between gut microbiota and rheumatoid arthritis

Figure 2 presented the results of the forward MR analysis. Fourteen bacterial taxa were found to be associated with RA. Eight of them were positively correlated with RA, including Order *MollicutesRF9* (OR=1.17; p_{IVW} <0.01), Class *Alphaproteobacteria* (OR=1.20; p_{IVW} <0.01), Class *Betaproteobacteria* (OR=1.13; p_{IVW} =0.04), Family *Bacteroidaceae* (OR=1.17; p_{IVW} <0.01), Genus *Adlercreutzia* (OR=1.16; p_{IVW} <0.01), Genus *Bacteroides* (OR=1.17; p_{IVW} <0.01), Genus *Butyricimonas* (OR=1.09; p_{IVW} =0.03), and Genus *Holdemanella* (OR=1.10; p_{IVW} =0.03).

On the other hand, six bacterial taxa were negatively correlated with RA, including Order *Desulfovibrionales* (OR=0.89; p_{IVW} =0.01), Order *Methanobacteriales* (OR=0.92; p_{IVW} <0.01), Class *Methanobacteria* (OR=0.92; p_{IVW} <0.01), Family *Desulfovibrionaceae* (OR = 0.87; p_{IVW} <0.01), Family *Methanobacteriaceae* (OR=0.92; p_{IVW} <0.01), and Genus *Butyrivibrio* (OR=0.95; p_{IVW} =0.02). Supplementary Table S1 provides information of the IVs used in the forward MR analysis, and it demonstrates that the associations were almost consistent in the WME analysis. It revealed that the associations in *Alphaproteobacteria* (p_{WME} =0.01), *Adlercreutzia* (P_{WME} =0.04), *Butyrivibrio* (p_{WME} =0.05) and *Holdemanella* (p_{WME} =0.01) were consistent with IVW ($p<0.05$).

The results of the reverse MR analysis, as shown in Figure 3, suggested no evidence of a causal effect from RA to the identified bacterial traits in forward MR analysis. But it suggested that RA might influence bacterial taxa including Genus *Anaerostipes* (OR=0.97; p_{IVW} =0.05), Genus *FamilyXIIIUCG001* (OR=0.96; p_{IVW} =0.03), Genus *Ruminiclostridium9* (OR=0.96; p_{IVW} =0.04), Genus *RuminococcaceaeUCG009* (OR=0.95; p_{IVW} =0.03), and Genus *Prevotella7* (OR=1.08; p_{IVW} =0.03). Details of the IVs used in reverse MR analysis are shown in Supplementary Table S2.

Sensitivity analyses

As shown in Supplementary Tables S1 and S2, the sensitivity analyses results indicated that there was no evidence of horizontal pleiotropy based on the MR-PRESSO test and the MR-Egger regression (both $p>0.05$). The analyses based on the IVW and MR-Egger regression showed that no heterogeneity was detected (both $p>0.05$).

Discussion

In this bidirectional MR study, we found 8 bacterial taxa such as *Alphaproteobacteria* that exhibited protective causal effects on the pathogenesis of RA. Conversely, we found negative association of 6 bacterial taxa such as *Methanobacteria*. Reverse MR revealed no evidence of causal effect of RA on the above bacteria.

Gut commensal bacteria play a crucial role in establishing a regular innate immune system (30). As the frontline of intestinal mucosal defense, innate immune cells in gut-associated lymphoid tissues (GALTs) have a primary function of recognizing pathogens and initiating an innate immune response (30). Pattern recognition receptor-microbe-associated molecular patterns (PRR-MAMP) lead to activation of a variety of signaling pathways which are critical for promoting mucosal barrier function and protecting the host from invading pathogens (31). Short-chain fatty acids

(SCFAs), metabolites of gut microbiota, play a role in regulating the immune system and inflammatory response (32). Gut microbiota shapes the structural development of GALTs and initiates their immune response via PRR-PAMP recognition and epigenetic modulators like SCFAs, promoting host defense (30).

Gut microbiota may have a regulatory role in the adaptive immune system of RA through lymphocyte subpopulations and cytokines (33). Immunological analysis of RA revealed various changes in T cell phenotype and function, such as an imbalance of T helper cell 17, regulatory cells, and helper innate lymphoid cells in peripheral blood (34). Toll-like receptors (TLRs) are essential pattern recognition receptors for pathogen-associated molecular patterns of microbiota and can be impaired by dysbiosis (35). A previous study revealed that TLR-2 and TLR-4 are the most important receptors in RA mediated by microbiota (35).

Recently, numerous studies found that RA is often accompanied by alterations in gut microbiota composition. Phylum *Proteobacteria*, which includes the Class *Alphaproteobacteria* and *Betaproteobacteria*, was more abundant in RA patients (36). This finding is consistent with our results, suggesting that the increased relative abundance of *Alphaproteobacteria* and *Betaproteobacteria* is causally associated with a higher risk of RA. Specifically, *Alphaproteobacteria* can directly combine with angiogenin, resulting in lethal damage to the integrity of the bacterial membrane and consequently promoted the dysbiosis (37).

Bacteroidaceae is an important bacterial taxon in the human body and some studies suggest its significant role in the pathogenesis of RA (14, 38, 39). The abundance of *Bacteroidaceae* was higher in collagen-induced arthritis (CIA) mice (38). Furthermore, RA patients were reported to have a higher abundance of *Bacteroides* (14). Our results align with these findings, indicating that the increased relative abundance of *Bacteroidaceae* and *Bacteroides* is causally associated with a higher risk of RA. Their integrase antigen promotes the recruitment and proliferation of CD8+ T cells in GALTs (39). However, one study contradicted our results which showed *Bacteroides* may be a protective factor in RA (36).

The abundance of *Adlercreutzia* was significantly higher in CIA mice models (40). A previous case-control study reported a positive correlation between *Adlercreutzia* and RA (41), which is consistent with our results. Our study suggested that *Adlercreutzia* is a detrimental factor in RA pathogenesis. High levels of *Adlercreutzia* are correlated with inflammation, as well as reduced circulating levels of non-essential amino acids that can promote bone health (42).

Our results showed that, compared with healthy controls, RA patients had higher relative abundances of *Butyricimonas* and *Holdemanella*, indicating their deleterious impact on RA. Some evidence supported our results. The gut microbiota at mucosal sites in RA may facilitate autoimmunity (43). Filamin A is targeted by T and B cell responses in 56% of RA patients, and it shares epitopes with proteins of *Butyricimonas* (43). This sequence homology may provide a connection between *Butyricimonas* and mucosal immunity in RA patients (43). One previous study found that *Holdemanella* may produce 3-hydroxyoctadecanoic acid, which is able to promote the anti-inflammatory properties of probiotics (44).

Our study demonstrated a causal relationship between specific bacterial taxa and RA, providing some new insights for RA treatment. Firstly, the identified bacterial taxa are informative and valuable when predicting patients' response to RA medicine. Gut microbiota can have a role in medicine metabolism. For example, a recent study revealed that oral methotrexate (MTX), a type of anti-rheumatic drug, can be metabolised by gut microbiota (45). MTX is able to alleviate joint oedema in mice by increasing the abundance of bacterial taxa such as *Bacteroides* and *Butyricimonas* (46). In addition, some bacterial taxa like *Romboutsia*, *Lactobacillus*, and *Adlercreutzia* can generate metabolites such as *O*-desmethylangolensin and SCFAs, which are necessary for *Lycium barbarum* polysaccharide in alleviating RA (47). Those bacterial taxa can possibly become indicators of certain RA medicine's effects. Secondly, the identified RA-related bacterial taxa are potential treatment target of RA. Antibiotics have been an integral part of traditional RA therapies, but the ability of antibiotics is limited and non-specific with a possible effect of gut dysbiosis (48). Probiotics are able to target specific bacterial taxa and reshape the equilibrium, providing new strategy for RA treatment (48). A recent trial found that *Lactobacillus casei* can improve disease activity of RA patients by reducing the inflammatory markers and cytokines levels (49). However, extensive efforts are still needed to improve the usage of probiotics in RA treatment.

Previous studies have reported a causal relationship between the gut microbiota and RA through MR analysis (50, 51). However, they did not distinguish the impact of specific bacterial taxa on human health. Besides, another study failed to find any causal effect between specific bacterial taxa and RA through MR analysis, possibly due to their small sample size including only 14,361 RA cases and 43,923 controls (52).

The main advantage of our study is that the implementation of the MR analysis reduced the interference from confounding factors and reverse causality, making it more convincing than observational studies. Besides, we used summary statistics of large-scale GWAS with exposure data from 18,340 individuals and outcome data from 31,313 RA cases. Large sample size and a series of sensitive analyses ensured the robustness and accuracy of the results. Nevertheless, our study also has several limitations. First, the majority of participants in the GWAS data were of European ancestry, but a small portion of participants in the gut microbiota data came from other races, which may cause bias. Second, bacterial taxa were analysed only at the genus level, not at more detailed levels such as species or strains.

In conclusion, this study employed a bidirectional, two-sample MR analysis using large-scale GWAS summary data to explore the causal effect between the gut microbiota and RA. We found that

Proteobacteria, *MollicutesRF9*, *Bacteroidaceae*, *Adlercreutzia*, *Butyricimonas* and *Holdemanella* were positively correlated with RA. In contrast, *Desulfovibrionales*, *Methanobacteriales*, and *Butyrivibrio* were negatively correlated. Our results provide strong evidence for the causal relationship between gut microbiota and RA, helping guide the prevention and diagnosis of the RA.

Acknowledgements

We wish to thank Chun'e Li for her support in the data collection.

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