Orthopedic Research and Reviews

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ORIGINAL RESEARCH

Investigating the Impact of Gut Microbiota on Gout Through Mendelian Randomization

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Background: The relationship between gout and gut microbiota has attracted significant attention in current research. However, due to the diverse range of gut microbiota, the specific causal effect on gout remains unclear. This study utilizes Mendelian randomization (MR) to investigate the causal relationship between gut microbiota and gout, aiming to elucidate the underlying mechanism of microbiome-mediated gout and provide valuable guidance for clinical prevention and treatment.

Materials and Methods: The largest genome-wide association study meta-analysis conducted by the MiBioGen Consortium (n=18,340) was utilized to perform a two-sample Mendelian randomization investigation on aggregate statistics of intestinal microbiota. Summary statistics for gout were utilized from the data released by EBI. Various methods, including inverse variance weighted, weighted median, weighted model, MR-Egger, and Simple-mode, were employed to assess the causal relationship between gut microbiota and gout. Reverse Mendelian randomization analysis revealed a causal association between bacteria and gout in forward Mendelian randomization analysis. Cochran's Q statistic was used to quantify instrumental variable heterogeneity.

Results: The inverse variance weighted estimation revealed that *Rikenellaceae* exhibited a slight protective effect on gout, while the presence of *Ruminococcaceae UCG_011* is associated with a marginal increase in the risk of gout. According to the reverse Mendelian Randomization results, no significant causal relationship between gout and gut microbiota was observed. No significant heterogeneity of instrumental variables or level pleiotropy was detected.

Conclusion: Our MR analysis revealed a potential causal relationship between the development of gout and specific gut microbiota; however, the causal effect was not robust, and further research is warranted to elucidate its underlying mechanism in gout development. Considering the significant association between diet, gut microbiota, and gout, these findings undoubtedly shed light on the mechanisms of microbiota-mediated gout and provide new insights for translational research on managing and standardizing treatment for this condition.

Keywords: Gout, Gut Microbiota, Mendelian Randomization, Diet

Introduction

Gout, a chronic disease characterized by the deposition of monosodium urate crystals, affects 41 million individuals globally with an incidence rate ranging from 0.58 to 2.89 per 1000 person-years,[1–3] placing a burden on individual health and healthcare systems. The condition can lead to painful inflammatory arthritis and other comorbidities, and is strongly associated with obesity, hyperlipidemia, type 2 diabetes, and other conditions. Additionally, it serves as an independent predictor of premature death.[4,5] In the pathogenesis of this disease, the deposition of urate salts promotes synovial inflammation (synovitis), leading to arthritis,[6] while elevated serum uric acid levels (hyperuricemia) play a crucial role in facilitating monosodium urate (MSU) crystal deposition and driving pain progression.[7] Therefore, it is imperative to lower uric acid levels in order to effectively prevent gout attacks.[8] In clinical practice, the hallmark of this disease is the abrupt onset of joint inflammation. Henceforth, the pivotal role lies in the natural immune pathway, particularly in the activation of NLRP3 inflammasome, which triggers the release of IL-1β and other pro-inflammatory cytokines.[9] Studies

Received: 9 February 2024
Accepted: 7 May 2024
Published: 13 May 2024

have demonstrated that long-chain fatty acids (C18) may contribute to the release of IL-1β during macrophage phagocytosis of MSU crystals.[10] Moreover, clinical evidence indicates a negative correlation between the dietary intake of omega-3 fatty acids and the incidence of acute gout attacks.[11] The aforementioned studies collectively suggest that dietary intake may be a contributing factor in the pathogenesis of gout inflammation.

The role of diet as a risk factor for gout has been well-documented, and dietary factors can significantly influence urate production.[1] Considering the pivotal role of gut microbiota in mediating the extensive impact of diet on human health and disease, it is plausible to hypothesize that alterations in the composition of gut microbiota may also exert an influence on the pathogenesis of gout.[12] The gut microbiota plays a pivotal role in the process of food digestion and exerts a significant influence on the overall metabolism of the human body.[13] Gut microbiota can also affect serum uric acid levels. Studies have shown that the Dietary Approaches to Stop Hypertension (DASH) diet can significantly reduce serum uric acid levels in patients with hyperuricemia.[14] Furthermore, studies investigating the impact of high-fiber diets on gout have suggested that these diets may exert an anti-inflammatory effect by augmenting acetate and other short-chain fatty acid production, thereby influencing neutrophil activity.[15] The findings suggest that dysbiosis of the gut microbiota, characterized by a decrease in microbial diversity and alterations in specific bacterial taxa, may be associated with an elevated risk of gout. The aforementioned studies provide valuable insights into the potential role of gut microbiota in the pathogenesis of gout. Further investigations are warranted to gain a more comprehensive understanding of the underlying mechanisms and to pinpoint potential therapeutic targets for the prevention and treatment of this prevalent ailment.

Gut microbiota and its metabolites have been observed to be implicated in the pathogenesis of metabolic diseases,[16,17] playing key roles in key biological processes such as metabolic interactions and host immune responses, such as polyamines, short-chain fatty acids (SCFAs), and aryl hydrocarbon receptor (AHR) ligands. It may affect the immune response and disease progression by interacting with host intestinal cells.[18] The pathogenesis of gout involves an inflammatory rheumatic condition characterized by arthritis and perturbed uric acid metabolism, which is widely acknowledged in the scientific community.[19] The intestinal tract serves as the principal pathway for uric acid excretion. Studies have demonstrated a reduction in both richness and diversity of gut microbiota among individuals with gout,[20] including a decrease in α diversity.[21] *Lactic acid bacteria* and *Pseudomonas* facilitate the excretion of uric acid in the intestines through the production of short-chain fatty acids.[22] The intestinal flora of hyperuricemic rats exhibits a decreased abundance of *Lactic acid bacteria, Streptococcus*, and *Clostridium*, which are involved in purine absorption and uric acid breakdown. Conversely, there is an increased abundance of *Proteus*, known for its secretion of xanthine dehydrogenase.[23] Although these studies have confirmed the dysbiosis of gut microbiota in gout patients, the causal relationship between gut microbiota dysbiosis and the development of gout remains unclear. Therefore, further investigation is necessary to elucidate the causal association between gout and gut microbiota.

In observational studies, the association between gut microbiota and gout is influenced by confounding factors such as age, environmental conditions, dietary patterns, and lifestyle choices.[24–26] Reaching effective control over these factors in observational studies thus presents a significant challenge. These conditions impose limitations on establishing causal relationships between gut microbiota and gout. Mendelian randomization (MR) represents a novel approach to investigate the causal association between gut microbiota and gout. The principle of MR can be employed to assess the causal relationship between exposure factors (such as gut bacteria) and outcomes (like gout) by leveraging genetic markers associated with the exposure, while satisfying specific assumptions.[27] Furthermore, the MR has been extensively employed to investigate the causal association between microbiota and diseases, such as rheumatoid arthritis.[28] However, two-sample MR employs single nucleotide polymorphism (SNP) data and independent genome-wide association study (GWAS) results to establish correlations and integrate them into a unified estimate of causality.[29] The present study employed a double-sample MR technique, utilizing aggregated statistics derived from MiBioGen's GWAS, to investigate the causal association between gut microbiota and gout.

Material and Methods

Overview of Research

The analysis employed a two-sample Mendelian randomization approach, treating each of the 196 gut microbiota species (Table S1) as an individual exposure factor while considering gout as the outcome. The assumptions for MR studies

necessitate the following: 1) a strong correlation between instrumental variables and exposure factors; 2) no correlation between instrumental variables and confounders; 3) the absence of direct association between the instrumental variable and the outcome, with its impact on the outcome solely reflected through exposure.

Data Sources

The gout data utilized in this study was derived from the comprehensive dataset compiled by the EBI database in 2021 (N=166,401). The intestinal flora data comes from the international alliance MiBioGen (N=18,340), which collected the data of 24S rRNA gene sequencing map and genotyping for 18,340 participants from 16 cohorts in the United States, Canada, Israel, Korea, Germany, Denmark, the Netherlands, Belgium, Sweden, Finland and the United Kingdom.[30]

Selection of Instrumental Variables

The initial screening of intestinal flora identified a total of 211 taxa, out of which 196 taxa were included in the experimental criteria after excluding 15 unknown taxa. These encompassed 9 phyla, 16 classes, 20 orders, 32 families, and 119 genera.

The SNPs of the 196 bacterial taxa were selected based on the following criteria: 1) Due to insufficient SNPs obtained from $p < 5\times10^{-8}$ screening, this criterion was revised and a threshold of $p < 1\times10^{-5}$ was used instead. 2) The LD criteria for selecting conforming SNPs from point 1) were as follows: $r^2 = 0.001$, kb = 10,000 to ensure independence of the obtained SNPs. 3) The F-statistic of each SNP was calculated, and instrumental variables with F >10 were chosen to mitigate weak instrumental bias.[31] Detailed data are available at Table S2 and Table S3.

Mendelian Randomization Studies and Sensitivity Analysis

In this study, the inverse variance weighted method (IVW) was employed to investigate the causal effect of intestinal flora on gout. To enhance the stability and reliability of experimental findings, four additional methods were utilized: weighted median method, weighted model method, MR-Egger method, and Simple-mod method. The IVW method, known for its high reliability, was chosen as the gold standard in cases where the causal effect varied across the five outcomes.

The Cochran's Q statistic was computed to quantify and assess the potential heterogeneity (Table S4), while the MR-Egger intercept test was employed to estimate pleiotropy levels. To ensure result reliability, a leave-one-out approach was utilized to examine the impact of each SNP on overall results and evaluate its heterogeneity.

Meanwhile, to mitigate errors arising from confounding factors in the experiment, we employed PhenoScanner (http://www.phenoscanner.medschl.cam.ac.uk) for querying instrumental variable SNPs during experimental selection. The objective was to ensure that all SNPs satisfied the three fundamental assumptions of the Mendelian randomization study2) 3) article.

Statistical Analysis

Considering the inclusion of n bacterial flora at various taxonomic levels, including phylum, class, family, order, species and genus, the significance threshold was adjusted using Bonferroni correction.[32] The p-values for the door, class, division, mesh, species and genus are calculated using the formula 0.05 / n as follows: 5.56×10^{-3}, 3.13×10^{-3}, 2.5×10^{-3}, 1.56×10^{-3} and 4.20×10^{-4}. In the experimental results, we observed a significant p-value below 0.05 after applying Bonferroni correction, indicating statistical significance. However, it should be noted that even though the p value was less than 0.05 but did not meet the criteria for Bonferroni correction, it was still considered to have potential statistical significance.

The experiments were primarily conducted using the Two-Sample-MR package (version 0.5.7) within the R software environment (version 4.3.1).

Results

Description of Instrumental Variables

The screening process involved the application of genome-wide significance thresholds ($p<1\times10^{-5}$), followed by LD tests to eliminate linkage disequilibrium, data coordination and harmonization, MR-PRESSO tests, and calculation of

F-values. Only SNPs with F-statistic values exceeding 10 were retained to ensure a strong correlation with the corresponding flora.

Causal Impact of Gut Microbiota on Gout

Inverse variance weighted estimates revealed a potential protective effect of *Rikenellaceae* against gout (OR= 0.9979, 95% CI: 0.9958–1.0000, P-value= 4.91×10^{-2}). Conversely, *Ruminococcaceae UCG_011* (OR= 1.0016, 95% CI: 1.0001–1.0031, P-value= 4.03×10^{-2}) was identified as a risk factor for gout. From Figure 1, it is evident that among the five tests conducted on *Rikenellaceae*, only the IVW method exhibits statistical significance with an OR value approaching unity. Although it demonstrates a certain degree of protective effect, the specific mechanism remains unknown. Similarly, *Ruminococcaceae UCG_011* displays a positive association with gout; however, the risk effect appears to be relatively weak.

Sensitivity Analysis

The sensitivity analysis of *Rikenellaceae* bacteria is depicted in Figure 2, while the corresponding analysis for *Ruminococcaceae UCG_011* bacteria is illustrated in Figure 3.

The Q test revealed no evidence of heterogeneity, and the horizontal pleiotropy test yielded negative results ($p>0.05$), the data specifics are outlined in Table S4, thereby bolstering the robustness of our findings.

Based on the findings presented in Figure 4, it can be observed that all five estimates of *Rikenellaceae* bacteria exhibit consistent trends, except for MR Egger. Although statistical significance was not achieved, this observation further

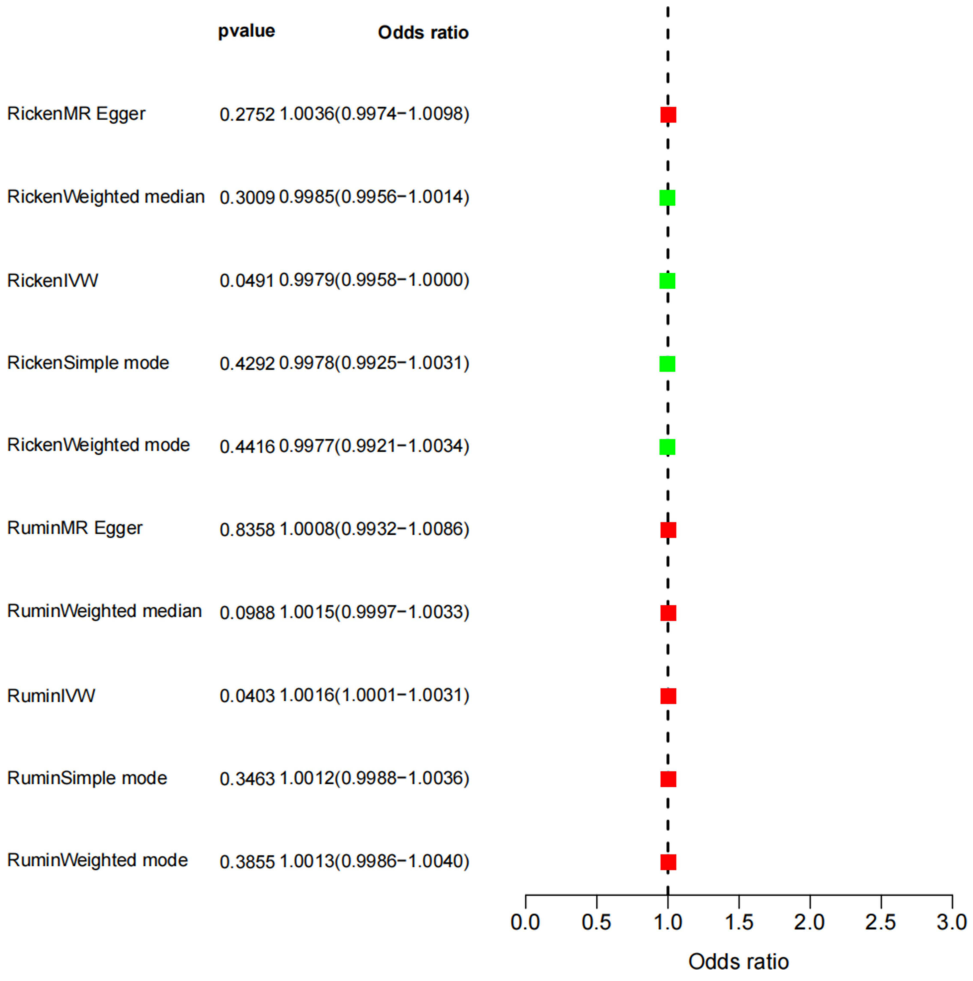

Figure 1 Causal impact of gut microbiota on gout.

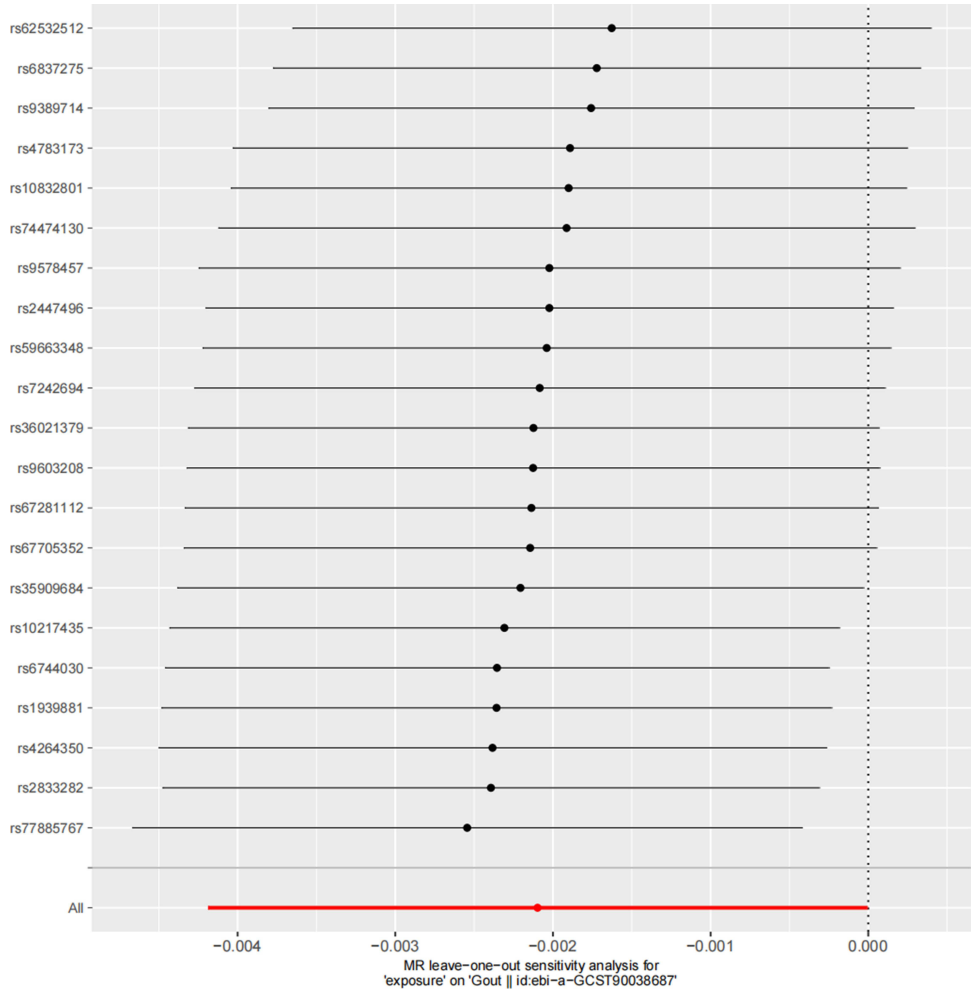

Figure 2 The sensitivity analysis of *Rikenellaceae* bacteria.

supports the current conclusion from an additional perspective. Furthermore, all identified causal effects of *Ruminococcaceae UCG_011* bacteria in Figure 5 demonstrate consistency.

The IVW method, considered as the benchmark in the experimental results of this study, demonstrated statistical significance with a p-value less than 0.05. Although it did not meet the Bonferroni correction for p-values, we still consider the estimated results to be statistically significant. However, out of the 196 bacterial groups included in this study, only two were identified as statistically significant for gout with an odds ratio (OR) value approaching unity. Further research is necessary to validate their specific causal effects.

Discussion

In this study, we utilized intestinal flora summary statistics from the largest GWAS meta-analysis conducted by the MiBioGen consortium and gout summary statistics from the EBI project open data to perform a two-sample MR analysis aimed at evaluating the causal relationship between intestinal flora and gout. Our findings suggest that *Rikenellaceae* has a protective effect on gout, while *Ruminococcaceae UCG_011* poses a risk for developing gout. However, due to its OR problem, our exploration of the causal relationship between intestinal flora and gout is still in its exploratory stage. By exploring microbial mechanisms, we indirectly confirmed that our estimated results are more reliable.

Currently, studies have confirmed a reduction in purine metabolism among patients with gout. Based on the study findings, the same group proposed that this reduced purine metabolism, along with an enhanced carbohydrate metabolism of intestinal flora, may lead to uric acid overload.[33] Currently, studies have confirmed a reduction in purine metabolism

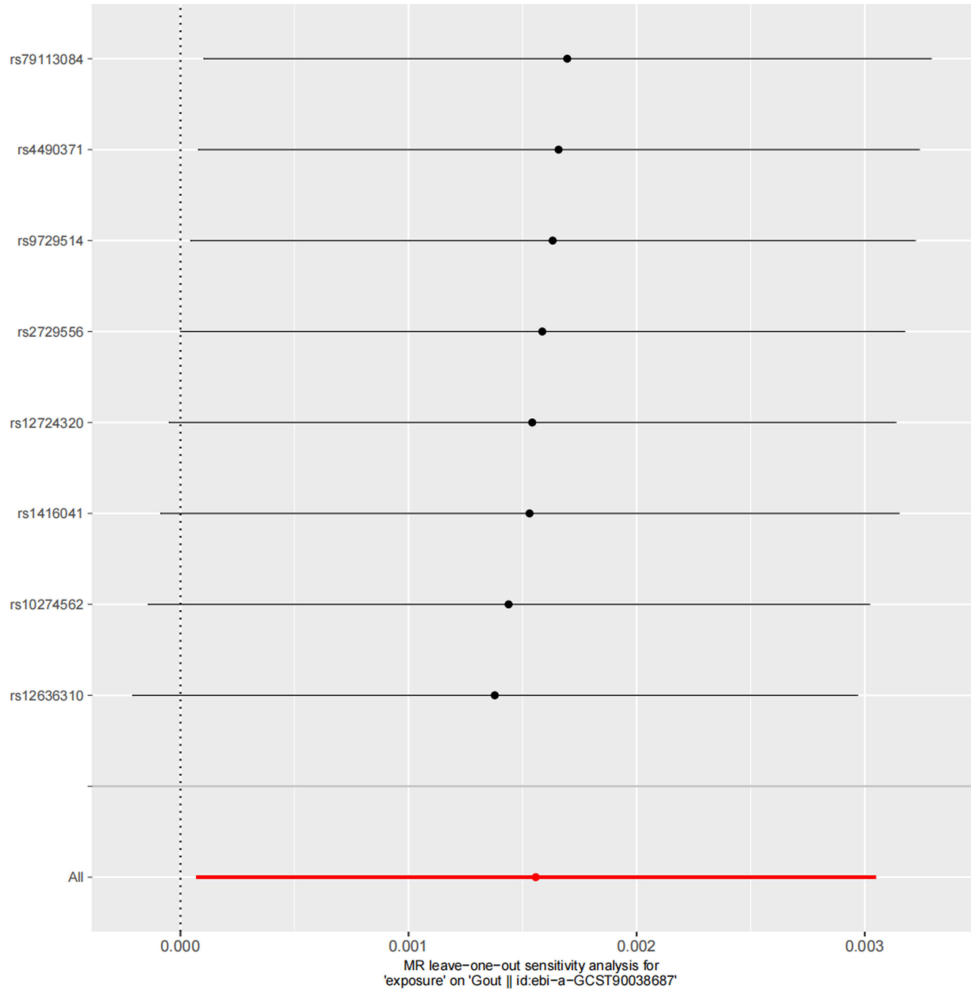

Figure 3 The sensitivity analysis of *Ruminococcaceae UCG_011* bacteria.

among patients with gout. Based on the study findings, the same group proposed that this reduced purine metabolism, along with an enhanced carbohydrate metabolism of intestinal flora, may lead to uric acid overload,[34] characterized by metabolic hyperuricemia and the deposition of urate crystals in and around the joint.[35] Among these, hyperuricemia resulting from purine metabolism dysfunction serves as a significant risk factor for the development of gouty inflammation.[36] Approximately 70% of uric acid is excreted via the kidney, while the remaining portion is primarily eliminated through feces or undergoes further metabolism by intestinal flora.[37] Guo observed a significant enrichment of xanthine dehydrogenase, a microorganism capable of converting purines into uric acid, in gout patients. Conversely, allantoinase, which facilitates the degradation of uric acid into urea, was notably reduced in these individuals, resulting in the accumulation of uric acid and exacerbation of gout symptoms.[38] Additionally, studies have demonstrated that *Enterobacteriaceae* play a crucial role in uric acid degradation among healthy individuals.[33]

Furthermore, accumulating evidence suggests that the intestinal microbiota not only contributes to purine metabolism and urate excretion but also plays a pivotal role in activating the NLRP3 inflammasome.[39–41] A previous study demonstrated significantly elevated expression levels of NLRP3, ASC, and caspase-1 in both gout and hyperuricemia groups compared to the control group[42]. Additionally, several researchers have reported that NLRP3 can mediate various programmed cell death pathways involved in inflammatory responses, thereby promoting the onset and progression of gout.[43]

Short-chain fatty acids play a crucial role as mediators utilized by intestinal flora to modulate the physiological function and immune response of the host. They possess the ability to sense and suppress inflammatory reactions,

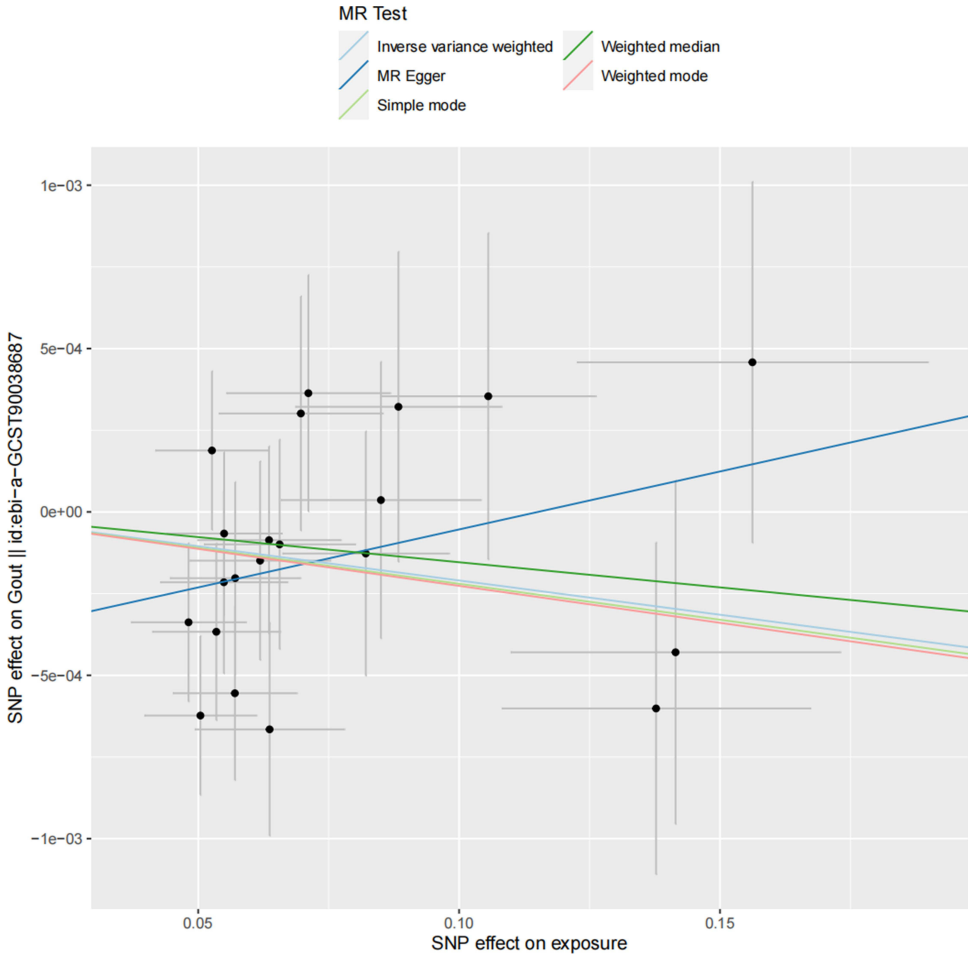

Figure 4 The five estimates of *Rikenellaceae* bacteria.

including the expression of adhesion molecules and inflammatory mediators, as well as leukocyte chemotaxis.[44] The major short-chain fatty acids involved are acetate, propionate, and butyrate.[45] Recent investigations have demonstrated that propionate can inhibit NLRP3 inflammasome activation by preventing apoptosis-associated speck-like protein (ASC) oligomerization and speck formation. Importantly, this inhibitory effect is independent of G protein-coupled receptor (GPCR) or histone deacetylase (HDAC) signaling pathways.[46] Notably, *Rikenellaceae* has been found to exhibit a negative correlation with proinflammatory cytokines and certain injury factors,[47,48] while also being capable of producing propionate.[49] Given that increased levels of propionate contribute to anti-inflammatory effects,[50] we hypothesize that *Rikenellaceae* may be involved in regulating NLRP3 inflammatory factor metabolism thereby influencing gout-related inflammation.

Furthermore, previous studies have demonstrated a positive correlation between *Rikenellaceae* and the products of amino acid metabolic pathways.[51] It has been established that plasma free amino acids play a crucial physiological role in the biosynthesis and catabolism of various metabolites, as well as acting as modulators for numerous metabolic pathways.[52] Specific amino acids are involved in purine biosynthesis and subsequent uric acid formation. For instance, glutamine, glycine, and serine contribute to increased uric acid production in individuals with gout.[53] Other investigations have indicated that serum glycine and aspartate may participate in purine nucleotide biosynthesis among patients with gout, thereby leading to purine metabolic disorders.[54] Additionally, Mahbub et al observed significantly elevated levels of serum alanine, isoleucine, phenylalanine, leucine, valine, tyrosine, and lysin e in individuals with gout.[55] Therefore, it is reasonable to hypothesize that plasma free amino acids play a pivotal role in the pathogenesis of gout.

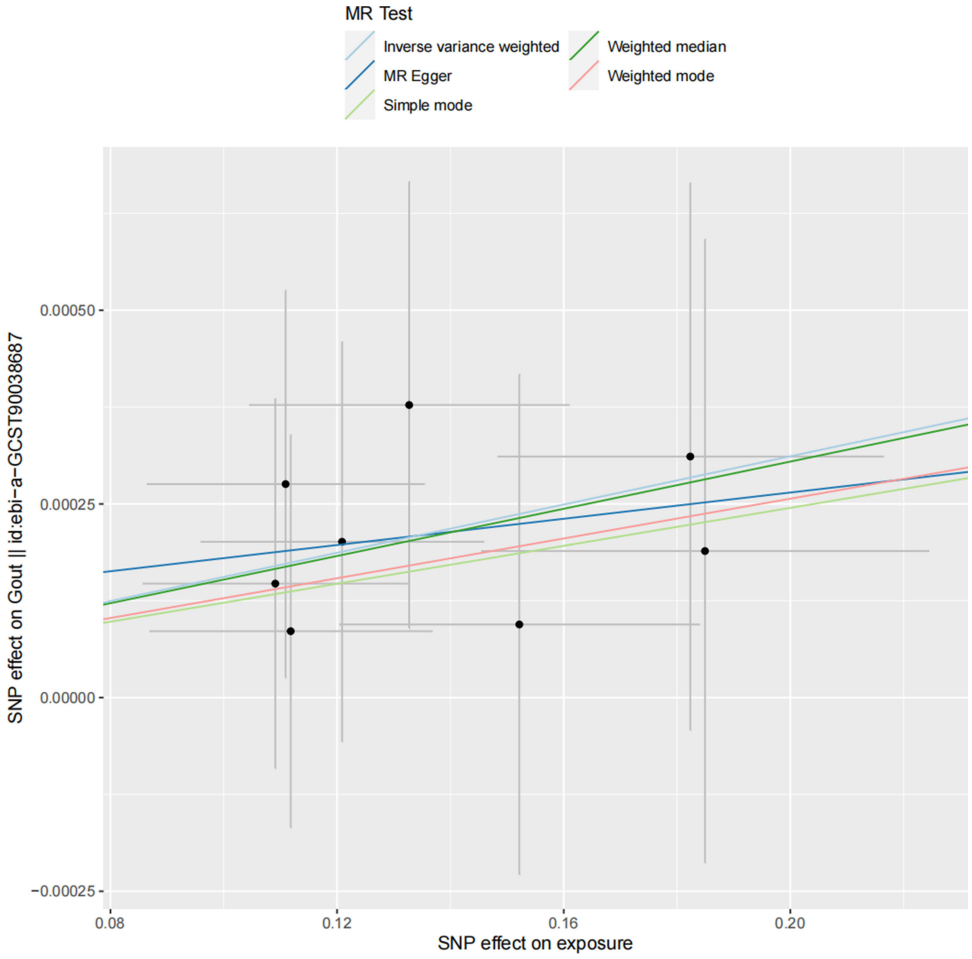

Figure 5 The five estimates of *Ruminococcaceae UCG_011* bacteria.

However, the relationship between *Rikenellaceae* and plasma free amino acids remains unclear, and further extensive research is warranted.

Numerous studies have demonstrated the association of *Rikenellaceae* with protection against cardiovascular and metabolic diseases linked to visceral fat.[56] In a murine model of anthracycline-induced cardiac toxicity, there was a reduced abundance of *Rikenellaceae_RC9_gut_group*.[57] Investigation on obese mice induced by high-fat diet revealed that Sabah polysaccharide could ameliorate obesity through upregulation of *Ricenellaceae_RC9_gut_group*.[58] Given the well-established interrelation between cardiovascular diseases, obesity, and gout,[2,59] these findings collectively support the notion that *Ricenellaceae* exhibits a protective tendency against cardiovascular and metabolic diseases associated with visceral fat, which aligns with our own results.

Ruminococcaceae is a gram-positive anaerobe. Molecular studies have demonstrated that the presence of glucan on the cell surface of *R. gnavus* strain ATCC 29149 is dependent on TLR4 receptor binding, indicating its pro-inflammatory properties in vitro by inducing secretion of inflammatory cytokines (TNF-α).[60] Moreover, an association between increased levels of *R. gnavus* and spondyloarthritis as well as systemic lupus erythematosus has been established.[61,62] In metabolic diseases, Yan found a strong correlation between *R. gnavus* and visceral fat along with positive correlations with metabolic indicators.[63] Additionally, in diabetes research, *R. gnavus* was identified as one of four consistently associated species with disease onset.[64] However, contradicting these findings, Shao observed lower enrichment levels of *Ruminococcaceae UCG_011* in the intestinal flora of patients with gout.[65] This inconsistency challenges our results; however, considering the overall context of the disease, we believe it still poses a risk effect on gout development due to limited explanatory power provided by SNPs as causal factors. Nonetheless, this does not completely negate the

possibility that *Ruminococcaceae UCG_011* may be implicated in increasing risk. In addition, Wang discovered that the genus *RuminococcaceaeUCG011* is a risk factor for gout.[66] This is consistent with the results of this study and strengthens the credibility of our results.

Due to differences in data, such as the inclusion of different participants, the other researchers have also found that *Escherichia Shigella, Lachnospiraceae NC2004 group, Family XIII AD3011 group, Coprococcus 3, Bifidobacteriales order* and *Bifidobacteriaceae family* are closely related to gout.[67,68] These findings could be mutually complementary with our results.

This study possesses several notable advantages. Firstly, in contrast to previous observational studies investigating the association between gut microbiota and gout, our analysis employed a more refined approach by examining the causal relationship between specific gut microbiota and gout at five taxonomic levels ranging from phylum to genus. Consequently, we identified potential gut microbiota that may exert an impact on gout development. This methodological advancement provides a conceptual framework for elucidating the mechanisms through which particular bacterial strains contribute to gout pathogenesis and offers valuable research insights. For instance, the increased abundance of *Rikenellaceae* and decreased abundance of *Ruminococcus* could potentially be linked to a high-fat diet, suggesting targeted interventions that can mitigate gout incidence associated with such dietary patterns. Secondly, leveraging state-of-The-art large-scale genome-wide association studies (GWAS) enabled us to analyze genetic data from a substantial number of samples, thereby enhancing the credibility of our findings compared to smaller randomized controlled trials. Furthermore, employing MR analysis helped circumvent confounding factors and provided novel perspectives for unraveling the intricate interplay between gut microbiota and gout.

Although this study supports the hypothesis of MR analysis, certain limitations should be acknowledged. The gout and gut microbiota database utilized in this study did not encompass Asian and African populations, thus generalizability to the global population cannot be guaranteed. Further investigations will be conducted to address this issue. Additionally, the stringent screening threshold employed may have excluded some SNPs with a causal relationship with gout. To provide additional theoretical evidence supporting the gut-gout axis mechanism, correlation studies at the species level with a larger sample size are warranted.

Conclusion

In summary, our study has unveiled the advantageous and precarious gut microbiota associated with gout. When combined with existing research, it becomes evident that the gut microbiota plays a pivotal role in both the development and management of gout, thereby holding significant implications for its clinical prevention and treatment. Moreover, diet-induced systemic metabolic pathways, energy balance, and alterations in gut microbiota also offer novel insights into the mechanisms and potential interventions for comprehending the progression of gout. In conclusion, the implementation of dietary modifications accompanied by effective regulatory mechanisms may represent a viable strategy for mitigating the high prevalence of gout. These findings present new avenues for our subsequent animal experiments to explore the impact of diet on intervention and its potential mechanisms based on gut microbiota.

Data Sharing Statement

The dataset utilized in this study is available for download from the MiBioGen repository(https://mibiogen.gcc.rug.nl/), and EBI repository(https://www.ebi.ac.uk/).

Ethical Statement

According to Article 32 of the Measures for Ethical Review of Life Science and Medical Research Involving Human Beings adopted by the National Science and Technology Ethics Committee of the People's Republic of China, ethical review can be exempted because the data used in this study do not cause any harm to human beings, do not involve any sensitive personal information or commercial interests, and the databases selected are open and legal.

Acknowledgments

The authors extend their sincere gratitude to the participants and investigators of the EBI study. Furthermore, the authors would like to express their appreciation to the MiBioGen consortium for providing access to the gut microbiota GWAS summary statistics.

Funding

This research was funded by TCM Science and Technology Project of Shandong Province, grant number 2019-0798.

Disclosure

The authors report no conflicts of interest in this work.

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