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ORIGINAL RESEARCH

Mitochondrial Function, Gut Microbiota, and Gout Risk Among Individuals of European Descent: A Mendelian Randomization Study of a Mediated Relationship

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Purpose: Gout, a common form of arthritis, is characterised by recurrent episodes of severe inflammation owing to the accumulation of monosodium urate crystals in the joints and tissues, resulting from elevated serum uric acid levels. While the roles of gut microbiota and mitochondria in gout have been studied, their causal interactions remain unclear. The purpose of this study is to investigate the interplay between gut microbiota and mitochondrial biological function in the pathogenesis of gout.

Methods: This study utilized Mendelian randomization to explore causal links between mitochondrial biological function, gut microbiota, and gout, by leveraging data from genome-wide association studies. Bidirectional causal effects of mitochondrial biological functions on gout and serum uric acid levels were analysed; moreover, the causal effects of gut microbiota on gout and uric acid levels were evaluated through mediation analysis of the gut microbiota in the pathway linking mitochondrial biological function with gout.

Results: A causal relationship was found between mitochondrial biological function and gout mediated by gut microbiota. The NAD-dependent protein deacylase sirtuin-5 mediated 18.24% of the total effect on the adverse effects of gout by reducing creatinine degradation I. Calcium uptake protein 3 had a substantial impact on mitigating the negative effects of serum uric acid by decreasing the abundance of the order Burkholderiales and class Betaproteobacteria, which accounted for 16.52% and 15.83%, respectively, of the overall effect.

Conclusion: This analysis elucidated the complex relationships between mitochondrial biological function, gut microbiota, and gout, providing novel perspectives for gout prevention and treatment. Further investigations will enhance our understanding of the interactions between these biological processes and guide future intervention strategies.

Keywords: Mendelian randomisation, mitochondrial biological function, gut microbiota, gout, serum uric acid

Introduction

Gout, a common type of arthritis, is characterized by repeated episodes of severe inflammation triggered by the accumulation of monosodium urate crystals within joints, tendons, and other tissues owing to high levels of serum uric acid (SUA). The growing clinical burden and rising prevalence of gout have intensified the search for modifiable risk factors and novel therapeutic targets.[1] In recent years, accumulating evidence has indicated that mitochondrial function and gut microbiota are critical regulators of purine metabolism, inflammatory responses, and overall metabolic homeostasis. These factors are closely involved in the pathogenesis of gout. At the cellular level, mitochondria are the primary site for oxidative phosphorylation and oxygen reduction, generating most of the ATP essential for cellular activities and serving as a major source of reactive oxygen species. As they supply up to 80% of cellular energy, mitochondria are

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closely associated with metabolic diseases and have become crucial targets for the prevention and treatment of various disorders.[2] Moreover, impaired mitochondrial function disrupts intestinal homeostasis, consequently altering the composition of the gut microbiota. These microbial changes affect purine metabolism, leading to abnormal uric acid production and excretion. This imbalance further stimulates the release of inflammatory mediators that are closely associated with gout development.[3,4] Therefore, we hypothesize that gut microbiota may serve as a key mediator linking mitochondrial dysfunction to gout onset. These complex interrelationships warrant further in-depth investigation. However, the mechanisms underlying the interaction between mitochondrial function and gut microbiota in influencing gout risk remain poorly understood. Elucidating these relationships is crucial for developing novel interventions for gout prevention and management.

Establishing causal relationships between the gut microbiota or mitochondrial biofunction and gout through randomized controlled trials in humans is challenging, primarily because of constraints such as limited strain screening and the complexity of reducing mitochondrial function levels.[5] Consequently, current investigations depend heavily on observing the gut microbiota composition and alterations through fecal sampling, along with indirect interventions such as administration of prebiotics,[6] and serum urate inhibitors, including allopurinol or benzbromarone.[7]

Considering these constraints, Mendelian randomization (MR) offers unique opportunities to strengthen causal inferences. In conventional epidemiological research, establishing causal relationships between exposures and outcomes is challenging owing to the presence of unmeasurable confounding variables and potential reverse causality, which limit the ability to infer causal relationships. MR studies capitalize on data derived from genome-wide association studies (GWAS) and utilize single-nucleotide polymorphisms (SNPs) as instrumental variables (IVs) to assess causal relationships between risk factors and diverse outcomes. These models offer a robust framework by mitigating the influence of confounding factors and altering the direction of causal inferences. Accordingly, this study aims to clarify the causal relationships between mitochondrial function, gut microbiota, and gout (including serum uric acid levels) by performing MR analysis using GWAS data from individuals of European ancestry. Specifically, we examine the bidirectional causal effects of mitochondrial function on gout and SUA levels. We also explore the mediating role of gut microbiota in the pathway linking mitochondrial function to gout. By improving our understanding of these interactions, this research aims to provide novel insights into potential therapeutic strategies for gout prevention and treatment.[8]

Materials and Methods

Study Design

This study encompassed three primary components (Figure 1): first, an analysis of the bidirectional causal effects between mitochondrial biological function and both gout and serum uric acid (SUA) levels (IA and IB); second, an investigation into the causal effects of gut microbiota on gout and SUA (IC); and third, a mediation analysis exploring the role of gut microbiota in the pathways linking mitochondrial biological function to gout and SUA (ID). MR relies on three core assumptions, as illustrated in Figure 2. First, the IVs should demonstrate a strong association with exposure (the relevance assumption). Second, the IVs must remain independent of any confounding factors in the exposure-outcome relationship (the independence assumption). Third, the IVs should influence the outcome solely through exposure (the exclusion-restriction assumption).[9] Based on this paradigm, we searched for SNPs that were linked to exposure across the whole genome ($P < 5 \times 10^{-8}$) and excluded those in linkage disequilibrium (LD) ($r2 < 0.001$, distance < 1,000 kb).[10] This threshold effectively controls the false-positive rate due to multiple testing, while ensuring that the selected SNPs have sufficient statistical certainty for the corresponding phenotypes. The remaining SNPs were aggregated into the GWAS database for outcome assessment, and each SNP was assessed for potential violations of assumptions 2 and 3 using PhenoScanner (http://www.phenoscanner.medschl.cam.ac.uk).[11] This can screen whether each SNP was significantly associated with other phenotypes that may be associated with gout or SUA levels. If significant pleiotropy was found, we excluded the SNP from the main analysis or tested it in sensitivity analyses to ensure that the final causal inference was not affected by pleiotropy bias. We also assessed the biological plausibility of the selected SNPs and evaluated potential pleiotropic effects by consulting relevant databases and literature. These steps aimed to minimize the risk that the instrumental variables could influence gout through pathways unrelated to gut microbiota. We

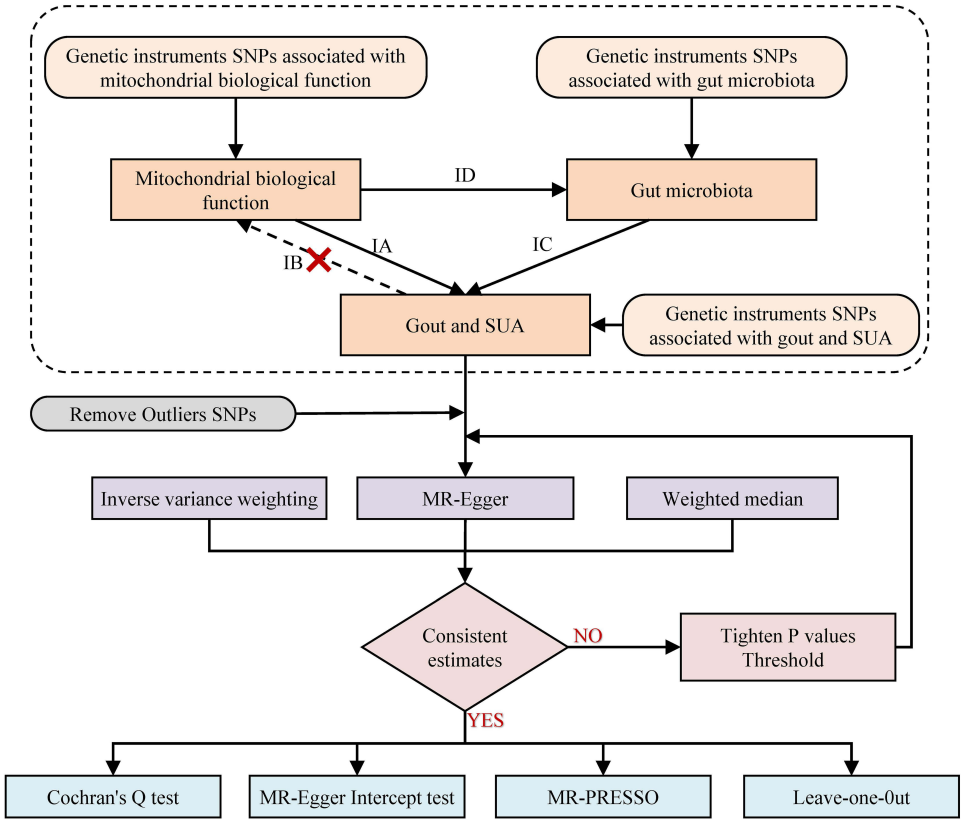

Figure 1 Research flow chart of the Mendelian randomisation study.

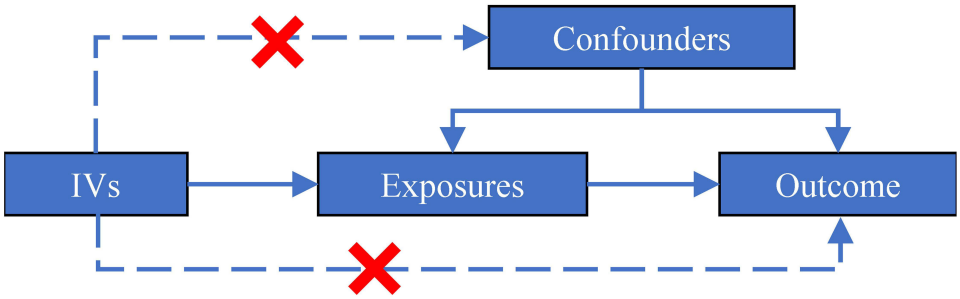

Figure 2 The three core assumptions in the study design.

are confident that these methods substantially reduced potential biases and enhanced the reliability of our results. This study employed MR to investigate causal links between mitochondrial function, gut microbiota, and gout using GWAS data from European-ancestry populations.

Data Sources

Mitochondrial biological functions and associated GWAS were derived from the INTERVAL study.[12] This study, involving a blood donation drive that included approximately 50,000 participants, was designed to explore the health effects of blood donation and the relationship between plasma protein levels and the genome. The results revealed mechanisms underlying the genetic control of plasma proteins. In total, 1,927 genotype-protein associations were identified in that study. It provided new information about how proteins are controlled and how disease susceptibility loci function (Table S1).[12] Genetic data for the gut microbiome were sourced from the latest GWAS summary data. This

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study used genetic data to screen for genetic markers and analyse European samples via LD trimming, principal component analysis of genotype data, microbial DNA extraction, and Illumina sequencing of faecal samples. This study ultimately determined the taxonomic composition of the microbiome using MetaPhlAn2 (Table S2).[13] The GWAS dataset on gout contained information on both gout and urate levels. The summary statistics of the GWAS from the UK BioBank included 389,404 European individuals for whom urate levels were recorded.[14] Publicly available summary-level GWAS data were also obtained from the UK BioBank. Another GWAS of gout included 484,598 individuals, comprising 6,810 individuals diagnosed with gout and 477,788 individuals serving as controls.[15]

MR Analysis

Five methods were used to ascertain the causal correlation between mitochondrial physiological functions and gout, including inverse variance weighting (IVW), MR-Egger regression, simple mode, weighted mode, and weighted median. The "TwoSampleMR" package in R was employed to display MR data, including scatter plots, forest plots, and sensitivity analysis plots. The IVW approach is commonly used as the main reliability indicator in MR analyses. A favourable conclusion is typically determined using a significance criterion of $P < 0.05$.

We assessed heterogeneity using Cochran's Q test, where a significance level of $P < 0.05$ indicated the presence of heterogeneity in the investigations. If the instrumental variable (IV) affected the outcome through factors unrelated to the exposure variable, the presence of pleiotropy was suggested, which could compromise the independence and exclusivity of the outcomes. We utilized the MR-Egger intercept test to assess pleiotropy and ensure result reliability. A P-value < 0.05 indicates the presence of pleiotropy in the data. We also utilised the "leave-one-out" method to perform sensitivity analyses, which involves eliminating outcomes linked to particular SNPs, evaluating whether they deviate significantly from the norm, and analysing the consistency of the outcomes following the removal of each SNP. We employed the MR-PRESSO test to remove the variant in question. Subsequently, we conducted IVW regression to identify horizontal pleiotropic outliers. We also employed R programming language to create a funnel plot, enabling us to evaluate the symmetry of single-nucleotide polymorphisms (SNPs) and assess the reliability of our results.[16–21] The research flowchart is presented in Figure 1. Finally, we utilised the TwoSampleMR (version 0.4.25) and MR-PRESSO (version 1.0) packages available in R (version 4.3.2) to perform MR analysis.

Results

Genetic Instruments and Strength of the IVs

In total, 830 SNPs were utilised as IVs for mitochondrial biological functions, with a significance threshold of $P < 5\times10^{-6}$. Subsequently, 4,146 SNPs linked to 412 gut microbiota were identified at a significance level of $P < 1\times10^{-5}$. Lastly, 30 and 318 SNPs were used as IVs for gout and SUA, respectively, with a significance threshold of $P < 5 \times 10^{-8}$ (Additional file 1: Tables S3–S6).

Bidirectional Causal Effect of Mitochondrial Biological Function on Gout and SUA

First, we identified the causal relationship between mitochondrial biological function and gout (Table S7, Figure 3). We identified five SNP datasets that displayed a strong correlation with gout: phenylalanine-tRNA ligase (odds ratio [OR]: 0.9982; 95% confidence interval [CI]: 0.9969 to 0.9994; P = 0.0047), [pyruvate dehydrogenase (acetyl-transferring)] kinase isozyme 1 (OR: 0.9988; 95% CI: 0.9978 to 0.9998; P = 0.0214), NAD-dependent protein deacylase sirtuin-5 (OR: 0.9989; 95% CI: 0.9980 to 0.9999; P = 0.0243), cytochrome c oxidase assembly factor 3 homolog (OR: 1.0016; 95% CI: 1.0005 to 1.0026; P = 0.0043), and cytochrome c oxidase subunit 5B (OR: 0.9989; 95% CI: 0.9979 to 0.9999; P = 0.0306) (Supplementary Fig. S1, Table 1). Furthermore, we also identified the causal relationship between mitochondrial biological function and SUA (Table S8, Figure 4). Four SNP datasets showed a strong and consistent connection with SUA: 4-hydroxy-2-oxoglutarate aldolase (OR: 1.0189; 95% CI: 1.0056 to 1.0323; P = 0.0051), Lon protease homolog (OR: 0.9917; 95% CI: 0.9843 to 0.9991; P = 0.0276), NADH dehydrogenase [ubiquinone] iron-sulfur protein 4 (OR: 1.0050; 95% CI: 1.0014 to 1.0087; P = 0.0065), and calcium uptake protein 3 (OR: 0.9903; 95% CI: 0.9827 to 0.9980; P = 0.0137) (Supplementary Fig. S2, Table 2). However, MR

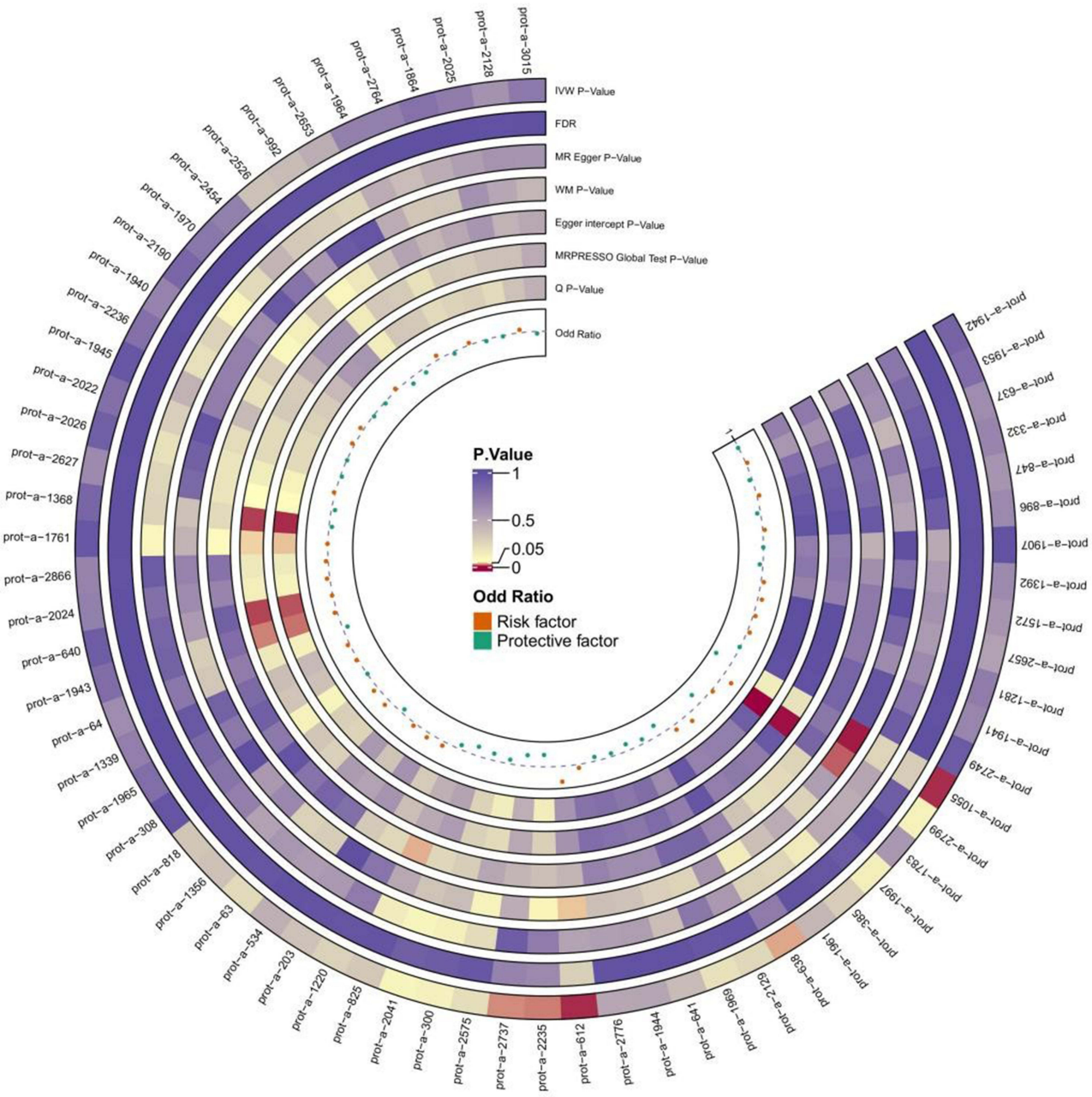

Figure 3 GWAS dataset of SNPs demonstrating robust associations with mitochondrial function and gout.

analyses revealed that 39S ribosomal protein L33 (OR: 0.0282; 95% CI: 0.0008 to 0.0.9964; P = 0.0498) and phenylalanine-tRNA ligase (OR: 0.0093; 95% CI: 0.0001 to 0.6951; P = 0.0336) exhibited an inverse causal relationship with the genetic prediction of gout; 39S ribosomal protein L33 (OR: 0.8413; 95% CI: 0.7211 to 0.9816; P = 0.0281) and ADP-ribose pyrophosphatase (OR:1.2116; 95% CI: 1.0231 to 1.4349; P = 0.0261) have a causal relationship with SUA (Table 3).

Bidirectional Causal Effect of the Gut Microbiota on Gout and SUA Levels

The results revealed seven gut microbiota and 14 gut bacterial pathways that were associated with gout: creatinine degradation I (OR: 1.0019; 95% CI: 1.0003 to 1.0036; P = 0.0232), superpathway of fucose and rhamnose degradation (OR: 1.0017; 95% CI: 1.0002 to 1.0031; P = 0.0229), galacturonate degradation I (OR: 1.0026; 95% CI: 1.0005 to 1.0047;

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Table 1 Causal Effects of Mitochondrial Biological Functions on Gout

ID	Name	IVW		$P_{heterogeneity}$	$P_{Egger\ intercept}$	$P_{pleiotropy}$
		OR (95% CI)	P			
prot-a-612	Cytochrome c oxidase assembly factor 3 homolog	1.0016 (1.0005–1.0026)	0.0043	0.5948	0.4312	0.6070
prot-a-1055	Phenylalanine–tRNA ligase	0.9982 (0.9969–0.9994)	0.0047	0.1381	0.7644	0.2040
prot-a-2235	[Pyruvate dehydrogenase (acetyl-transferring)] kinase isozyme 1	0.9988 (0.9978–0.9998)	0.0214	0.1409	0.3785	0.2540
prot-a-2737	NAD-dependent protein deacylase sirtuin-5	0.9989 (0.9980–0.9999)	0.0243	0.4613	0.4272	0.4740
prot-a-638	Cytochrome c oxidase subunit 5B	0.9989 (0.9979–0.9999)	0.0306	0.9547	0.6825	0.9530

P = 0.0160), heme biosynthesis II (OR: 1.0024; 95% CI: 1.0005 to 1.0043; P = 0.0155), glutamate degradation V (OR: 1.0022; 95% CI: 1.0002 to 1.0041; P = 0.0306), superpathway of phospholipid biosynthesis I (OR: 0.9978; 95% CI: 0.9960 to 0.9995; P = 0.0124), polyisoprenoid biosynthesis (OR: 0.9985; 95% CI: 0.9972 to 0.9998; P = 0.0216), superpathway of heme biosynthesis from glycine (OR: 1.0014; 95% CI: 1.0004 to 1.0024; P = 0.0081), purine nucleotides degradation II (OR: 0.9984; 95% CI: 0.9968 to 1.0000; P = 0.0450), peptidoglycan biosynthesis IV (OR: 1.0019; 95% CI: 1.0003 to 1.0036; P = 0.0225), superpathway of L-tyrosine biosynthesis (OR: 1.0012; 95% CI: 1.0000 to 1.0024; P = 0.0446), thiamin salvage II (OR: 0.9979; 95% CI: 0.9961 to 0.9997; P = 0.0205), superpathway of pyrimidine ribonucleosides degradation (OR: 1.0014; 95% CI: 1.0001 to 1.0026; P = 0.0339), superpathway of ubiquinol-8 biosynthesis (OR: 1.0015; 95% CI: 1.0001 to 1.0030; P = 0.0428), family Lactobacillaceae (OR: 0.9992; 95% CI: 0.9984 to 1.0000; P = 0.0487), family Clostridiales_noname (OR: 1.0023; 95% CI: 1.0007 to 1.0040; P = 0.0054), genus Odoribacter (OR: 0.9983; 95% CI: 0.9965 to 1.0000; P = 0.0493), genus Paraprevotella (OR: 0.9986; 95% CI: 0.9972 to 0.9999; P = 0.0421), genus Dorea (OR: 1.0022; 95% CI: 1.0001 to 1.0042; P = 0.0434), Bacteroides_ovatus (OR: 0.9986; 95% CI: 0.9972 to 1.0000; P = 0.0496), and Bacteroides_thetaiotaomicron (OR: 1.0023; 95% CI: 1.0004 to 1.0043; P = 0.0196) (Supplementary Fig. S3, Table 4, Figure 5).

We also identified seven gut microbiota and nine gut bacterial pathways that were associated with SUA levels: glycolysis III from glucose (OR: 0.9853; 95% CI: 0.9717 to 0.9991; P = 0.0370), glycogen biosynthesis I from ADP-D-glucose (OR: 1.0147; 95% CI: 1.0004 to 1.0293; P = 0.0436), superpathway of glycerol degradation to 1,3-propanediol (OR: 1.0116; 95% CI: 1.0017 to 1.0216; P = 0.0211), lipid IVA biosynthesis (OR: 0.9806; 95% CI: 0.9659 to 0.9955; P = 0.0111), superpathway of polyamine biosynthesis I (OR: 0.9680; 95% CI: 0.9497 to 0.9867; P = 0.0008), phosphatidylglycerol biosynthesis I (OR: 0.9807; 95% CI: 0.9655 to 0.9961; P = 0.0144), 5-aminoimidazole ribonucleotide biosynthesis I (OR: 1.0258; 95% CI: 1.0075 to 1.0443; P = 0.0055), pyrimidine deoxyribonucleotides de novo biosynthesis IV (OR: 1.0217; 95% CI: 1.0010 to 1.0429; P = 0.0403), superpathway of L-lysine, L-threonine and L-methionine biosynthesis II (OR: 1.0162; 95% CI: 1.0013 to 1.0314; P = 0.0332), class Betaproteobacteria (OR: 1.0141; 95% CI: 1.0002 to 1.0282; P = 0.0470), genus Roseburia (OR: 1.0189; 95% CI: 1.0007 to 1.0373; P = 0.0419), genus Erysipelotrichaceae_noname (OR: 0.9913; 95% CI: 0.9833 to 0.9994; P = 0.0352), order Burkholderiales (OR: 1.0147; 95% CI: 1.0009 to 1.0287; P = 0.0365), Parabacteroides johnsonii (OR: 0.9933; 96% CI: 0.9867 to 0.9999; P = 0.0474), Haemophilus parainfluenzae (OR: 1.0157; 97% CI: 1.0040 to 1.0275; P = 0.0084), and Bacteroides uniformis (OR: 0.9638; 98% CI: 0.9406 to 0.9875; P = 0.0029) (Supplementary Fig. S4, Table 5, Figure 6).

Mediation Analysis

We also analysed the gut microbiota as a mediator of the pathway from mitochondrial biological functions to gout and SUA. The results showed that the NAD-dependent protein deacylase sirtuin-5 (SIRT5) mediated 18.24% of the adverse effects of gout by reducing creatinine degradation I. The results indicated that calcium uptake protein 3 had

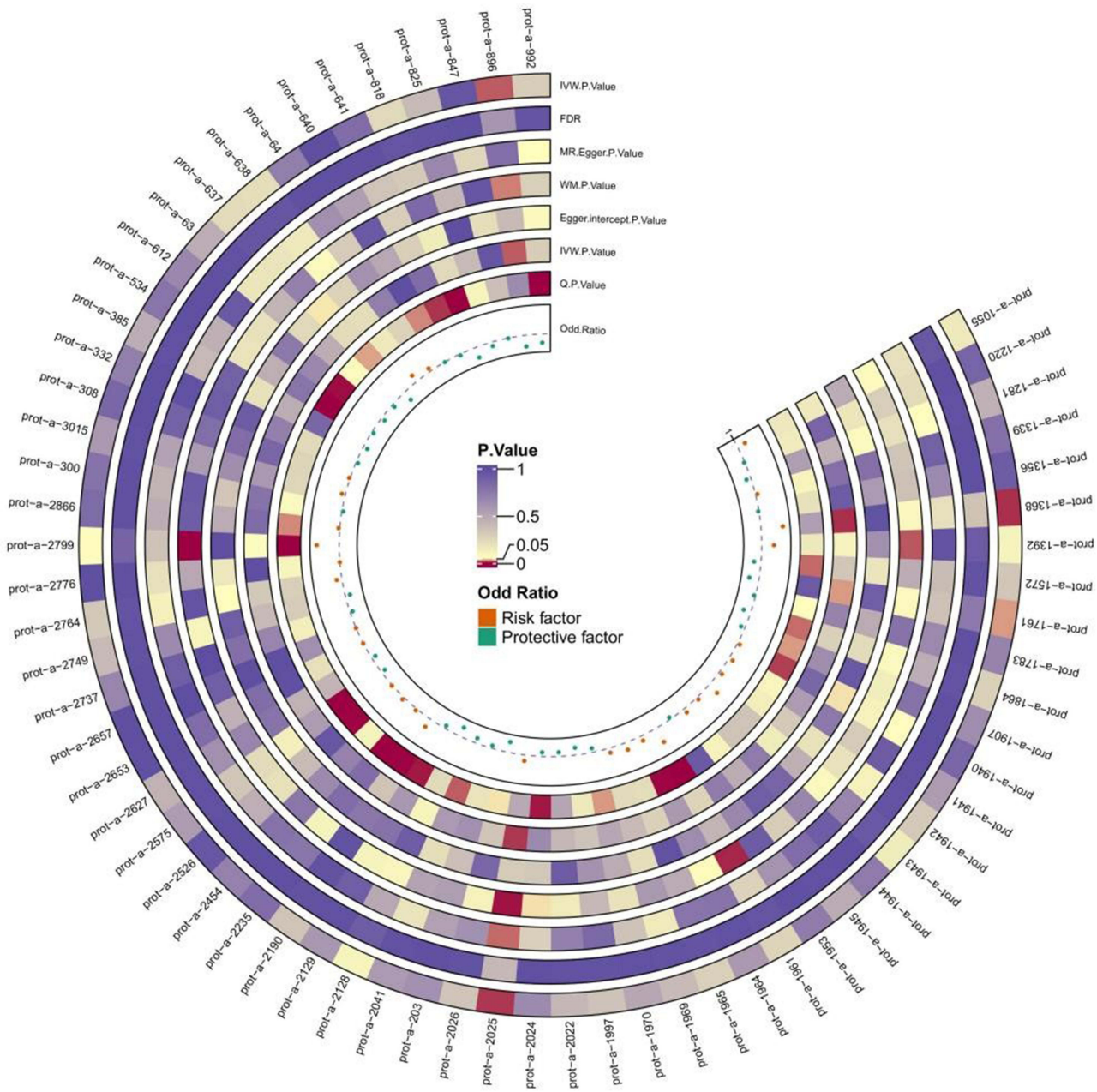

Figure 4 GWAS dataset of SNPs demonstrating robust associations with mitochondrial function and SUA.

a substantial impact on mitigating the negative consequences of SUA by decreasing the abundance of the order Burkholderiales and class Betaproteobacteria, which accounted for 16.52% and 15.83%, respectively, of the overall effect (Figure 7).

Sensitivity Analysis

Several sensitivity analyses were employed to assess and address potential pleiotropy in the causal estimates. Based on the MR-Egger regression intercept approach, the findings suggested that genetic pleiotropy did not introduce bias into the results. Additionally, the MR-PRESSO analysis indicated the absence of horizontal pleiotropy in the MR study. Cochran's Q-test and funnel plots revealed no heterogeneity or asymmetry among the SNPs concerning their causal relationships (Supplementary Fig. S5). The impact of each SNP on the overall causal estimates was validated using

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Table 2 Causal Effects of Mitochondrial Biological Functions on SUA Levels

ID	Name	IVW		$P_{heterogeneity}$	$P_{Egger\ intercept}$	$P_{pleiotropy}$
		OR (95% CI)	P			
prot-a-1368	4-hydroxy-2-oxoglutarate aldolase	1.0189 (1.0056–1.0323)	0.0051	0.5475	0.9783	0.6050
prot-a-2025	NADH dehydrogenase [ubiquinone] iron-sulfur protein 4	1.0050 (1.0014–1.0087)	0.0065	0.4933	0.3114	0.4000
prot-a-896	Calcium uptake protein 3	0.9903 (0.9827–0.9980)	0.0137	0.6503	0.4014	0.6850
prot-a-1761	Lon protease homolog	0.9917 (0.9843–0.9991)	0.0276	0.9245	0.9105	0.9550

Table 3 Causal Effects of Gout and SUA on Mitochondrial Biological Functions

Exposure	ID.outcome	Outcome	Method	Pval	OR/β(95% CI)
Gout	prot-a-1942	39S ribosomal protein L33	IVW	0.0498	−3.5679 (−7.1322, −0.0036)
Gout	prot-a-1055	Phenylalanine–tRNA ligase	IVW	0.0336	−4.6730 (−8.9824, −0.3637)
SUA	prot-a-1942	39S ribosomal protein L33	IVW	0.0281	0.8413 (0.7211, 0.9816)
SUA	prot-a-2129	ADP-ribose pyrophosphatase	IVW	0.0261	1.2116 (1.0231, 1.4349)

Table 4 Causal Effects of Gut Microbiota on Gout

Name	IVW		$P_{heterogeneity}$	$P_{Egger\ intercept}$	$P_{pleiotropy}$
	OR (95% CI)	P			
Creatinine degradation I	1.0019 (1.0003–1.0036)	0.0232	0.4385	0.2199	0.4890
Superpathway of fucose and rhamnose degradation	1.0017 (1.0002–1.0031)	0.0229	0.7637	0.4118	0.7740
Alacturonate degradation I	1.0026 (1.0005–1.0047)	0.0160	0.9678	0.6439	0.9760
Heme biosynthesis II	1.0024 (1.0005–1.0043)	0.0155	0.1626	0.5028	0.1700
glutamate degradation V	1.0022 (1.0002–1.0041)	0.0306	0.3124	0.5123	0.3270
Superpathway of phospholipid biosynthesis I	0.9978 (0.9960–0.9995)	0.0124	0.7445	0.4312	0.7650
Polyisoprenoid biosynthesis	0.9985 (0.9972–0.9998)	0.0216	0.4797	0.8897	0.5310
Superpathway of heme biosynthesis from glycine	1.0014 (1.0004–1.0024)	0.0081	0.2917	0.8443	0.3440
Purine nucleotides degradation II	0.9984 (0.9968–1.0000)	0.0450	0.6247	0.9208	0.6510
Peptidoglycan biosynthesis IV	1.0019 (1.0003–1.0036)	0.0225	0.3279	0.4259	0.1250
Superpathway of L-tyrosine biosynthesis	1.0012 (1.0000–1.0024)	0.0446	0.1192	0.6401	0.1330
Thiamin salvage II	0.9979 (0.9961–0.9997)	0.0205	0.9695	0.8011	0.9680
Superpathway of pyrimidine ribonucleosides degradation	1.0014 (1.0001–1.0026)	0.0339	0.6972	0.8854	0.7240
Superpathway of ubiquinol-8 biosynthesis	1.0015 (1.0001–1.0030)	0.0428	0.8886	0.9440	0.9090
Family Lactobacillaceae	0.9992 (0.9984–1.0000)	0.0487	0.5068	0.5692	0.4810
Family Clostridiales noname	1.0023 (1.0007–1.0040)	0.0054	0.5267	0.2989	0.5850
Genus Odoribacter	0.9983 (0.9965–1.0000)	0.0493	0.5531	0.7367	0.5520
Genus Paraprevotella	0.9986 (0.9972–0.9999)	0.0421	0.6237	0.6934	0.6530
Genus Dorea	1.0022 (1.0001–1.0042)	0.0434	0.8752	0.6322	0.8650
Bacteroides ovatus	0.9986 (0.9972–1.0000)	0.0496	0.9693	0.6947	0.9730
Bacteroides thetaiotaomicron	1.0023 (1.0004–1.0043)	0.0196	0.4311	0.4778	0.4550

a leave-one-out analysis (Supplementary Fig. S6). Following the removal of each SNP, we systematically re-conducted MR analyses for the remaining SNPs, which yielded consistent results that underscored the significance of all SNPs in establishing the causal relationship.

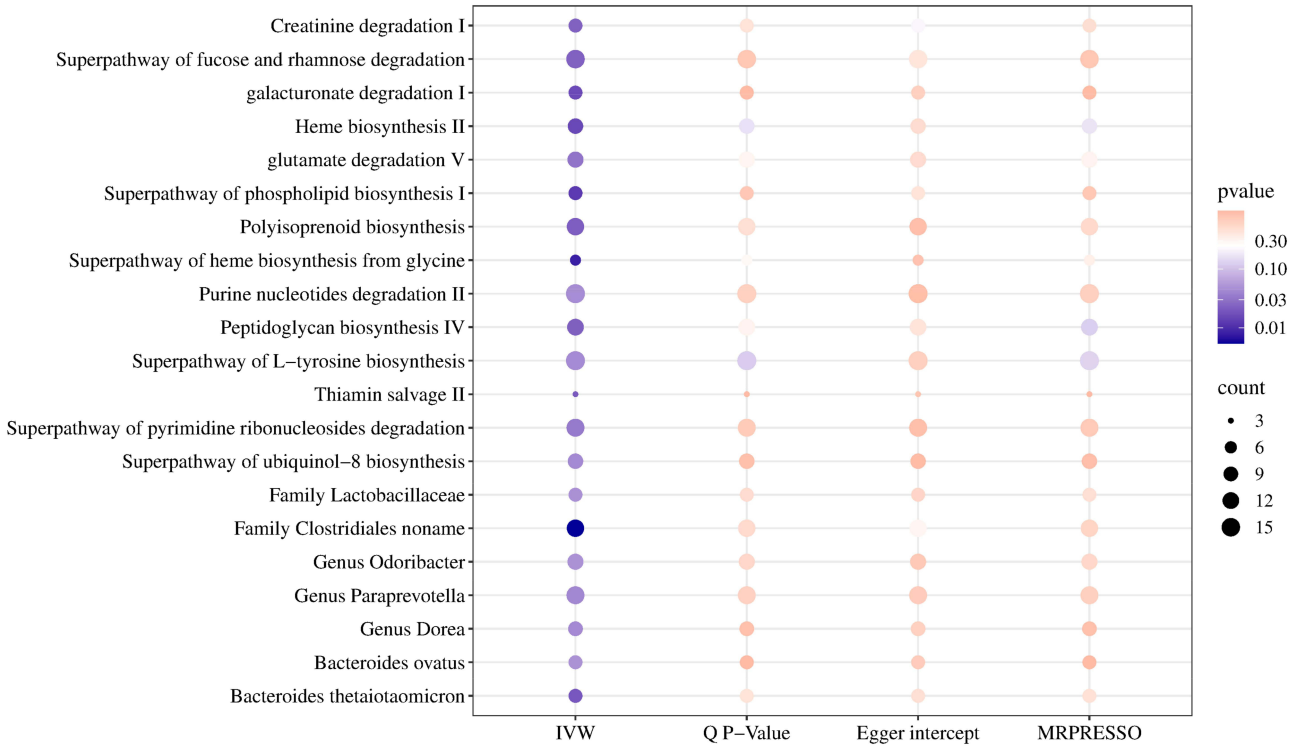

Figure 5 Mendelian randomisation results of the causal effects between gut microbiota and gout.

Discussion

This study systematically evaluated the causal associations between mitochondrial biological functions and the gut microbiota in relation to gout. Our results suggest that the SIRT5 may contribute to the adverse effects of gout by impairing creatinine degradation I. In contrast, calcium uptake protein 3 appears to mitigate the adverse effects of

Table 5 Causal Effects of Gut Microbiota on SUA Levels

Name	IVW		$P_{heterogeneity}$	$P_{Egger\ intercept}$	$P_{pleiotropy}$
	OR (95% CI)	P			
Glycolysis III from glucose	0.9853 (0.9717–0.9991)	0.0370	0.8254	0.4522	0.8040
Glycogen biosynthesis I from ADP-D-glucose	1.0147 (1.0004–1.0293)	0.0436	0.6075	0.2896	0.6130
Superpathway of glycerol degradation to 1,3-propanediol	1.0116 (1.0017–1.0216)	0.0211	0.3655	0.1357	0.3520
Lipid IVA biosynthesis	0.9806 (0.9659–0.9955)	0.0111	0.5164	0.9793	0.5880
Superpathway of polyamine biosynthesis I	0.9680 (0.9497–0.9867)	0.0008	0.3425	0.3178	0.4540
Phosphatidylglycerol biosynthesis I	0.9807 (0.9655–0.9961)	0.0144	0.1283	0.5584	0.1460
5-aminoimidazole ribonucleotide biosynthesis I	1.0258 (1.0075–1.0443)	0.0055	0.7481	0.4547	0.7680
Pyrimidine deoxyribonucleotides *de novo* biosynthesis IV	1.0217 (1.0010–1.0429)	0.0403	0.8178	0.9918	0.8240
Superpathway of L-lysine, L-threonine and L-methionine biosynthesis II	1.0162 (1.0013–1.0314)	0.0332	0.4015	0.3450	0.3920
Class Betaproteobacteria	1.0141 (1.0002–1.0282)	0.0470	0.3661	0.1735	0.3930
Genus *Roseburia*	1.0189 (1.0007–1.0373)	0.0419	0.0628	0.3238	0.0980
Genus Erysipelotrichaceae noname	0.9913 (0.9833–0.9994)	0.0352	0.5517	0.9307	0.5760
Order Burkholderiales	1.0147 (1.0009–1.0287)	0.0365	0.3815	0.1582	0.4040
Parabacteroides johnsonii	0.9933 (0.9867–0.9999)	0.0474	0.7452	0.5431	0.7590
Haemophilus parainfluenzae	1.0157 (1.0040–1.0275)	0.0084	0.3028	0.6368	0.3440
Bacteroides uniformis	0.9638 (0.9406–0.9875)	0.0029	0.1648	0.5870	0.2560

https://doi.org/10.2147/ORR.S513204

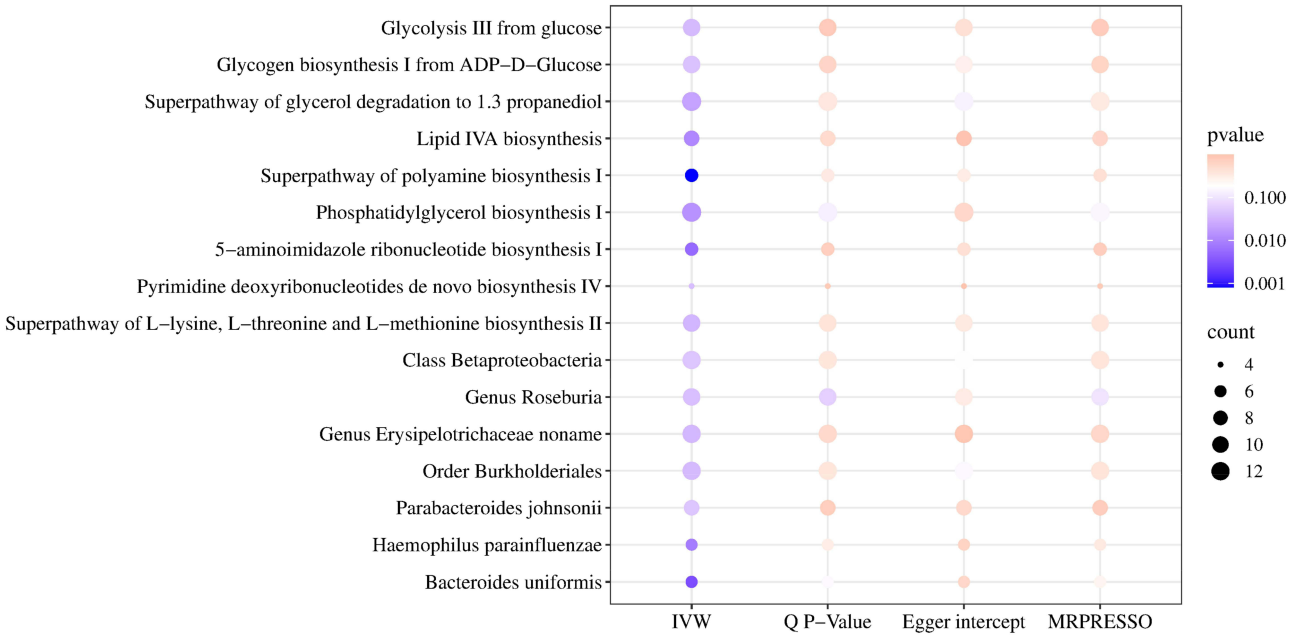

Figure 6 Mendelian randomisation results of the causal effects between gut microbiota and SUA levels.

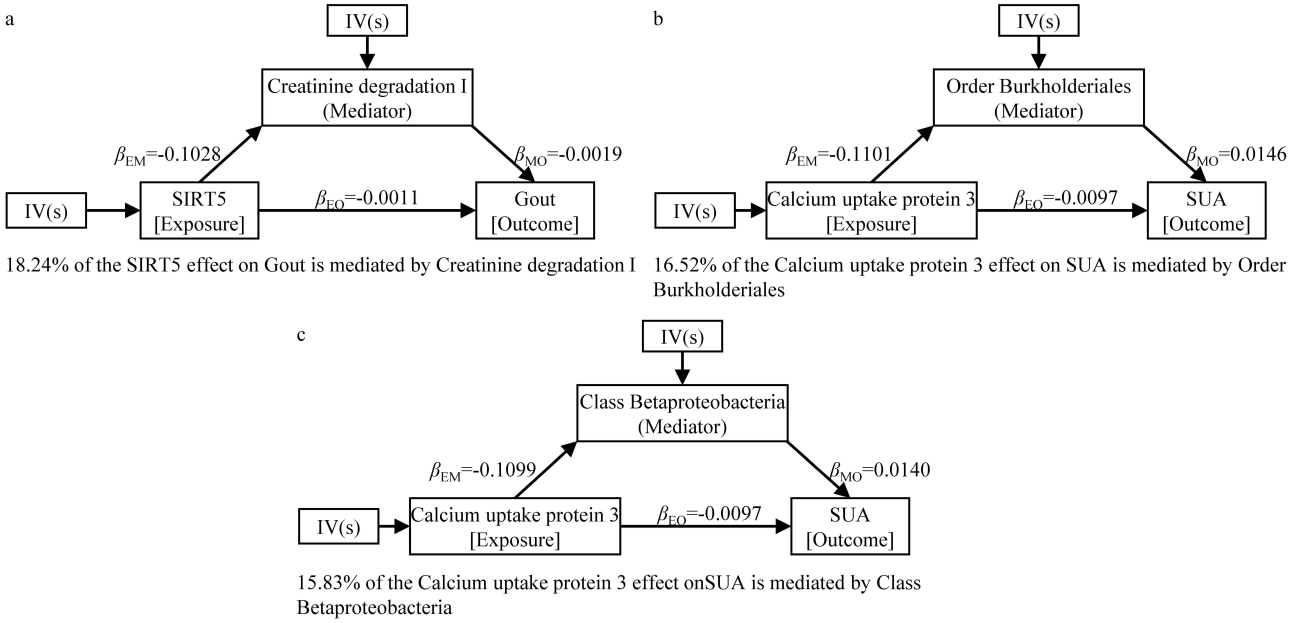

Figure 7 Regulatory effects of mitochondrial function on stroke via gut microbiota. (**a**) Effect of SIRT5 on gout mediated by creatinine degradation I. (**b**) Effect of calcium uptake protein 3 on SUA mediated by bacteria of the order Burkholderiales. (**c**) Effect of calcium uptake protein 3 on class Betaproteobacteria-mediated SUA.

elevated SUA levels by reducing the abundance of the order Burkholderiales and class Betaproteobacteria. Multiple sensitivity analyses excluded gene pleiotropy and heterogeneity bias, confirming the robustness and consistency of our findings. Although previous studies have separately explored the links between mitochondrial biological functions and gout, as well as between gut microbiota and gout,[22–24] a detailed investigation into the mediating role of gut microbiota in gout induced by mitochondrial biological functions remains lacking. The available evidence to date stems largely from observational research, which could be influenced by confounding causes. Therefore, our objective was to elucidate the

causal relationship between mitochondrial function and gout. Using MR analysis, we examined the association between mitochondrial biological functions and gout using available GWAS data to ascertain whether the causal relationship between them was mediated by the gut microbiota. Our findings suggest that genetically predicted gout is correlated with an increased risk of mitochondrial dysfunction, which is mediated by the gut microbiota.

Disruptions in mitochondrial function can disturb the internal environment of the intestine, resulting in elevated oxidative stress and changes in metabolite profiles. These alterations can affect the composition and diversity of the gut microbiota.[25] Previous studies have demonstrated a measurable (and significant) link between mitochondrial dysfunction and the gut microbiota. For example, exposure to harmful substances such as deoxynivalenol (DON) has been shown to impair mitochondrial function, resulting in disruption of the internal mitochondrial environment. Studies have shown that mitochondrial dysfunction leads to damage and apoptosis in intestinal epithelial cells, thereby compromising the integrity and functionality of the intestinal barrier. Further, abnormal mitochondrial function may affect the production of adhesion junction proteins by intestinal epithelial cells, further exacerbating intestinal barrier disruption. Abnormal mitochondrial function could be a significant mechanism underlying intestinal barrier impairment and its impact on the composition and diversity of the intestinal flora. Further studies will help to better understand this relationship and develop interventions to protect intestinal barrier function.[26]

Altered mitochondrial function can potentially result in changes to the mitochondrial membrane potential and the release of mitochondrial reactive oxygen species, influencing the onset and progression of gout. SIRT5 is involved in regulating the acetylation state of mitochondrial proteins, affecting biological processes such as energy metabolism, oxidative stress, and inflammatory responses. The importance of SIRT5 in metabolism, disease, and cellular physiology has attracted much attention.[27] Creatinine is a metabolite normally produced from creatine phosphate in the muscles. It is released from the muscles into the bloodstream and is then excreted out of the body through the kidneys. Consequently, the kidneys are the primary organ of creatinine metabolism and excretion. Creatinine degradation I refers to the process of creatinine degradation. Although no direct metabolic relationship is known between creatinine and uric acid, both are associated with renal function and metabolism. Therefore, investigating whether creatinine degradation is associated with gout development is worthwhile.[28] Furthermore, impaired SIRT5 function can potentially enhance the breakdown of creatinine, leading to an increased risk of gout. This can be achieved by regulating metabolic processes, the immune system, and the production of naturally occurring substances inside the body.

Mitochondria, the energy supply stations of the cell, function as important nodes in the intracellular calcium signalling network. Mitochondrial calcium uptake is crucial for normal cellular physiological functions, such as stimulating ATP production, inhibiting autophagy, modulating intracytoplasmic calcium signalling, and regulating cell death. Unidirectional transporters on the inner mitochondrial membrane facilitate the movement of calcium ions from the cytoplasm into the mitochondrial matrix, driven by membrane potential.[29] Among these regulators, Calcium uptake protein 3, or mitochondrial calcium uptake protein 3 (MICU3), is a member of the mitochondrial calcium-regulatory protein family. MICU3 plays a significant role in controlling mitochondrial calcium ion uptake. MICU3 facilitates the uptake of calcium ions into mitochondria, thereby influencing intracellular calcium levels and associated cellular processes. MICU3 function may be intricately linked to synaptic activity and neuronal signalling pathways in neurons. Previous studies have demonstrated that MICU3 contributes significantly to the regulation of cellular functions and intracellular signalling cascades.[30–32]

We found a potential positive causal association between MICU3 and SUA in our MR study, suggesting that the tissue-specific regulation of MICU3 could be linked to the initiation and advancement of various diseases. The disease types in which aberrant expression or dysfunction of MICU3 in specific tissues may be implicated include neurological, metabolic, and cardiac disorders.[33] These findings align with the outcomes of our study. We also found that MICU3 dysfunction can increase SUA levels by increasing the abundance of bacteria from the order Burkholderiales and class Betaproteobacteria. Species within the order Burkholderiales are recognized for posing significant risks to patients in intensive care units and to individuals with chronic lung disease.[34] Specifically, bacteria within the genus Burkholderia exhibit considerable diversity and variability, with some species, such as Burkholderia pseudomallei, capable of causing illness in healthy individuals.[35] The class Betaproteobacteria also includes some species harmful to humans; for example, Bordetella pertussis, and some species belonging to the genus Neisseria can cause human diseases. In conclusion, gut

microbiota-mediated mitochondrial dysfunction is an important mechanism in gout pathogenesis. A thorough understanding of the interplay between the gut microbiota and mitochondrial function could unveil novel pathways involved in gout development and present fresh targets for its prevention and treatment.

Although the associations identified in this study suggest a potential causal link, we acknowledge that the observed genetic effect sizes were relatively small, raising concerns about clinical relevance. However, small effect sizes are common in complex traits, where phenotypic variation is shaped by the combined effects of multiple genetic and environmental factors. Importantly, even modest genetic effects may become clinically meaningful when interacting with other risk factors or accumulating over time. Therefore, future studies should investigate the interactions between these genetic variants and additional biological pathways, as well as the influence of lifestyle and environmental exposures, to better understand how small genetic effects may translate into meaningful clinical outcomes.

Our study has several strengths. First, it offers a unique research perspective by investigating the causal relationship between mitochondrial biological functions, gut microbiota, and gout using an MR approach. Compared with observational studies, the advantage of the MR design lies in its ability to directly discern causality, thereby mitigating confounding and reverse causality issues. Second, by leveraging data from genomic association studies, our in-depth analysis of the association among mitochondrial biological function, intestinal flora, and gout provides crucial support for research in related domains. Third, through a systematic assessment of the effects of mitochondrial biological functions on gout, it elucidates the potential impact of mitochondrial dysfunction on intestinal flora, offering novel insights into gout pathogenesis. These findings suggest a significant causal relationship between disrupted mitochondrial function and gout development mediated by alterations in the gut microbiota.

Despite the significant contributions of this study, we acknowledge the following limitations. First, the limited availability of GWAS data and the considerable heterogeneity or multi-directionality of certain results may weaken the robustness of some conclusions, particularly those drawn from single data sources. Additionally, our study focused exclusively on participants of European descent and did not explore the influence of sexual dimorphism on genetic characteristics. Consequently, our findings may not be generalizable to other ethnic populations, the necessitating validation in diverse cohorts. MR studies exploring causal associations between exposures and outcomes with genetic variants can attenuate confounding and reverse causation and are suitable for assessing long-term health effects. However, genetic pleiotropy, population structure and insufficient strength of instrumental variables may still affect the results. Combined with prospective cohort or experimental studies, the effects of short-term interventions and personalized treatment can be further validated, providing pragmatic evidence for precision medicine. Finally, although our Mendelian randomization analysis identified potential causal links between gut microbiota composition and gout, we acknowledge that multiple external factors—such as diet, lifestyle habits, and medication use—can influence gut microbial communities and confound the observed associations. Specifically, dietary patterns, alcohol consumption, physical activity, and the use of antibiotics or urate-lowering therapies are known to shape gut microbiota and may modify gout risk. In this study, although we employed genetic instruments to mitigate confounding, the inability to directly adjust for these lifestyle and pharmacological variables represents a notable limitation. Therefore, future studies should aim to incorporate more comprehensive measurements and implement stratified or sensitivity analyses to account for these potential confounders. Such efforts would provide a clearer understanding of the complex interplay between genetic predispositions, gut microbiota alterations, and environmental factors in the development of gout. Furthermore, future research should advance along several key dimensions. First, systematic studies in larger, multi-center, and more ethnically diverse cohorts are needed to verify the external validity and generalizability of the current findings and to identify potential population-specific risk factors and mechanisms. Second, there is an urgent need to integrate multi-omics data—such as genomics, transcriptomics, proteomics, metabolomics, and single-cell histology—into a multi-level analytical framework spanning cellular, tissue, and systemic levels. This would allow for a more comprehensive investigation of the complex interactions between mitochondrial function, gut microbiota, and uric acid metabolism, and elucidate the underlying molecular mechanisms involved in the development and progression of gout. Based on these findings, the direct effects of key molecules, microbiota, and their metabolites on uric acid metabolism and gout pathogenesis should be validated through functional studies, including in vivo and in vitro experiments, to provide robust biological evidence supporting the results of observational and MR analyses. Finally,

potential therapeutic targets suggested by this study, including gut microbiota modulation and mitochondrial function restoration, should be systematically evaluated in terms of safety and efficacy through interventional and clinical studies, particularly exploring their synergistic effects when combined with conventional therapies. A multidisciplinary and multi-strategy approach is expected to promote the development of novel diagnostic tools and targeted interventions, enriching our understanding of gout pathogenesis and supporting precision prevention and treatment of gout and related metabolic disorders.

Conclusion

In conclusion, our analysis elucidated the complex relationships between mitochondrial function, gut microbiota, and gout among individuals of European ancestry, offering novel perspectives for gout prevention and treatment. This MR analysis presented in this study offers valuable insights into the causal relationships among mitochondrial biological function, gut microbiota, and gout. Disruptions in mitochondrial function potentially disturb the intestinal environment, impacting the composition and diversity of the gut microbiota, thereby influencing gout onset. These results underscore the intricate interplay between cellular bioenergetics, intestinal flora, and metabolic disorders like gout. Future research should thus prioritize elucidation of the mechanistic pathways linking mitochondrial dysfunction, gut microbiota alterations, and gout pathogenesis, with the goal of developing therapeutic strategies and personalised interventions to manage this prevalent arthritic condition. However, further research in more diverse populations is warranted to improve our understanding of these biological processes and inform future intervention strategies.

Abbreviations

SIRT5, NAD-dependent protein deacylase sirtuin-5; GWAS, genome-wide association study; IV, instrumental variable; IVW, inverse variance weighting; LD, linkage disequilibrium; MR, Mendelian randomisation; OR, odds ratio; SNP, single nucleotide polymorphism; SUA, serum uric acid.

Ethics

All data used in this study were obtained from a publicly available, de-identified secondary database that had been reviewed and approved by its original ethics committee during the data collection and release phases. Personally identifiable information was adequately protected and removed, ensuring that no individual could be identified. We consulted with the Ethics Committee/Institutional Review Board (IRB) of our institution regarding this study. Given that this study involved only secondary analysis of publicly available, de-identified data, did not entail new data collection, and imposed no interventions on human subjects, the Ethics Committee determined that it was eligible for exemption from further review. In addition, this project (No. ZHY2022-131) has passed the ethical review (HZYLLBA2022029).

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests in this work.

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