Research article

Causal effects of gut microbiota on the risk of urinary tract stones: A bidirectional two-sample mendelian randomization study

Yongdong Pan, Jingyi Su, Shengnan Liu, Yueyan Li, Guofeng Xu [*]

Xinhua Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

ARTICLE INFO

ABSTRACT

Background: Recent studies increasingly suggest notable changes in both the quantity and types of gut microbiota among individuals suffering from urinary tract stones. However, the causal relationship between GMB and urinary tract stone formation remains elusive, which we aim to further investigate in this research through Mendelian Randomization (MR) analysis.

Materials and methods: Single nucleotide polymorphisms (SNPs) associated with the human GMB were selected from MiBioGen International Consortium GWAS dataset. Data on urinary tract stone-related traits and associated SNPs were sourced from the IEU Open GWAS database. To investigate the causal relationships between gut microbiota and urinary tract stones, Mendelian Randomization (MR) was applied using genetic variants as instrumental variables, utilizing a bidirectional two-sample MR framework. This analysis incorporated various statistical techniques such as inverse variance weighting, weighted median analysis, MR-Egger, and the maximum likelihood method. To ensure the reliability of the findings, a range of sensitivity tests were conducted, including Cochran's Q test, the MR-Egger intercept, leave-one-out cross-validation, and examination of funnel plots.

Results: The results revealed the causal relationship between the increase in the abundance of 10 microbial taxa, including Genus-Barnesiella (IVW OR $= 0.73$, 95%CI 0.73–0.89, P $= 2.29 \times 10^{-3}$) and Genus-Flavonifractor (IVW OR $= 0.69$, 95%CI 0.53–0.91, P $= 8.57 \times 10^{-3}$), and the decreased risk of urinary tract stone formation. Conversely, the development of urinary tract stones was observed to potentially instigate alterations in the abundance of 13 microbial taxa, among which Genus-Ruminococcus torques group was notably affected (IVW OR $= 1.07$, 95%CI 0.64–0.98, P $= 1.86 \times 10^{-3}$). In this context, Genus-Clostridium sensustricto1 exhibited a bidirectional causal relationship with urinary tract stones, while the remaining significant microbial taxa demonstrated unidirectional causal effects in the two-sample MR analysis. Sensitivity analyses did not identify significant estimates of heterogeneity or pleiotropy.

Conclusion: To summarize, the results of this study suggest a likely causative link between gut microbiota and the incidence of urinary tract stones. This insight opens up potential pathways for discovering biomarkers and therapeutic targets in the management and prevention of urolithiasis. However, further in-depth research is warranted to investigate these associations.

* Corresponding author.
  E-mail address: 18556355759@163.com (G. Xu).

1. Introduction

Urinary tract stones are a commonly encountered urological condition, affecting an estimated 3%–20% of the global population [1]. According to available data, expenditures by the United States for addressing kidney stones through preventive and treatment measures exceeded $5 billion in the year 2005 [2]. Furthermore, as the incidence of kidney stones and its associated risk factors (obesity, diabetes) continue to rise, it is projected that annual expenditures will increase by $1.24 billion by the year 2030 [3]. Simultaneously, urinary tract stones contribute to the etiology of diseases including renal dysfunction [4] and cardiovascular issues [5]. In recent years, minimally invasive treatment methods for urolithiasis have rapidly advanced, accompanied by gradual improvements in treatment outcomes. However, there has been minimal progress in pharmacological treatment over the past three decades [6]. Urinary tract stones are prone to recurrence, with approximately one-third of initial sufferers experiencing relapse, and an even greater risk of recurrence among those who have already relapsed [7]. Consequently, delving into the pathophysiological underpinnings that lead to the formation of urinary tract stones becomes crucial. This exploration is vital for developing innovative strategies in pharmacological treatment.

In a healthy state, the human gastrointestinal tract hosts a vast number of bacteria, forming a unique ecosystem known as the intestinal microbiota [8]. This intestinal microbiota, also referred to as the gut microbiota (GMB), actively participates in modulating human metabolism and immune activities, contributing significantly to maintaining homeostasis. Furthermore, accumulating research indicates a link between gut microbiota and the onset and advancement of various illnesses, such as metabolic syndromes, autoimmune conditions, and cancers [9]. The gut-kidney axis concept has recently become more prominent, spurring heightened investigative attention on the gut microbiota's contribution to urinary tract stone development [10]. Research findings demonstrate notable changes in the gut microbiota's diversity and composition among urinary tract stone patients, suggesting an imbalance in the microbial ecosystem and the associated disruption of the gut barrier [11,12]. Compromised gut barrier integrity can lead the imbalanced gut microbiota to potentially incite systemic inflammation, oxidative stress, and immune system malfunctions through the release of metabolites like lipopolysaccharides and gut-derived uremic toxins. These processes can worsen the occurrence and progression of urinary tract stone formation [13]. Studies have underscored a linkage between gut microbiota and the incidence of urinary tract stones, implying that gut microbiota could be a contributing pathogenic element in their formation. Nonetheless, in-depth and thorough biomedical and observational investigations are essential to ascertain the precise causal relationship of gut microbiota with urinary tract stone development and to pinpoint the specific bacterial species that elevate the risk of their formation. Such research endeavors are recognized for their high costs, intensive labor requirements, and complex control demands.

Mendelian randomization, known for its efficacy in causal inference, employs single nucleotide polymorphisms as genetic instrumental variables to replicate the conditions of randomized controlled trials. This strategy significantly reduces the impact of confounding factors and reverse causality, enabling extensive exploration of genetic associations with diseases across large populations [14,15]. Importantly, given that gut microbiota does not change an individual's DNA sequence [16], Mendelian randomization is a valuable tool to thoroughly investigate the link between urinary tract stones and gut microbiota.

This approach significantly contributes to further elucidating how the GMB influences the occurrence, progression, and treatment of urinary tract stones.

Hence, the objective of this study is to clarify the possible causative influence of the gut microbiota on the likelihood of urinary tract stone formation, employing MR for this analysis. Furthermore, the investigation will delve into the reciprocal nature of these causal interactions, aiming to provide a comprehensive insight into the intricate interplay between the gut microbiota and urinary tract stones.

2. Methods

This research considered each bacterial group within the GMB as a unique variable for exposure or outcome analysis. To delve into the reciprocal causative dynamics between urinary tract stones and gut microbiota, we implemented a two-way MR analysis using integrated data from extensive GWAS. Our approach encompassed four different MR techniques: IVW, MR-Egger regression analysis, the application of the weighted median strategy, and ML evaluation. Moreover, we engaged in an array of sensitivity checks, including but not limited to Cochran's Q test, Egger intercept examination, and the MR PRESSO global assessment. The aim of these thorough sensitivity checks was to meticulously probe for any heterogeneity and the existence of horizontal pleiotropy within the MR findings, thereby solidifying the validity and trustworthiness of our conclusions.

2.1. Selection of GMB-related SNPs

SNPs related to human GMB were chosen as IVs from the MiBioGen International Consortium's GWAS dataset. This study constituted a large-scale association research involving 18,340 participants across 24 cohorts [17]. Participants in this study were drawn from a range of countries, namely the United Kingdom, South Korea, Germany, the United States, Sweden, Israel, Finland, Denmark, Belgium, the Netherlands, and Canada. The majority of participants, specifically in 16 cohorts (N = 13,266), had European ancestral origins. Within the 24 distinct cohorts, 17 of these (encompassing 13,804 participants) consisted of individuals whose average age fell between 50 and 62 years. The mapping analysis for quantitative microbiota-associated genetic loci (mbQTL) across each cohort considered only those taxonomic units that appeared in more than 10% of the samples. Altogether, this comprehensive analysis included 211 different taxonomic units. The mapping analysis for binary trait loci (mbBTL) considered taxonomic units occurring at a frequency between 10% and 90% in the eligible samples.

For the selection of SNPs in each classification group, a rigorous filtering methodology was employed: 1) SNPs linked with GMB were identified as instrumental variables (IVs) using a genome-wide significance level of 5×10^{-5}; 2) Strict selection criteria, including a linkage disequilibrium cutoff of $r^2 < 0.001$ and a maximum distance criterion of 10,000 kb, were imposed to guarantee IVs' independent selection under specific parameters; 3) The F statistic for each SNP was computed, and those with $F < 10$ were excluded to reduce the risk of weak instrument bias [18]; 4) Palindromic SNPs, which presented incompatible genotypes or median allele frequencies, were discarded during the process of harmonization.

2.2. Selection of urinary tract stones occurrence related SNPs

We gathered all the relevant traits associated with urinary system stones by downloading the Urolithiasis Dataset: finn-b-N14_UROLITHIASIS from the IEU Open GWAS project, accessible at https://gwas.mrcieu.ac.uk/datasets/finn-b-N14_UROLITHIASIS/. The GWAS dataset includes data related to urinary tract stones, and covers various phenotypes, including "kidney stones, ureter stones, and bladder stones". The research involved a total of 218,792 individuals, comprising 5347 individuals with the condition under study and 213,445 control subjects. Notably, all participants in this research had a similar genetic heritage, with each being of European ancestry.

2.3. Statistical analysis

2.3.1. Two-sample mendelian randomization (MR) analysis

As depicted in Fig. 1, our analysis involved estimating the causative link between the gut microbiota and urinary tract stone formation by applying the traditional Mendelian Randomization model: βcausal effect = βZY/βZX (where βZX and βZY represent the regression coefficients of gut microbiota SNPs and urinary system stone SNPs, respectively). In Mendelian Randomization analysis, genetic variations are utilized as IVs to deduce causative connections between exposure factors and outcome variables. To ensure effective and unbiased estimation, three critical assumptions need to be satisfied in forward MR analysis, as exemplified below: (1) The IV must have a genuine association with the GMB (in this study, genetic associations were defined as $p < 5 \times 10^{-5}$); (2) It is essential that the chosen instrumental variable remains unaffected by any confounding elements associated with both the gut microbiota and the occurrence of urinary system stones; (3) The IVs should not directly affect urinary system stones but should influence them solely through their association with GMB.

To determine the potential causative effects of GMB on the prevalence of urinary tract stones, this study integrated a multitude of SNPs in a detailed bidirectional two-sample MR analysis. This analysis was conducted using a quartet of sophisticated techniques: IVW, MR-Egger regression, weighted median analysis, and the ML approach. In instances where GMB categories demonstrated P-values below 0.05 in the preliminary MR analysis with IVW, an expanded array of six diverse methods was utilized. These included not only IVW, MR-Egger, and weighted median, but also ML, simple mode, and weighted mode techniques. This comprehensive and meticulously executed strategy was designed to validate the precision, all-encompassing nature, and repeatability of our investigative findings.

Within the array of four MR techniques used, the IVW outcomes were regarded as the principal MR estimates, and their congruence with the results of other MR methods was evaluated. During the forward MR analysis, the Bonferroni adjustment was utilized to regulate the level of significance. The modified significance threshold according to Bonferroni for a set containing n bacterial taxa is calculated as 0.05/n. As an example, at the phylum level in MR results, the adjusted threshold for p-values was established at 0.0056 (0.05 divided by 9). Bacterial taxa are deemed to hold nominal significance if their p-value post-Bonferroni correction falls below 0.05. In reverse MR analysis and additional tests, bi-directional p-values under 0.05 were treated as statistically significant.
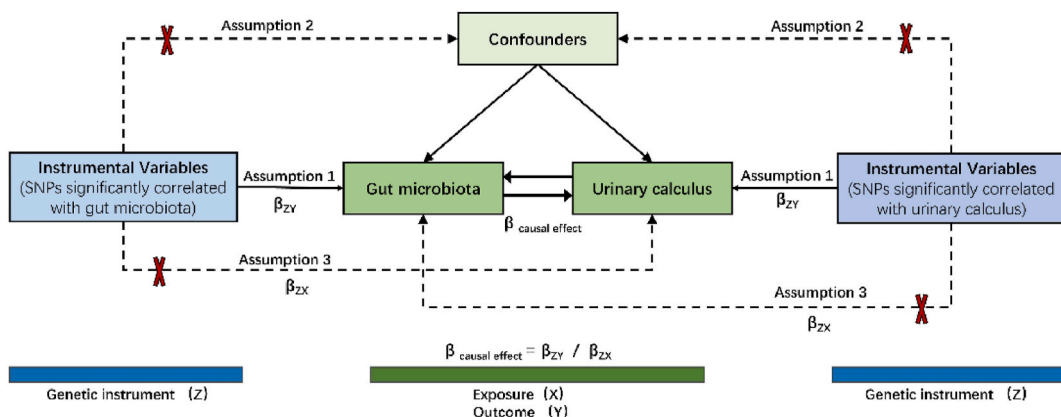

Fig. 1. An overview of the study design. SNP, single nucleotide polymorphisms.

2.3.2. Sensitivity analysis

Cochran's Q test was employed to assess the heterogeneity of the MR results, where a significance level of $p < 0.05$ would indicate significant heterogeneity in SNP effect estimates. We assumed the absence of horizontal pleiotropy when the intercept of the MR-Egger analysis approached zero, and the results of both IVW and MR-Egger analyses were consistent. To identify and address potential outliers that might independently influence the observed causal relationships, we conducted a leave-one-out analysis. Additionally, we utilized the Mendelian Randomization-Pleiotropy Residual Sum and Outlier (MR-PRESSO) test, which evaluates overall horizontal pleiotropy by comparing the observed distances between all SNPs and the regression line (sum of squared residuals) with the expected distances under the null hypothesis of no horizontal pleiotropy. The results of the MR-PRESSO global test are presented, and if heterogeneity or horizontal pleiotropy was detected, MR estimates were recalculated using the outlier SNPs identified by MR-PRESSO.

For the MR analysis, we utilized the "TwoSampleMR," "mr-raps," and "MR-PRESSO" packages in R (version 4.2.1), ensuring the application of robust and well-established tools and methods for causal inference in our study.

3. Results

To identify significant SNPs, our strategy incorporated several stages, initiating with a genome-wide significance criterion ($p < 1 \times$
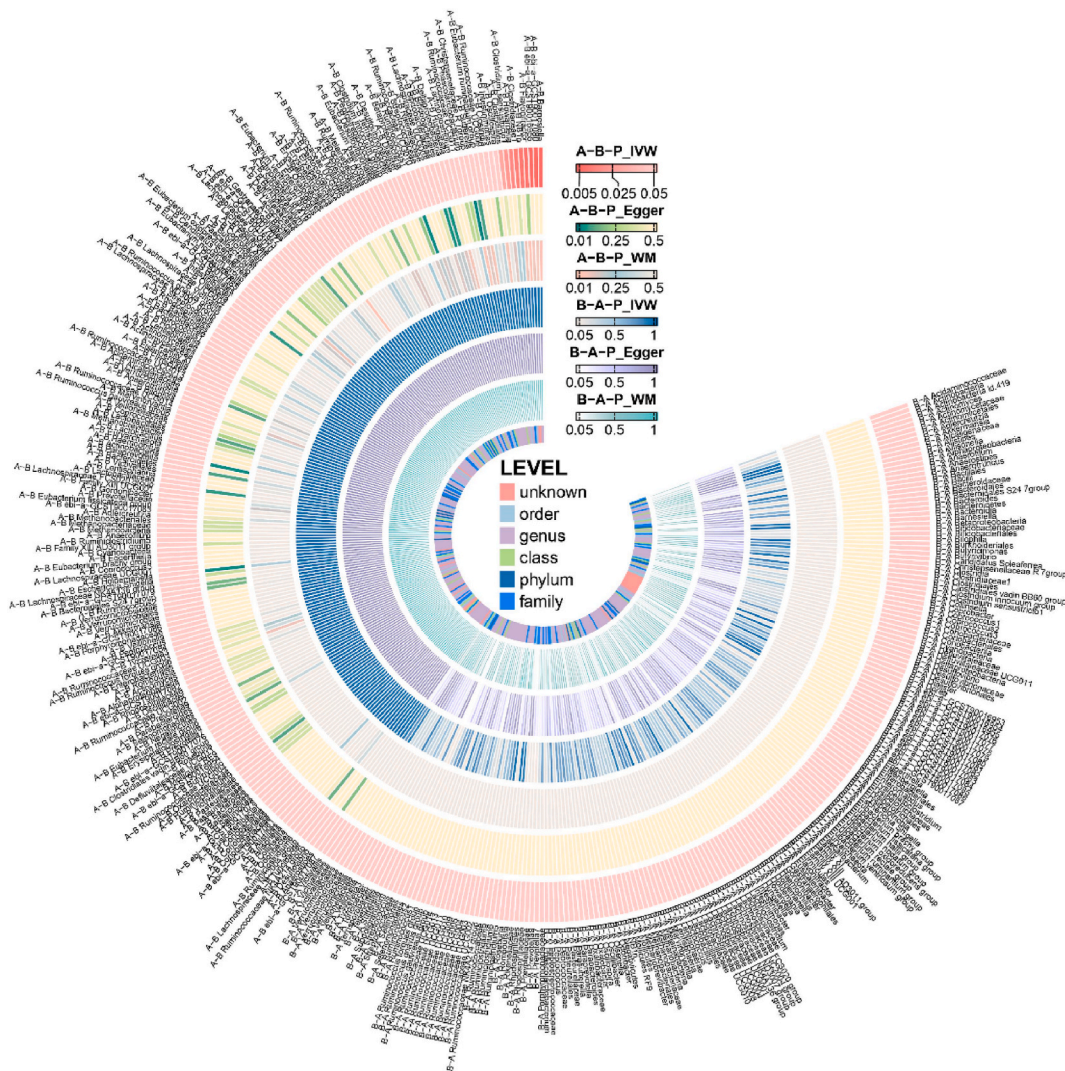
Fig. 2. Assessment of the relationships between GMB and urolithiasis using MR. 'A-B' indicates the analysis with GMB as the influencing agent and urolithiasis as the resultant condition, whereas 'B-A' represents the reverse scenario, suggesting urolithiasis as the influencing factor. Concentric circles illustrate estimates from three different MR methodologies: inverse variance weighted, MR-Egger, and weighted median, each providing unique insights in the bidirectional analysis. Color intensity variations in the graphic signify the range of p-values, offering a visual depiction of each estimate's statistical relevance. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

10⁻⁵). Following this, we undertook LD testing, harmonization, MR-PRESSO evaluation, and validation through F statistics. SNPs classified as outliers in the MR-PRESSO global test (p < 0.05) were not included in our final analysis. This careful selection process led to the removal of these SNPs. Importantly, all remaining SNPs exhibited F statistics exceeding 10, signifying a robust correlation between instrumental variables and their respective bacterial taxa. For the forward MR analysis, we included 204 bacterial taxa, while the reverse MR analysis included 203 bacterial taxa, with each category having multiple SNPs ranging from 3 to 27. To interpret the causal effects in our bidirectional MR analysis, we focused on the intersection of 194 bacterial taxa, and we summarized all the results of bidirectional MR in Fig. 2. Additionally, significant results from the IVW method are presented in Fig. 3. For a comprehensive list of the retained SNPs and associated statistical data, please refer to Supplementary Tables S3 and S4. These tables provide detailed information on the SNPs included in our analysis and the corresponding results.
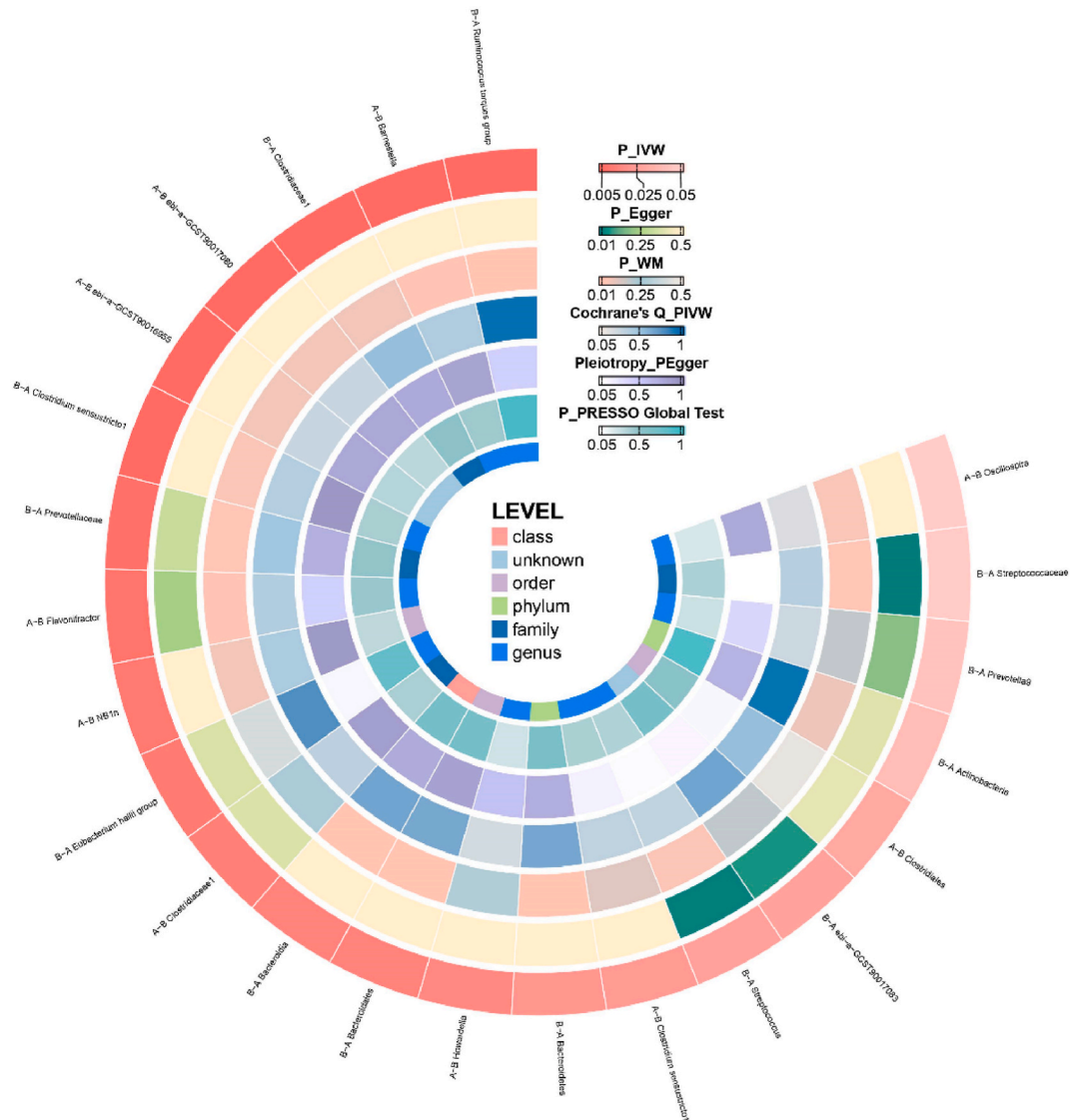
Fig. 3. This diagram presents MR findings and the outcomes of sensitivity assessments for the notable correlations between GMB and urolithiasis. The notation 'A-B′ represents GMB as the influencing factor and urolithiasis as the resultant event, with 'B-A′ illustrating the inverse relationship. The graphic transitions from external to internal rings, detailing estimates derived via diverse MR approaches, including inverse variance weighted, MR-Egger, and weighted median methods, complemented by sensitivity evaluations like Cochran's Q test, Egger intercept, and MR-PRESSO global test in the bidirectional MR framework. The gradations of color within the figure correlate with the p-value magnitudes, providing a visual tool to interpret the statistical impact of both the MR estimates and sensitivity analysis outcomes. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.1. Causal impact of GMB on urolithiasis

In the forward MR IVW analysis, a total of 10 bacterial taxa exhibited a significant association with urolithiasis where P < 0.05 (refer to Table 1 and Fig. 4). Supplementary Table S5 provides detailed results for all analytical methods for these 10 taxa. Unfortunately, ebi-a-GCST90017080 and ebi-a-GCST90016955 were categorized as unknown taxa in the original study. Among the remaining 8 bacterial taxa, no statistically significant causal relationship with urinary tract stones was observed post strict Bonferroni correction. However, we stated that there exists a notable causal effect of Genus-Barnesiella (IVW OR = 0.73, 95% CI 0.73–0.89, P = 2.29×10^{-3}) and Genus-Flavonifractor (IVW OR = 0.69, 95% CI 0.53–0.91, P = 8.57×10^{-3}) on urolithiasis within the urinary system. Initially, at the genus classification level, applying the Bonferroni adjustment leads to a rigorous threshold, with the IVW findings for both Genus-Barnesiella and Genus-Flavonifractor approaching this stringent threshold. Additionally, the outcomes from alternative MR methodologies, such as WM, MR-Egger, ML, simple mode, and weight mode, corroborate the IVW findings (as seen in Fig. 5: A-J). Notably, as depicted in Fig. 4, all 10 studied taxa indicate a potential causal link with a reduced incidence of urolithiasis. The MR-Egger outcomes for Genus-Clostridium sensustricto1 and Order-Clostridiales show a deviation from the results of the other five MR methods, yet the IVW outcomes for the remaining taxa are in harmony with those derived from the other five MR methodologies.

3.2. Causal impact of urolithiasis on GMB

In the IVW analysis of reverse MR, a total of 13 taxonomic group classifications demonstrated P < 0.05, as presented in Table 2 and Fig. 6. Detailed results for all analytical methods for all 13 taxonomic groups can be found in Supplementary Table S6. Notably, Genus-Clostridium sensustricto1 exhibited P < 0.05 in the IVW analysis conducted in both casual directions, suggesting a bidirectional causal relationship between Genus-Clostridium sensu stricto 1 and urinary tract stones. In contrast, the remaining significant taxonomic groups indicated unidirectional causal relationships. Among the 13 taxonomic groups, the impact of urolithiasis on the Genus-Ruminococcus torques group was the most significant (IVW OR = 1.07, 95% CI 0.64–0.98, P = 1.86×10^{-3}). Moreover, as illustrated in Fig. 7 (A-M), the IVW analysis results were consistent with those obtained from the other three MR analysis methods.

3.3. Sensitivity analysis

In order to reduce possible biases, a thorough array of techniques was implemented to evaluate the sensitivity of the bidirectional MR analysis. Additionally, these methods were used to thoroughly examine the potential of pleiotropy affecting each phenotype IV. In the forward MR analysis, no outliers that could be causally associated with urinary tract stones were detected among the IVs of the 10 taxonomic groups. Similarly, in the reverse MR analysis, no outliers that could be causally associated with the 13 taxonomic groups were found among the urolithiasis IVs.

Tables 3 and 4 illustrate that the Cochrane's Q tests applied to both MR-Egger and IVW methods did not show significant heterogeneity, as all p-values were above 0.05. Symmetry was observed in the funnel plots, as detailed in Supplementary Figs. S1 and S2. The MR-Egger regression intercepts approached zero, implying a lack of horizontal pleiotropy. Additionally, leave-one-out analysis, depicted in Supplementary Figs. S3 and S4, confirmed that omitting any individual SNP from the analysis would not substantially change the outcome. The global test conducted by MR-PRESSO also did not yield any abnormal outcomes. Therefore, the findings derived from these comprehensive sensitivity analyses emphasize the steadfastness and dependability of our bidirectional MR analysis.

4. Discussion

As of now, this research stands as the inaugural bidirectional MR investigation assessing the causal influence of GMB on the likelihood of developing urinary tract stones. This analysis was based on a comprehensive GMB gene dataset and the largest available GWAS dataset. The study's outcomes elucidated a direct causal connection between a reduction in urinary stone formation risk and the presence of 10 specific microbial taxa, as evidenced by an odds ratio (OR) less than 1. Moreover, the research highlighted that the development of urinary tract stones might actively contribute to variations in the prevalence of 13 distinct microbial taxa. Notably,

Table 1
Significant MR results by IVW method. SNP, single nucleotide polymorphisms; βcasual effect, the effect size of the exposure on urolithiasis; SE, standard errors; OR, odds ratio; IVW, inverse-variance weighted.

Exposure	Method	Number of SNPs	$\beta_{casual\ effect}$	SE	P-value	OR
Genus-Barnesiella	IVW	13	−0.31	0.10	2.29×10^{-3}	0.73
ebi-a-GCST90017080	IVW	13	−0.20	6.50×10^{-2}	2.66×10^{-3}	0.82
ebi-a-GCST90016955	IVW	13	−0.20	6.51×10^{-2}	2.67×10^{-3}	0.82
Genus-Flavonifractor	IVW	5	−0.37	0.14	8.57×10^{-3}	0.69
Order-NB1n	IVW	12	−0.16	6.28×10^{-2}	1.03×10^{-2}	0.85
Family-Clostridiaceae1	IVW	10	−0.29	0.12	1.30×10^{-2}	0.74
Genus-Howardella	IVW	9	−0.16	6.80×10^{-2}	1.61×10^{-2}	0.85
Genus-Clostridium sensustricto1	IVW	7	−0.28	0.13	2.36×10^{-2}	0.75
Order-Clostridiales	IVW	13	−0.23	0.11	3.09×10^{-2}	0.79
Genus-Oscillospira	IVW	8	−0.25	0.13	4.96×10^{-2}	0.77

Exposure	Method	nSNP		OR(95%CI)	P-value
Genus-Barnesiella	IVW	13		0.73(0.60 to 0.89)	0.002
ebi-a-GCST90017080	IVW	13		0.82(0.72 to 0.93)	0.003
ebi-a-GCST90016955	IVW	13		0.82(0.72 to 0.93)	0.003
Genus-Flavonifractor	IVW	5		0.69(0.53 to 0.91)	0.009
Order-NB1n	IVW	12		0.85(0.75 to 0.96)	0.010
Family-Clostridiaceae1	IVW	10		0.74(0.59 to 0.94)	0.013
Genus-Howardella	IVW	9		0.85(0.74 to 0.97)	0.016
Genus-Clostridium sensustricto1	IVW	7		0.75(0.59 to 0.96)	0.024
Order-Clostridiales	IVW	13		0.79(0.64 to 0.98)	0.031
Genus-Oscillospira	IVW	8		0.77(0.60 to 1.00)	0.050

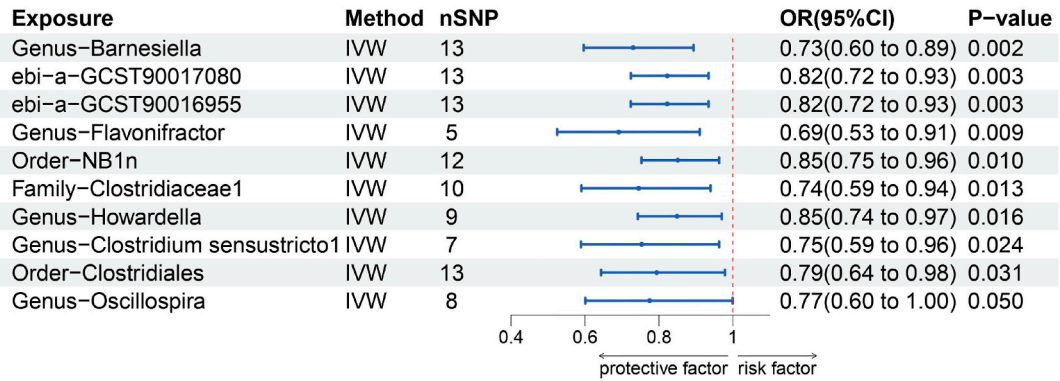0.4   0.6   0.8   1

protective factor   risk factor

Fig. 4. The figure illustrates the magnitude of influence each notable gut microbiota has on the development of urolithiasis. An odds ratio less than 1 implies a protective effect against urolithiasis, whereas an odds ratio greater than 1 suggests a contributory role in the disease's pathogenesis.
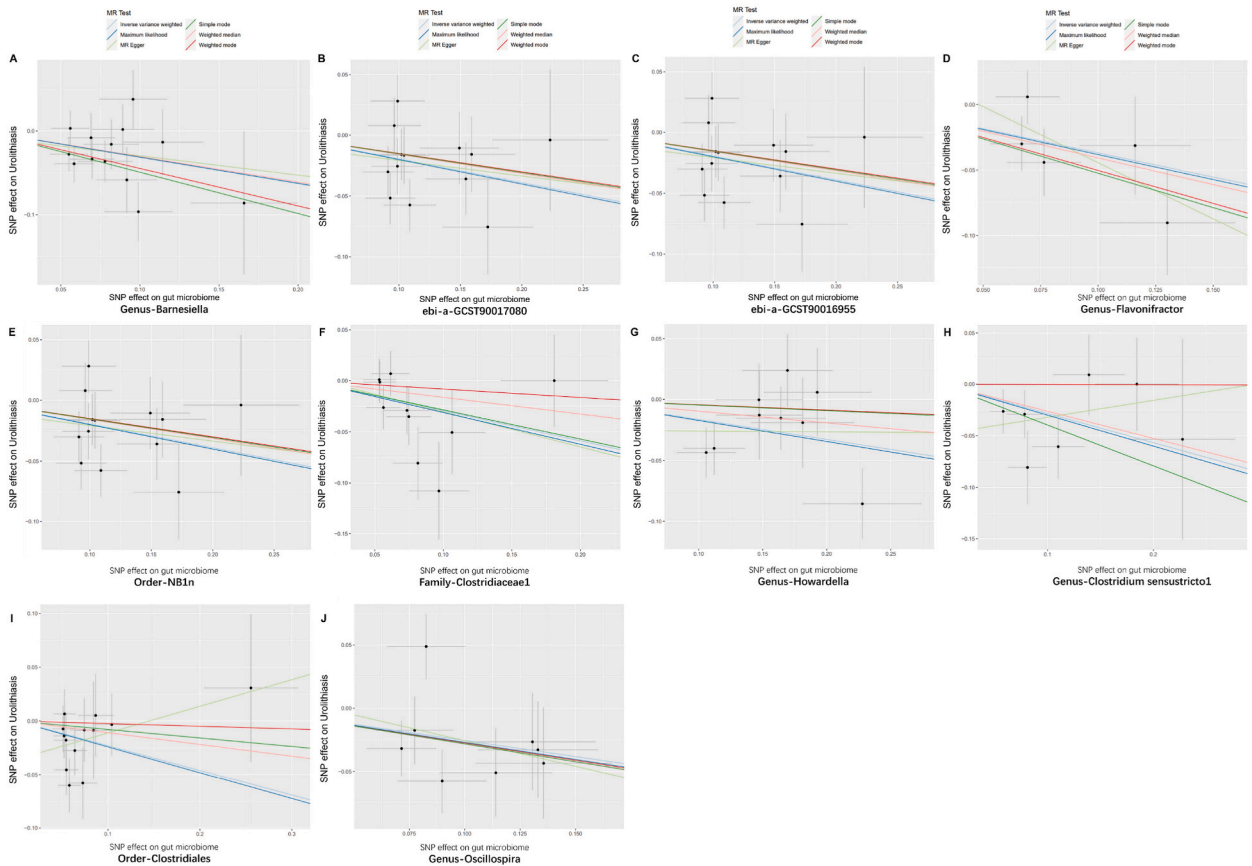

Fig. 5. Scatter diagrams representing the MR investigations that link 10 distinct gut bacterial groups with the likelihood of developing urolithiasis. In these analyses, SNP stands for single nucleotide polymorphisms, and MR denotes Mendelian randomization.

there is a mutual causal relationship between Genus-Clostridium sensu stricto 1 and urinary stone formation, while the remaining microbial taxa indicate unidirectional causal relationships in the bidirectional MR analysis. These findings illuminate the crucial influence of GMB in the development of urinary tract stones and offer pertinent insights that are instrumental for future research directions.

In recent years, there has been a gradual increase in the global incidence of urinary tract stones, driven by the growing population affected by related diseases such as diabetes and hypertension, as well as the changing trends associated with global warming [1,19]. Urinary tract stones, commonly referred to as urolithiasis, are capable of causing a range of complications such as urinary blockage, infections, discomfort, and potentially irreversible damage to the kidneys, thereby posing a substantial concern for public health [20].

Table 2

Significant reverse MR results by IVW method. SNP, single nucleotide polymorphisms; βcasual effect, the effect size of the exposure on urolithiasis; SE, standard errors; OR, odds ratio; IVW, inverse-variance weighted.

Exposure	Method	Number of SNPs	$\beta_{casual\ effect}$	SE	P-value	OR
Genus-Ruminococcus torques group	IVW	23	7.00×10^{-2}	2.25×10^{-2}	1.86×10^{-3}	1.07
Fanily-Clostridiaceae1	IVW	21	-7.72×10^{-2}	2.54×10^{-2}	2.42×10^{-3}	0.93
Genus-Clostridium sensustricto1	IVW	21	-7.42×10^{-2}	2.63×10^{-2}	4.82×10^{-3}	0.93
Family-Prevotellaceae	IVW	23	-6.57×10^{-2}	2.45×10^{-2}	7.44×10^{-3}	0.94
Genus-Eubacterium hallii group	IVW	22	5.93×10^{-2}	2.34×10^{-2}	1.12×10^{-2}	1.06
Class-Bacteroidia	IVW	23	-5.43×10^{-2}	2.21×10^{-2}	1.42×10^{-2}	0.95
Order-Bacteroidales	IVW	23	-5.31×10^{-2}	2.21×10^{-2}	1.64×10^{-2}	0.95
Class-Bacteroidetes	IVW	22	-5.22×10^{-2}	2.22×10^{-2}	1.89×10^{-2}	0.95
Genus-Streptococcus	IVW	22	5.52×10^{-2}	2.49×10^{-2}	2.65×10^{-2}	1.06
ebi-a-GCST90017083	IVW	19	-7.61×10^{-2}	3.46×10^{-2}	2.76×10^{-2}	0.93
Phylum-Actinobacteria	IVW	23	4.56×10^{-2}	2.23×10^{-2}	4.13×10^{-2}	1.05
Genus-Prevotella9	IVW	20	-6.60×10^{-2}	3.28×10^{-2}	4.40×10^{-2}	0.94
Family-Streptococcaceae	IVW	22	4.75×10^{-2}	2.42×10^{-2}	4.92×10^{-2}	1.05

Outcome	Method	nSNP		OR(95%CI)	P-value
Genus−Ruminococcus torques group	IVW	23		1.07(1.03 to 1.12)	0.002
Fanily−Clostridiaceae1	IVW	21		0.93(0.88 to 0.97)	0.002
Genus−Clostridium sensustricto1	IVW	21		0.93(0.88 to 0.98)	0.005
Family−Prevotellaceae	IVW	23		0.94(0.89 to 0.98)	0.007
Genus−Eubacterium hallii group	IVW	22		1.06(1.01 to 1.11)	0.011
Class−Bacteroidia	IVW	23		0.95(0.91 to 0.99)	0.014
Order−Bacteroidales	IVW	23		0.95(0.91 to 0.99)	0.016
Class−Bacteroidetes	IVW	22		0.95(0.91 to 0.99)	0.019
Genus−Streptococcus	IVW	22		1.06(1.01 to 1.11)	0.027
ebi−a−GCST90017083	IVW	19		0.93(0.87 to 0.99)	0.028
Phylum−Actinobacteria	IVW	23		1.05(1.00 to 1.09)	0.041
Genus−Prevotella9	IVW	20		0.94(0.88 to 1.00)	0.044
Family−Streptococcaceae	IVW	22		1.05(1.00 to 1.10)	0.049

0.7 0.8 0.9 1 1.1

protective factor   risk factor

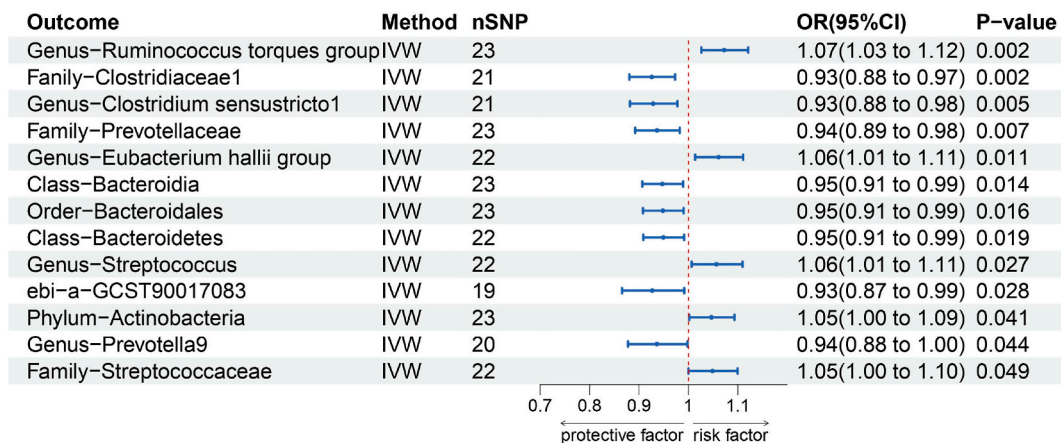**Fig. 6.** This figure illustrates the impact of urolithiasis on each of the significant GMB. An odds ratio value < 1 indicates a decreasing causal effect, while a value > 1 suggests the causal pathogenic incremental impact.

Therefore, comprehending the pathophysiology of urolithiasis and implementing preventive measures to mitigate its occurrence are crucial.

The gut microbiota, representing the most expansive microbial ecosystem within the human body, plays an active role in influencing the metabolism of diverse substances and energy. A decrease in the diversity of gut microbiota is linked to several health conditions, including obesity, diabetes, and inflammatory bowel disease, each recognized as a risk factor for the development of urinary tract stones [21–23]. Furthermore, an increasing amount of research indicates a distinctive imbalance in the gut microbiota of individuals suffering from urinary tract stones [24]. This indicates a strong link between the gut microbiota and the development of urinary stone disease, yet confirming a direct causal relationship continues to be an area of active research. Our study offers a substantial chance to deepen our comprehension of the possible causative link between gut microbiota and the occurrence of urinary tract stones.

One intriguing microbial community in this context is Genus-Clostridium sensu stricto 1, which exhibits a bidirectional causative relationship. In our study, we observed that urinary tract stones lead to a decrease in the abundance of Genus-Clostridium sensu stricto 1, while the reduction in the abundance of Genus-Clostridium sensu stricto 1 further promotes the risk of developing urinary tract stones, resembling a "positive-feedback" regulatory mechanism for urinary tract stones. Previous research has established the significance of Genus-Clostridium sensu stricto 1 as one of the vital anaerobic bacteria in the gut [25]. Its member, Clostridium butyricum, is known to produce butyrate through fermentation, a short-chain fatty acid that plays a crucial role in intestinal health by providing energy and maintaining overall gut mucosal health [26]. Puddu et al. [27] discovered that the abundance of butyrate in patients with kidney stones was 2.3 times lower compared to the control group (p = 0.04). However, further research is necessary to confirm the existence of this "positive-feedback" loop in the human body and whether interrupting this loop can mitigate the occurrence and progression of urinary stone disease.

In the forward MR analysis, while none of the 10 microbial taxa reached the Bonferroni-corrected threshold, it's important to consider the stringent criteria for genus-level taxa. Notably, Genus-Barnesiella (IVW OR = 0.73, 95% CI 0.73–0.89, p = 2.29×10^{-3}) and Genus-Flavonifractor (IVW OR = 0.69, 95% CI 0.53–0.91, p = 8.57×10^{-3}) exhibited a more significant causal effect on urinary tract stones. Barnesiella plays a crucial physiological role in the human body by assisting in the decomposition and absorption of
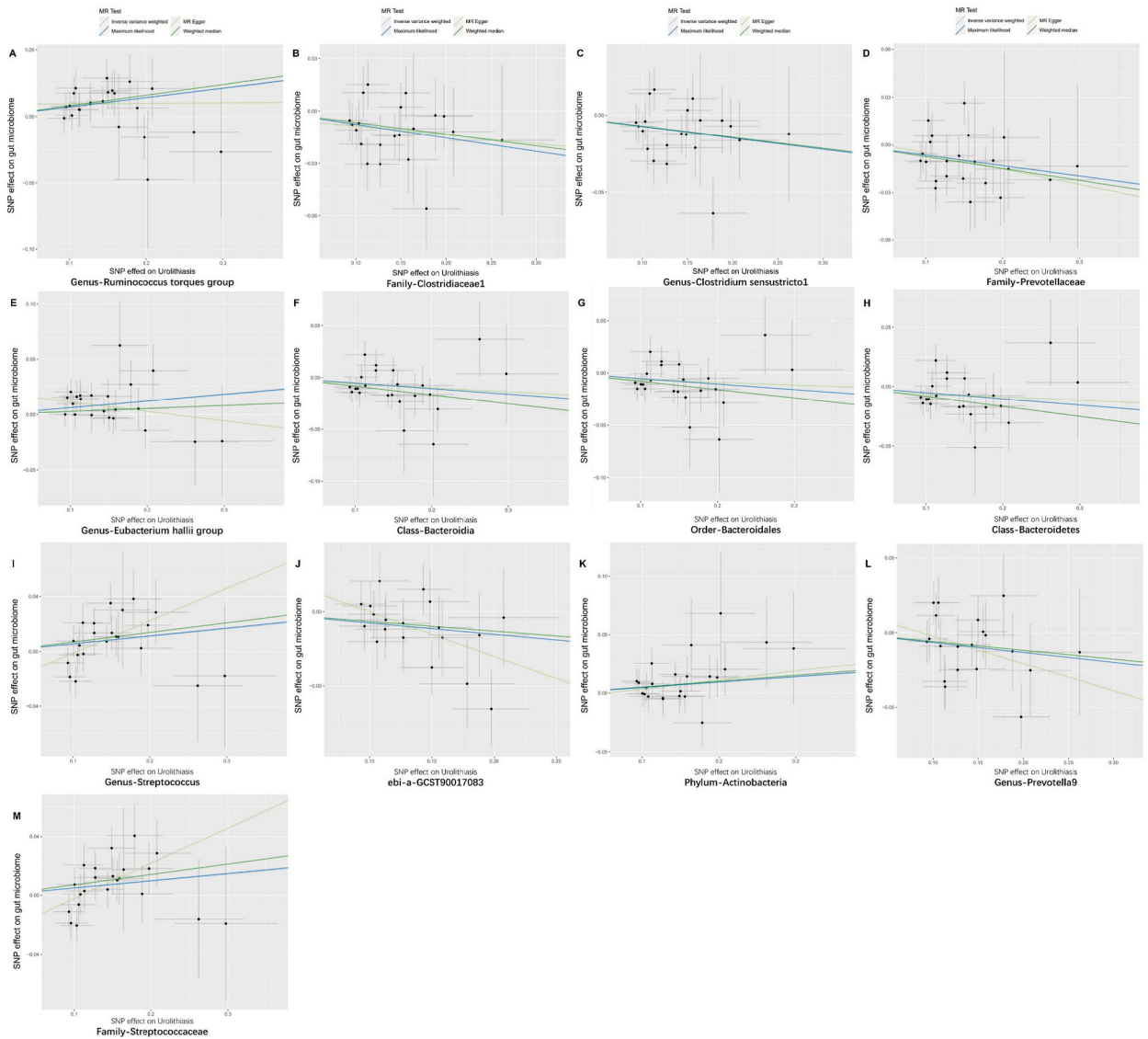
Fig. 7. Scatter diagrams illustrating the results of reverse MR studies exploring the relationship between urolithiasis and 13 different taxa of GMB. In these charts, SNP refers to single nucleotide polymorphisms, and MR signifies Mendelian randomization.

Table 3
Sensitivity analysis results for forward MR.

Exposure	Q-Egger	P-value	Q-IVW	P-value	Egger-Intercept	P-value	P-PRESSO Global Test
Genus-Barnesiella	12.28	0.42	12.24	0.35	-6.45×10^{-3}	0.85	0.44
ebi-a-GCST90017080	14.23	0.29	14.16	0.22	-7.93×10^{-3}	0.82	0.33
ebi-a-GCST90016955	14.28	0.28	14.21	0.22	-7.97×10^{-3}	0.82	0.35
Genus-Flavonifractor	4.07	0.40	3.25	0.36	4.13×10^{-2}	0.45	0.46
Order-NB1n	14.21	0.22	14.28	0.28	-1.96×10^{-3}	0.95	0.32
Family-Clostridiaceae1	10.02	0.35	9.99	0.27	3.77×10^{-3}	0.89	0.39
Genus-Howardella	10.56	0.23	10.09	0.18	-2.50×10^{-2}	0.59	0.24
Genus-Clostridium sensustricto1	6.82	0.34	4.22	0.52	-4.84×10^{-2}	0.17	0.39
Order-Clostridiales	10.93	0.54	7.82	0.73	-3.59×10^{-2}	0.11	0.54
Genus-Oscillospira	10.18	0.18	10.07	0.12	1.50×10^{-2}	0.80	0.21

Table 4
Sensitivity analysis results for reverse MR.

Outcome	Q-Egger	P-value	Q-IVW	P-value	Egger-Intercept	P-value	P-PRESSO Global Test
Genus-Ruminococcus torques group	17.35	0.63	17.43	0.68	−0.003438148	0.77	0.95
Family-Clostridiaceae1	18.68	0.48	18.76	0.54	−0.003714886	0.79	0.53
Genus-Clostridium sensustricto1	21.30	0.44	21.42	0.49	0.004440989	0.73	0.40
Family-Prevotellaceae	17.91	0.59	22.49	0.37	−0.026271479	0.04	0.49
Genus-Eubacterium hallii group	21.19	0.33	21.19	0.39	−0.000612152	0.97	0.80
Class-Bacteroidia	12.86	0.88	15.47	0.80	0.019723385	0.12	0.70
Order-Bacteroidales	21.50	0.25	22.48	0.26	0.015889976	0.38	0.70
Class-Bacteroidetes	11.77	0.95	12.37	0.95	0.009131151	0.45	0.68
Genus-Streptococcus	19.51	0.49	23.49	0.32	−0.024679994	0.06	0.37
ebi-a-GCST90017083	12.29	0.78	14.64	0.69	0.029828835	0.14	0.70
Phylum-Actinobacteria	18.56	0.61	18.59	0.67	−0.002047055	0.86	0.94
Genus-Prevotella9	12.91	0.91	13.05	0.93	−0.004356778	0.71	0.27
Family-Streptococcaceae	18.47	0.62	18.57	0.67	−0.003741666	0.75	0.39

complex polysaccharides, such as cellulose. It has the capacity to engage with other probiotic bacteria, resulting in the suppression of detrimental bacterial proliferation and the sustenance of a harmonious microbial ecosystem in the intestinal tract [28,29]. Barnesiella may also be associated with immune system regulation. In the research by Presley and colleagues [30], elevated concentrations of Barnesiella in the colonic regions of mice were found to be associated with heightened levels of marginal zone B cells and invariant natural killer T cells in both the spleen and liver [31]. Flavonifractor, a Gram-negative bacterium, derives its name from flavonoid compounds closely linked to human health through dietary intake. In the gastrointestinal tract, Flavonifractor species are involved in the biochemical processing of flavonoid compounds. This metabolism can generate substances with antibacterial, antioxidant, and anti-inflammatory properties [32]. In an investigative study led by Shiyun Luo and colleagues [33], a comparative analysis revealed a significantly higher presence of Flavonifractor bacteria in individuals from a normal control group, in contrast to those diagnosed with arterial sclerosis. The study further delved into the therapeutic potential of Flavonifractor plautii, demonstrating its efficacy in fortifying the elastic fiber network within vascular structures. This reinforcement effectively mitigates the elevated pulse wave velocity, a common complication in arterial sclerosis. The underlying mechanism involves the targeted suppression of MMP-2 (matrix metalloproteinase-2), coupled with the inhibition of key inflammatory markers such as MCP-1 (monocyte chemoattractant protein-1) and NF-κB (nuclear factor kappa-B), illustrating a multifaceted approach in vascular health improvement.

In the context of the forward MR analysis, microbial groups such as Family-Clostridiaceae1 and Genus-Oscillospira may exert an influence on the inflammatory process by modulating the host's immune response and inflammation levels [34–36]. Previous research has highlighted the role of inflammatory immune responses in contributing to the development of Randall's plaques and the subsequent formation of calcium-based kidney stones [37]. Furthermore, numerous families and genera within the Order-Clostridiales may be associated with lipid metabolism, including the metabolism and regulation of fatty acids [38]. Some research suggests that certain fatty acid supplements can enhance GMB diversity, benefiting conditions like neurological disorders and alcoholic liver disease [39]. Additionally, the intake of supplements rich in N-3 fatty acids has been demonstrated to effectively reduce critical risk factors associated with stone formation, notably including the conditions of hypercalciuria and hyperoxaluria [40]. However, the mechanistic contributions of these microbial groups to urological stone formation remain unclear, necessitating further investigation.

In the reverse MR analysis, urinary tract stones were found to impact the abundance of 13 bacterial taxa. While it may not be definitive that the 12 bacterial taxa, excluding Genus-Clostridium sensustricto1, influence the course of urinary tract stones, it is worth exploring whether changes in the abundance of these bacterial taxa can alter the overall composition and diversity of the GMB, thereby affecting the etiology of stones or causing other complications. In the detailed findings of a reverse MR study, a notable emphasis was placed on the Genus-Ruminococcus torques group, which exhibited a statistically significant impact (IVW OR = 1.07, 95% CI 0.64–0.98, P = 1.86×10^{-3}). This group of bacteria has been identified as a contributing factor in a range of health disorders, prominently including hypertension, Crohn's disease, Graves' disease, and irritable bowel syndrome, indicating its broad relevance in medical research and clinical diagnostics [41–44]. A meta-analysis conducted by Tianhui Yuan et al. [24] revealed that individuals with kidney stones exhibited a decreased relative abundance of Prevotella9 compared to healthy individuals, consistent with our study, while the relative abundance of Bacteroides increased, contradicting our results. These findings emphasize the need for comprehensive and in-depth research into the GMB.

In evaluating the scope and applicability of our study, it is crucial to acknowledge certain inherent limitations. The cornerstone of our analysis, the GWAS data, was drawn exclusively from subjects with European ancestry. This demographic specificity inherently limits the extrapolation of our findings to diverse global populations, particularly those of non-European descent. As such, extrapolating these results to broader, more varied demographic groups warrants additional scrutiny and caution. Secondly, our IVs were derived from GWAS meta-analysis, which confines our ability to explore non-linear relationships and stratified effects. Although existing observational studies do not offer conclusive evidence regarding the linearity of the impact of the GMB on the risk of urinary tract stones, the potential for non-linear models cannot be dismissed. Furthermore, our analysis was constrained due to the lack of detailed categorization regarding the types of kidney stones, such as distinctions between calcium oxalate and uric acid stones, which restricted the scope of our subgroup analysis capabilities. Despite these constraints, our causal estimates have been reinforced through a rigorous set of sensitivity analyses. However, in order to develop a more complete and nuanced understanding of how the GMB influences the formation of urinary tract stones, it is imperative to undertake additional investigative efforts. Future research should

focus on validating the observed effects and delving into the complexities of potential non-linear interactions within this framework.

5. Conclusion

In conclusion, our research has successfully demonstrated a cause-and-effect link between the GMB and the development of urolithiasis using bidirectional Mendelian Randomization analysis. We have pinpointed 10 bacterial groups, including the genera Barnesiella and Flavonifractor, as influencers in the risk of forming urinary tract stones. Additionally, 13 bacterial groups, notably the Genus-Ruminococcus torques group and the Family-Clostridiaceae, have been identified as being affected by urolithiasis. These identified microbial entities could be key in developing new biomarkers and exploring innovative therapeutic and preventive strategies for urinary tract stone conditions. We encourage healthcare professionals and researchers to prioritize monitoring the GMB in urolithiasis management, as this could unveil additional risk predictors and potentially beneficial taxonomic groups. This represents a significant clinical implication of our study.

Ethics declarations

Additional review or approval by an ethics committee was not needed for this study because the data is already publicly available, and its ethical approval was granted for the original study.

Data availability statement

Data included in article/supplementary material/referenced in article.

CRediT authorship contribution statement

Yongdong Pan: Writing – original draft, Software, Resources, Methodology, Investigation, Conceptualization. **Jingyi Su:** Validation, Software, Formal analysis, Conceptualization. **Shengnan Liu:** Validation, Resources. **Yueyan Li:** Visualization, Investigation, Data curation, Conceptualization. **Guofeng Xu:** Writing – review & editing, Writing – original draft, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We would like to express our gratitude to the participants and investigators involved in the GWAS utilized in the present study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e25704.

References

[1] K. Stamatelou, D.S. Goldfarb, Epidemiology of kidney stones, Healthcare (Basel) 11 (3) (2023). http://10.3390/healthcare11030424.
[2] E.S. Hyams, B.R. Matlaga, Economic impact of urinary stones, Transl. Androl. Urol. 3 (3) (2014) 278–283. http://10.3978/j.issn.2223-4683.2014.07.02.
[3] J.A. Antonelli, N.M. Maalouf, M.S. Pearle, et al., Use of the National Health and Nutrition Examination Survey to calculate the impact of obesity and diabetes on cost and prevalence of urolithiasis in 2030, Eur. Urol. 66 (4) (2014) 724–729. http://10.1016/j.eururo.2014.06.036.
[4] M. Zhe, Z. Hang, Nephrolithiasis as a risk factor of chronic kidney disease: a meta-analysis of cohort studies with 4,770,691 participants, Urolithiasis 45 (5) (2017) 441–448. http://10.1007/s00240-016-0938-x.
[5] H.Y. Chen, C.J. Chang, Y.C. Yang, et al., Renal stones and gallstones correlated with the ten-year risk estimation of atherosclerotic cardiovascular disease based on the pooled cohort risk assessment of males aged 40-79, J. Clin. Med. 12 (6) (2023). http://10.3390/jcm12062309.
[6] L.F. Fontenelle, T.D. Sarti, Kidney stones: treatment and prevention, Am. Fam. Physician 99 (8) (2019) 490–496.
[7] L.E. Vaughan, F.T. Enders, J.C. Lieske, et al., Predictors of symptomatic kidney stone recurrence after the first and subsequent episodes, Mayo Clin. Proc. 94 (2) (2019) 202–210. http://10.1016/j.mayocp.2018.09.016.
[8] J. Shen, M.S. Obin, L. Zhao, The gut microbiota, obesity and insulin resistance, Mol. Aspect. Med. 34 (1) (2013) 39–58. http://10.1016/j.mam.2012.11.001.
[9] Y. Jiao, L. Wu, N.D. Huntington, et al., Crosstalk between gut microbiota and innate immunity and its implication in autoimmune diseases, Front. Immunol. 11 (2020) 282. http://10.3389/fimmu.2020.00282.
[10] Y.Y. Chen, D.Q. Chen, L. Chen, et al., Microbiome-metabolome reveals the contribution of gut-kidney axis on kidney disease, J. Transl. Med. 17 (1) (2019) 5. http://10.1186/s12967-018-1756-4.
[11] M. Mehta, D.S. Goldfarb, L. Nazzal, The role of the microbiome in kidney stone formation, Int. J. Surg. 36 (Pt D) (2016) 607–612. http://10.1016/j.ijsu.2016.11.024.
[12] F. Mahmoodpoor, Y. Rahbar Saadat, A. Barzegari, et al., The impact of gut microbiota on kidney function and pathogenesis, Biomed. Pharmacother. 93 (2017) 412–419. http://10.1016/j.biopha.2017.06.066.

[13] A. Ticinesi, A. Nouvenne, T. Meschi, Gut microbiome and kidney stone disease: not just an Oxalobacter story, Kidney Int. 96 (1) (2019) 25–27. http://10.1016/j.kint.2019.03.020.

[14] H. Thomas, Mendelian randomization reveals causal effects of the gut microbiota, Nat. Rev. Gastroenterol. Hepatol. 16 (4) (2019) 198–199. http://10.1038/s41575-019-0133-y.

[15] M. Luo, J. Cai, S. Luo, et al., Causal effects of gut microbiota on the risk of chronic kidney disease: a Mendelian randomization study, Front. Cell. Infect. Microbiol. 13 (2023) 1142140. http://10.3389/fcimb.2023.1142140.

[16] J. Zheng, D. Baird, M.C. Borges, et al., Recent developments in mendelian randomization studies, Curr Epidemiol Rep 4 (4) (2017) 330–345. http://10.1007/s40471-017-0128-6.

[17] A. Kurilshikov, C. Medina-Gomez, R. Bacigalupe, et al., Large-scale association analyses identify host factors influencing human gut microbiome composition, Nat. Genet. 53 (2) (2021) 156–165. http://10.1038/s41588-020-00763-1.

[18] B.L. Pierce, H. Ahsan, T.J. Vanderweele, Power and instrument strength requirements for Mendelian randomization studies using multiple genetic variants, Int. J. Epidemiol. 40 (3) (2011) 740–752. http://10.1093/ije/dyq151.

[19] A. Trinchieri, E. Croppi, E. Montanari, Obesity and urolithiasis: evidence of regional influences, Urolithiasis 45 (3) (2017) 271–278. http://10.1007/s00240-016-0908-3.

[20] T. Dhondup, W. Kittanamongkolchai, L.E. Vaughan, et al., Risk of ESRD and mortality in kidney and bladder stone formers, Am. J. Kidney Dis. 72 (6) (2018) 790–797. http://10.1053/j.ajkd.2018.06.012.

[21] C.A. Lozupone, J.I. Stombaugh, J.I. Gordon, et al., Diversity, stability and resilience of the human gut microbiota, Nature 489 (7415) (2012) 220–230. http://10.1038/nature11550.

[22] R. Siener, Nutrition and kidney stone disease, Nutrients 13 (6) (2021). http://10.3390/nu13061917.

[23] S. Larsen, K. Bendtzen, O.H. Nielsen, Extraintestinal manifestations of inflammatory bowel disease: epidemiology, diagnosis, and management, Ann. Med. 42 (2) (2010) 97–114. http://10.3109/07853890903559724.

[24] T. Yuan, Y. Xia, B. Li, et al., Gut microbiota in patients with kidney stones: a systematic review and meta-analysis, BMC Microbiol. 23 (1) (2023) 143. http://10.1186/s12866-023-02891-0.

[25] L. Ma, Q. Shen, W. Lyu, et al., Clostridium butyricum and its derived extracellular vesicles modulate gut homeostasis and ameliorate acute experimental colitis, Microbiol. Spectr. 10 (4) (2022) e0136822. http://10.1128/spectrum.01368-22.

[26] N. Cassir, S. Benamar, B. La Scola, Clostridium butyricum: from beneficial to a new emerging pathogen, Clin. Microbiol. Infect. 22 (1) (2016) 37–45. http://10.1016/j.cmi.2015.10.014.

[27] A. Puddu, R. Sanguineti, F. Montecucco, et al., Evidence for the gut microbiota short-chain fatty acids as key pathophysiological molecules improving diabetes, Mediat. Inflamm. 2014 (2014) 162021. http://10.1155/2014/162021.

[28] R. Daillère, M. Vétizou, N. Waldschmitt, et al., Enterococcus hirae and Barnesiella intestinihominis facilitate cyclophosphamide-induced therapeutic immunomodulatory effects, Immunity 45 (4) (2016) 931–943. http://10.1016/j.immuni.2016.09.009.

[29] G.A. Weiss, C. Chassard, T. Hennet, Selective proliferation of intestinal Barnesiella under fucosyllactose supplementation in mice, Br. J. Nutr. 111 (9) (2014) 1602–1610. http://10.1017/s0007114513004200.

[30] L.L. Presley, B. Wei, J. Braun, et al., Bacteria associated with immunoregulatory cells in mice, Appl. Environ. Microbiol. 76 (3) (2010) 936–941. http://10.1128/aem.01561-09.

[31] A. Gomez, D. Luckey, C.J. Yeoman, et al., Loss of sex and age driven differences in the gut microbiome characterize arthritis-susceptible 0401 mice but not arthritis-resistant 0402 mice, PLoS One 7 (4) (2012) e36095. http://10.1371/journal.pone.0036095.

[32] A. Mikami, T. Ogita, F. Namai, et al., Oral administration of Flavonifractor plautii attenuates inflammatory responses in obese adipose tissue, Mol. Biol. Rep. 47 (9) (2020) 6717–6725. http://10.1007/s11033-020-05727-6.

[33] S. Luo, Y. Zhao, S. Zhu, et al., Flavonifractor plautii protects against elevated arterial stiffness, Circ. Res. 132 (2) (2023) 167–181. http://10.1161/circresaha.122.321975.

[34] J. Yang, Y. Li, Z. Wen, et al., Oscillospira - a candidate for the next-generation probiotics, Gut Microb. 13 (1) (2021) 1987783. http://10.1080/19490976.2021.1987783.

[35] J.M. Shapiro, M.R. de Zoete, N.W. Palm, et al., Immunoglobulin A targets a unique subset of the microbiota in inflammatory bowel disease, Cell Host Microbe 29 (1) (2021) 83–93.e83. http://10.1016/j.chom.2020.12.003.

[36] J. Zhou, J. Luo, S. Yang, et al., Different responses of microbiota across intestinal tract to Enterococcus faecium HDRsEf1 and their correlation with inflammation in weaned piglets, Microorganisms 9 (8) (2021). http://10.3390/microorganisms9081767.

[37] S.R. Khan, B.K. Canales, P.R. Dominguez-Gutierrez, Randall's plaque and calcium oxalate stone formation: role for immunity and inflammation, Nat. Rev. Nephrol. 17 (6) (2021) 417–433. http://10.1038/s41581-020-00392-1.

[38] Z.R. Ouyang, X.R. Niu, W.G. Wang, et al., The role of short-chain fatty acids in Clostridioides difficile infection: a review, Anaerobe 75 (2022) 102585. http://10.1016/j.anaerobe.2022.102585.

[39] P. Louis, H.J. Flint, Formation of propionate and butyrate by the human colonic microbiota, Environ. Microbiol. 19 (1) (2017) 29–41. http://10.1111/1462-2920.13589.

[40] A.L. Rodgers, R. Siener, The efficacy of polyunsaturated fatty acids as protectors against calcium oxalate renal stone formation: a review, Nutrients 12 (4) (2020). http://10.3390/nu12041069.

[41] M. Joossens, G. Huys, M. Cnockaert, et al., Dysbiosis of the faecal microbiota in patients with Crohn's disease and their unaffected relatives, Gut 60 (5) (2011) 631–637. http://10.1136/gut.2010.223263.

[42] C. Zhang, F. Chen, Y. Shen, et al., Sleep apnea is associated with the increase of certain genera of Ruminococcaceae and Lachnospiraceae in the gut microbiome of hypertensive patients, Expet Rev. Respir. Med. 16 (11–12) (2022) 1247–1256. http://10.1080/17476348.2022.2147509.

[43] J. Cao, N. Wang, Y. Luo, et al., A cause-effect relationship between Graves' disease and the gut microbiome contributes to the thyroid-gut axis: a bidirectional two-sample Mendelian randomization study, Front. Immunol. 14 (2023) 977587. http://10.3389/fimmu.2023.977587.

[44] J. Yang, P. Wang, T. Liu, et al., Involvement of mucosal flora and enterochromaffin cells of the caecum and descending colon in diarrhoea-predominant irritable bowel syndrome, BMC Microbiol. 21 (1) (2021) 316. http://10.1186/s12866-021-02380-2.