Assessing the Relationship between Gut Microbiota and Kidney Stones: A Two-Sample Mendelian Randomization Analysis

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Abstract

Background There is increasing evidence that gut microbiota is associated with the risk of kidney stones diseases (KSD), but whether a causal relationship exists remains unclear. We used the Mendelian randomization (MR) method to assess the potential causal relationship between gut microbiota and KSD risk.

Methods Genetic tool variables for the gut microbiota were identified from a genome-wide association study (GWAS) of 18340 participants. Summary statistics for KSD were derived from GWAS, including 484,598 cases and 480,873 normal controls. We used the inverse variance weighting (IVW) method as the primary analysis method. To test the robustness of our results, we further performed the weighted-median method, MR-Egger regression, and MR pleiotropy residual sum and outlier test. Finally, reverse MR analysis was performed to evaluate the possibility of reverse causation.

Results We identified suggestive associations between four bacterial traits and the risk of KSD (odds ratio (OR): 0.82, 95% confidence interval (CI): 0.69-0.98, p= 0.032 for class. *Lentisphaeria*; OR：0.79, 95% CI：0.66-0.96, p=0.018 for genus. *Oscillibacter*; OR：0.82, 95% CI：0.68-0.98, p=0.034 for order. *Victivallales*; OR：1.26, 95% CI：1.03-1.53, p=0.022 for genus. *Olsenella*). The results of sensitivity analyses for these bacterial traits were consistent. We did not find statistically significant associations between KSD and these four bacterial traits in the reverse MR analysis.

Conclusions Our systematic analysis provides evidence supporting a potential causal relationship between four gut microbiota taxa （including class. *Lentisphaeria,* genus. *Oscillibacter,* order. *Victivallales* and genus. *Olsenella*） and KSD risk. More studies are needed to demonstrate how gut microbiota influences KSD development.

Keywords: kidney stone disease, gut microbiota, mendelian randomization, single nucleotide polymorphism

1 Introduction

Kidney stones diseases (KSD) are one of the most common diseases in urology. Kidney stones affect about 15% of the population and 50% of patients relapse within 5 years of the first stone attack[1]. At present, it is believed that there are many factors affecting the formation of kidney stones, such as genetic, environmental factors, dietary habits and so on. In addition, many systemic diseases such as diabetes, hypertension and metabolic syndrome are also strongly associated with the risk of kidney stones[2]. The symptoms of patients with kidney stones are often back pain, hematuria, in addition to nausea, vomiting, fever, hydronephrosis and even renal insufficiency. The resulting urinary tract obstruction, infection and other complications will promote the formation of stones. With the development of minimally invasive treatment, the treatment of renal calculi has made great progress. However, the increasing prevalence of the disease has resulted in a economic burden on both patients and the community[3].

Trillions of microbes in our adult gut are known as the gut microbiota (GMB), a complex and evolving microbial community that functions like a metabolic organ. The imbalance of gut microbiota can also lead to the metabolic disorder of gut microbiota, and then affect the immune function[4]. Diet as a major factor of metabolism, is generally considered to be an important factor in the occurrence of KSD. GMB is also an important regulator of diet metabolism. Recent advances in human microbiome sequencing have led to important breakthroughs that describe how GMB can be made by fermenting fibers, carbohydrates and proteins, produces a wide range of metabolites that have a wide range of health effects[5].

Although gut microbiota has been related with KSD, the causal nature is elusive[6]. Mendelian Randomization (MR) analysis is a statistical method designed to infer potential causality from observational association outcomes[7]. MR uses genetic variants associated with exposure as a surrogate for exposure to assess the relationship between the surrogate and the outcome. In recent years, MR analysis has been used to assess the potential causal relationship between the gut microbiota and disease-risking genes [8-10]. Although Liu et al have done some research on the relationship between gut microbiota and kidney stones in their article, they found no cause-and-effect relationship between calcium oxalate and kidney stones[11]. So far, there is an urgent need to investigate the potential causal relationship between gut microbiota and the risk of KSD.

In this study, to explore the potential causal relationship between gut microbiota and KSD, and to identify specific pathogenic bacteria taxa, we conducted a two-sample MR study based on summary data from genome-wide association study (GWAS).

2 Methods and materials

2.1 Study design

The main goal of this study is to investigate the causal relationship between gut microbiota and KSD. The overall design of the present study is presented in **Fig.1**. This study utilizes gut microbiota as an exposure factor and selects single nucleotide polymorphisms (SNPs) as instrumental variables (IVs) based on a threshold of $p<1\times10^{-5}$. We conducted forward MR analysis using KSD as the outcome. Then, we perform reverse MR analysis using gut microbiota as the outcome [12-13].

In this study, MR analysis must meet three main assumptions: (1)Relevance assumption: IVs are strongly associated with exposure and are independent of each other; (2) Exclusive assumption: IVs are irrelevant to the outcome; and (3) Independence assumption: IVs do not relate to the confusion factor.

2.2 Data sources

The summary statistics for human gut microbiome we used in this study were obtained from the most recent GWAS meta-analysis, which included 18,340 participants from 24 cohorts. Detailed of the study has been described elsewhere [14-15]. Briefly, this study conducted association analyses by coordinating 16S rRNA gene sequencing profiles and genotyping data from diverse cohorts spanning multiple countries including the USA, Canada, Germany, Denmark, the Netherlands, Belgium, Sweden, Finland, the UK, and others. The analyses adjusted for various factors like age, sex, technical covariates, and genetic principal components to elucidate correlations. As the present study was based on public summary data, no additional ethics approval or consent to participate was required. KSD data are derived from GWAS studies and meta-analyses in European cohorts of EstBB, FinnGen and Lifelines, including 484,598 patients and 480,873 normal controls. The KSD case definition encompassed any participant with an International Classification of Diseases code for nephrolithiasis in their electronic health record, resulting in a cumulative incidence that reflects lifetime risk across the large, population-based biobanks. Controls were defined as individuals without any such record. The details of the data sources in this MR study are shown in **Table 1**[16].

2.3 Selection of instrumental variables (IVs)

We first removed 15 bacterial traits without specific name, leaving 196 bacterial traits, including 9 Phylum, 16 Class, 20 Order, 32 Family and 119 Genus. Then, we selected the instrumental variables at $p<1\times10^{-5}$. In order to obtain IVs from independent loci, we set the linkage disequilibrium (LD) threshold at $R^2<0.001$ and clumping distance = 10,000 kb in 1000 Genomes EUR data using "Two Sample MR" packages. Single nucleotide polymorphisms (SNPs) with the lowest *p*-value for the associated trait were retained for clumping with 196 bacterial traits [17]. A total of 2699 independent SNPs were found to be associated with 196 bacterial traits. Finally, we also computed the *F* statistics for each SNP to eliminate genetic variations with weak statistical power ($F < 10$). The SNPs obtained above were used as IVs to study the effects of gut microbiota on KSD. To assess whether KSD has a causal impact on the gut microbiota, we employed a genome-wide significance threshold ($p < 5 \times 10^{-8}$) to screen for SNPs associated with KSD. These SNPs were used as IVs in reverse MR analysis, with KSD as the exposure and the gut microbiota as the outcome. The statistical methods used in reverse MR analysis were the same as those used in forward MR analysis.

2.4 Statistical analysis

In this study, inverse variance Weighted (IVW), Weighted median (WM), Simple mode and Weighted mode were used to verify the causality between gut microbiota and KSD [18]. IVW is considered the standard method of analysis, which assumes that all instrumental variables are valid, and the effect size of the causal exposure was calculated by using the

standard error of each instrumental variable as the reciprocal of the weights. However, IVW regression did not take intercept terms into account, so we also adopted MR-Egger, WM, Simple mode, and Weighted mode for analysis [19]. If the above five methods give similar results for causal inference, the results are considered reliable. Heterogeneity between SNPs was detected using Cochran's Q [20], if there was significant heterogeneity (p<0.05). In addition, we used MR-PRESSO method to detect SNP outliers. If abnormal values are detected, they are deleted and the analysis is re-performed. MR-Egger intercept was used to test for the presence of horizontal pleiotropy of SNPs, and if the test was statistically significant (p<0.05), it indicated that there was significant pleiotropy in the analysis results. Sensitivity testing using the leave-one-out method can assess whether the results of MR change significantly when each IV is excluded in turn [21].

2.5 Data analysis

All statistical analyses were performed in R, version 4.3.1. Univariate Mendelian randomization analyses were primarily conducted using the "Two Sample MR" software package (version 0.5.7). MR-PRESSO tests were performed using the "MRPRESSO" package, p<0.05 was considered as significant difference.

3 Results

3.1 Main results of the 196 bacterial traits with the risk of KSD

The F-statistics for the 196 bacterial traits were all above 10, suggesting less possibility to suffer from weak instrument bias. The MR results of the associations between all 196 bacterial traits and the risk of KSD are presented in Additional file 1: **Table S1**. Briefly, we observed suggestive evidence for 5 bacterial traits to be associated with the risk of KSD using IVW method.

In particular, we found the genetically predicted class. *Lentisphaeria* were negatively associated with KSD [odds ratio (OR): 0.84; 95% confidence interval (CI): 0.70-1.00; p=0.049] in the IVW method (**Fig.2A**). The association between class. *Lentisphaeria* and KSD remained stable in the weighted-median method (OR: 0.89; 95% CI: 0.70-1.13; p=0.341). Furthermore, the MR-PRESSO test detected no outliers, and the results were consistent with those of the primary method (p=0.590). In the MR-Egger regression, there was no evidence of directional pleiotropic effects (intercept p-value=0.613). As for genus. *Oscillibacter*, it was also negatively associated with the risk of KSD in IVW method (OR: 0.80; 95% CI: 0.67-0.96; p=0.017) (**Fig.2B**). The results from the weighted-median method consistent (OR：0.82；95%CI：0.63, 1.06；p=0.132). Additionally, no evidence of directional pleiotropy was found in MR-Egger regression (intercept p-value = 0.089) and no outliers were detected with the MR-PRESSO test and the effect estimate was similar (p =0.035). As for order. *Victivallales*, it was also negatively associated with the risk of KSD in IVW method (OR：0.84；95% CI：0.70-1.00；p=0.049) (**Fig.2C**). The results from the weighted-median method consistent (OR：0.89；95%CI：0.70-1.14；p=0.363). The absence of outliers in the MR-PRESSO test, along with results consistent with the primary method (p=0.611), further supports the robustness of the findings. In the MR-Egger regression, there was no evidence of directional pleiotropic effects (intercept p-value=0.613).

In contrast, genus. *Olsenella* was positively associated with KSD risk using IVW method (OR：1.19；95% CI：1.02-1.39；p=0.029) (**Fig.2D**). In sensitivity analyses, the weighted median method produced similar estimates (OR：1.05；95%CI：0.85-1.30；p=0.655), though

with wider confidence intervals. Additionally, little evidence of directional pleiotropy was found in MR-Egger regression (intercept *p*-value = 0.043) and no outliers were detected with the MR-PRESSO test and the effect estimate was similar (*p* = 0.449). As for genus. *Escherichia. Shigella*, it was also positively associated with the risk of KSD in IVW method (OR：1.28；95% CI：1.02-1.61；*p*=0.034) (**Fig.2E**). The results from the weighted-median method consistent (OR：1.31；95%CI：0.95, 1.81；*p*=0.106). Furthermore, the MR-PRESSO test detected no outliers, with results aligning with the primary method(*p*=0.883). In the MR-Egger regression, there was no evidence of directional pleiotropic effects (intercept *p*-value=0.832).

To further assess the influence of potential directional pleiotropy on the causal effect estimates, we used the GWAS Catalog to scan the SNPs associated with these 5 bacterial traits and some SNPs were found to be accompanied with other traits. After excluding these pleiotropic SNPs, we recalculated the updated IV sets, and the associations of class. *Lentisphaeria*, genus. *Oscillibacter*, order. *Victivallales* and genus. *Olsenella* with the risk of KSD remained stable in the IVW method (OR：0.82；95% CI：0.69-0.98；*p*=0.032 for class. *Lentisphaeria*； OR：0.79；95% CI：0.66-0.96；*p*=0.018 for genus. *Oscillibacter*； OR：0.82；95% CI：0.68-0.98；*p*=0.034 for order. *Victivallales*； OR：1.26；95% CI：1.03-1.53；*p*=0.022 for genus. *Olsenella*)(**Fig.3**). However, the relationship between genus. *Escherichia. Shigella* and KSD was unstable (OR：1.31；95% CI：0.99, 1.73；*p*=0.061).

3.2 The result of reverse MR analysis

Finally, we evaluated the potential reverse associations of four bacterial traits and KSD using the reverse MR analyses. We did not find statistically significant associations between KSD and any of these four bacterial traits using IVW method (OR：0.99；95% CI：0.93, 1.05；*p*=0.732 for class. *Lentisphaeria*； OR：1.00；95% CI：0.96, 1.05；*p*=0.945 for genus. *Oscillibacter*； OR：0.99；95% CI：0.93, 1.05；*p*=0.732 for order. *Victivallales*； OR：0.99；95% CI：0.92, 1.06；*p*=0.728 for genus. *Olsenella*). The results were stable across sensitivity analyses, which are listed in **Table 2**.

4 Discussion

As a Mendelian randomization study, our findings are primarily hypothesis-generating and represent the first step in establishing causal inference from the gut microbiota to KSD. This study confirmed that the genus. *Olsenella* are risk factors for KSD, while the class. *Lentisphaeria*、genus. *Oscillibacter* and order. *Victivallales* have potential protective effects on KSD.

Kidney stone disease is a complex disease that can be caused by genetics and different environmental factors. Given that 75% of kidney stones are comprised of calcium oxalate, it is noteworthy that oxalate serves as an end product of amino acid metabolism, potentially exerting an influence on the structure and composition of the gut microbiota [22]. Various pathological changes in metabolism (for example, diabetes, obesity, CKD, CVD, metabolic syndrome, hypertension) are thought to be associated with stone formation[23-24]. The formation of oxalate has attracted the attention of the medicine because the human body mainly depends on the gut microbiota to maintain oxalate homeostasis. Siener and co-workers indicated that by increasing intestinal oxalate degradation, O. *formigenes* decreases the oxalate concentration that is available for absorption at constant rates in the intestine [25]. Therefore, it reduces oxalate excretion in urine and protects against formation of calcium

oxalate kidney stones. Little is known about the general role of gut microbiota in the pathophysiology of kidney stones. Stern published the first evidence that kidney stone former patients have a unique gut microbiota when compared to controls [26]. By the next generation sequencing, they observed that Bacteroides spp. were more abundant in kidney stone formers where Prevotella spp. were more abundant in the controls. Currently, the available data propose that in the future, manipulation of gut bacteria (e.g., colonization with O. formigenes) may present a novel therapeutic application in kidney stone former patients. Liu and co-workers indicated that there is no causal relationship was found between Oxalobacteriaceae and kidney stones. The relationship between the gut microbiota and kidney stones is not entirely dependent on the presence or absence of Oxalobacter/Oxalobacte formigenes [11].

In this study, genus. *Olsenella* is the only bacterial taxa identified that is positively associated with KSD. Genus. *Escherichia. Shigella* was a risk factor for KSD, but the relationship is not stable, when the polymorphic SNPs were excluded. However, no studies have reported genus. Olsenella's changed in patients with KSD. A recent paper by Colombo et al investigated subgingival biofilms in patients with periodontal disease and reported a large number of opportunistic species, such as gut bacteria, *Olsenella*, *Neisseria*, even *Clostridium difficile*, in addition to yeasts such as *Candida*, is associated with periodontitis [27]. Previous studies have shown that the relative abundance of *Escherichia. Shigella* in patients with kidney stones is higher than that in healthy individuals, and the overall effect size is moderate and significant （$Z = 3.23$，$P = 0.001$）. The percentage of KSD was 2.47 times higher than that of controls. *Escherichia. Shigella* was negatively correlated with urinary citrate ($r = -0.56$, $P<0.08$). It could bind free calcium and produce soluble Calcium citrate, which reduced the supersaturation of calcium salts and made it a urinary stone inhibitor. The risk of kidney stones increases with a decrease in citrate concentration [28].

Lentisphaeria has the ability to produce acid and gas, and has been found to be involved in the metabolism of polysaccharides and protein in the gut. One study found that the *Lentisphaeria* was more abundant in the gut of healthy people and less abundant in patients with inflammatory bowel disease and nonalcoholic fatty liver disease (NAFLD) [29]. *Oscillibacter*, an understudied group of anaerobic bacteria in the Clostridium group, belongs to Firmicutes, a family of rumen cocci, and is widely found in the gut of animals and humans. It is associated with obesity, emaciation, gallstones and chronic constipation, and has been shown to be associated with positive or negative changes in the course of the disease. *Oscillibacter* is a short-chain fatty acid bacterium that produces butyrate, which is considered a candidate for “Next-generation probiotics” because butyrate is an important parameter for screening “Next-generation probiotics” [30]. The order. Victivallales is a naturally occurring gut microbiota that plays an important role in ecosystems and interacts with other microorganisms. Current studies have shown that the gut microbiota mainly affects the development of kidney stones through metabolites. The *Victivallales*, which has been identified as a producer of short-chain fatty acid acid (SCFAs) , produces acetic acid, propionic acid and butyric acid, among others, which play an important role in oxalic acid metabolism and may shed light on its protective mechanism [31].

Many previous studies have suggested that patients with KSD are often accompanied by dysbiosis of the gut microbiota, but they are observational studies [32]. This study reinforces

the causal effect of gut microbiota on KSD by using genetic epidemiological methods. In addition, the F-statistic of IVs we used all satisfied the threshold of >10 which suggested that our analyses were less likely to suffer from weak instrument bias. We further performed a reverse MR analysis that excluded reverse causality. The study of causality will be the direction of future research on the role of intestinal flora in disease development. Today, many studies have focused on the role of certain gut bacteria in disease development using animal models. Our results may provide guidance for the selection of individual gut bacteria to study the role of the gut microbiota in the pathogenesis of KSD.

However, our study has some limitations. First, bacterial taxa are analyzed only at the genus level, not at a more specialized level, such as at the species or strain level. Second, although the majority of participants in GWAS were of European descent, participants of other ethnicities might have influenced the results. Therefore, generalizing our findings to other ethnic groups may be limited. Furthermore, the effects of the bacterial traits we reported were relatively weak, and no other independent KSD GWAS had sufficient sample sizes to validate our findings. Finally, the study was not able to stratify the severity of disease in different age groups and populations. Therefore, more extensive and detailed studies are needed to confirm our results in the future. To translate these genetic findings into clinical insights, a multi-stage research pathway is recommended. First, in vivo animal models are needed to confirm that manipulating these specific bacterial taxa alters calcium oxalate crystal formation. Second, prospective cohort studies can assess whether changes in these microbiotas over time predict KSD incidence in humans. Finally, understanding the underlying mechanisms—such as the role of bacterial-derived metabolites like oxalate, short-chain fatty acids, or immune-modulatory molecules—will be crucial for developing targeted therapies.

5 Conclusion

In conclusion, our MR analysis provides novel genetic evidence supporting a causal relationship between specific gut microbiota and KSD. Lentisphaeria, the genus. Oscillibacter and the order. Victivallales are efficacious protective factors against this disorder, whereas the genus. Olsenella may act as prominent risk factors for KSD. These findings are primarily hypothesis-generating and highlight the need for future experimental and mechanistic studies to validate these microbiota as potential therapeutic targets for the prevention of kidney stones.

Figure legends:

Fig.1 Overview of the analysis process of the causal relationship between the microbiota and KSD through MR analysis

Fig.2 Scatter plot of the associations of genetic variants with four bacterial traits and the risk of KSD

Fig.3 Forest plot of the associations between genetically determined 4 bacterial traits with the risk of KSD

Table 1 Details of the genome-wide association studies and datasets used in our analyses

Exposure or outcome	Sample size	Ancestry	Links for data download	PMID
Human gut microbiome	18,340 participants	Mixed	https:// mibiogen. gcc. rug. nl	33462485
Kidney stone	284,598 cases, 480,873 controls	European ancestry	http:// ftp. ebi. ac. uk/ pub/ datab ases/ gwas/ summary statistics/ GCST9 00160 01- GCST9 00170 00/ GCST9 0016564/	34741163

Table 2 Effect estimates of the associations of KSD with class. *Lentisphaeria*, genus. *Oscillibacter*, order. *Victivallales* and genus. *Olsenella* in the reverse MR analyses

Gut microbiome	Methods	N. SNPs	OR	95%CI	*P*-value	Intercept *P*-value
Class Lentisphaeria						
	IVW	19	0.99	0.93-1.05	0.732	
	Weighted median	19	0.99	0.90-1.08	0.766	
	MR-Egger	19	/	/	/	0.995
Genus Olsenella						
	IVW	18	0.99	0.92-1.06	0.728	
	Weighted median	18	0.94	0.85-1.04	0.217	
	MR-Egger	18	/	/	/	0.640
Genus Oscillibacter						
	IVW	19	1.00	0.96-1.05	0.945	
	Weighted median	19	1.02	0.95-1.08	0.616	
	MR-Egger	19	/	/	/	0.673
Order Victivallales						
	IVW	19	0.99	0.93-1.05	0.732	
	Weighted median	19	0.99	0.90-1.08	0.77	
	MR-Egger	19	/	/	/	0.995

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The original contributions presented in the study are included in the article Material. Further inquiries can be directed to the corresponding authors.

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Authors' contributions

Conceptualization, SW; Data curation, YC and SW; Formal analysis, SW and YC; Investigation, BF and TS; Methodology, SW and SX; Project administration, SW and JG; Software, SW and ZW; Validation, SW; Visualization, YC; Writing – original draft, SW; Writing – review & editing, YC and SZ. All authors read and approved the final manuscript.

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