Association between gut microbiota and diabetic nephropathy: a Mendelian randomization study

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Abstract

In recent years, with the improvement in living standards, the incidence of diabetes has been increasing year by year. Diabetic nephropathy (DN), as one of the most common complications of diabetes, also has an increasing incidence. Some existing clinical studies and reviews have revealed a connection between diabetic nephropathy and gut microbiota (GM), but whether there is a causal relationship between the two is still unclear. Exploring the causal relationship between diabetic nephropathy and gut microbiota can help with disease screening and provide new biomarkers. This study used a two-sample Mendelian randomization analysis, using 4,111 DN patients from the GWAS database and 308,539 control group members, to attempt to find gut microbiota categories among the 211 types that have a causal relationship with diabetic nephropathy. Further heterogeneity and sensitivity analysis were conducted to eliminate the influence of confounding factors on the experimental results. Ultimately, 15 types of gut microbiota were found to have a causal relationship with diabetic nephropathy, providing hints and new treatment directions for clinical research.

Introduction

Diabetic nephropathy (DN) is one of the most common microvascular complications of diabetes mellitus and is associated with increased morbidity and mortality in diabetic patients\(^1\). In China, the incidence and prevalence of diabetic nephropathy have dramatically risen in the past decade, with an estimated 24.3 million diabetic patients suffering from CKD\(^2\). Globally, there are 850 million people with diabetic nephropathy, primarily due to the significant increase in diabetes prevalence\(^3\). The clinical manifestations of diabetic nephropathy mainly involve varying degrees of proteinuria and progressive decline in kidney function, which can be staged based on the clinical course of type 1 diabetes. There are many pathways and mediators involved in the development and progression of DN\(^4\), including oxidative stress, angiotensin II, and inflammatory processes, which are recently considered to play an important role\(^5\). Hyperglycemia, hypertension, obesity, smoking, race, men, dyslipidemia, age, and genetic factors are the main risk factors for the development and progression of DN\(^6,7\).

The gut microbiota, which is the largest symbiotic microbial community in the human body, is often overlooked in its role. It is composed of bacteria, fungi, viruses, and protozoa, totaling 4 trillion microorganisms and 150,000 microbial genomes\(^8\). Numerous studies have suggested that specific gut microbiome patterns are associated with the development of certain chronic diseases in humans, such as nonalcoholic fatty liver disease, colorectal cancer, alcoholic hepatitis, and inflammatory bowel disease\(^9–13\). While recent research indicates a connection between the gut microbiota and diabetic nephropathy. Individuals with Type 2 diabetes (T2D), especially those with DN, exhibited significantly lower viral richness and diversity compared to control subjects. Various viral functions, particularly those involving phages that destroy host bacteria, were notably diminished in T2D and DN\(^14\). Nevertheless, it is still unknown whether there is a causal link between the gut microbiota and diabetic nephropathy.

Mendelian randomization (MR) is a statistical method based on whole genome sequencing data that can effectively reduce bias and reveal cause-and-effect relationships\(^15\). This method can verify whether there is a causal relationship between exposure and outcome. This study employed a two-sample MR analysis to investigate the causal relationship between gut microbiota and diabetic nephropathy using GWASs summary statistics obtained from the MiBioGen and FinnGen consortia.
Results

Characteristics of SNPs. The data on exposure factors is sourced from the Mibiogen database. The exposure data of gut microbiota is derived from 24 cohort studies conducted in the United States, Canada, Israel, South Korea, Germany, Denmark, the Netherlands, Belgium, Sweden, Finland, and the United Kingdom. The exposure data included 211 intestinal biological groups including Actinobacteria, Bacteroides, Clostridia, etc. The DN data on outcome factors is sourced from the FinnGen database, and included 4,111 DN patients and 308,539 control members (European), with a total number of SNPS of 18,708,278. After removing IVs in linkage disequilibrium, 5 taxa and 15 bacterial characteristics (2 classes, 4 families, 6 genera, 2 order, and 1 phylum) were included. In addition, we also collected more information about SNPs (such as effect alleles, beta, SE, and p values), and all F-statistics > 10. The information is shown in Supplementary Table S1.

Causal effect of gut microbiota on DN. At the genus level, a total of two gut microbiota were found to have a positive causal effect on the development of DN. These included Bacteroidia (OR = 1.419, CI = 1.119–1.799, p = 0.004), and Verrucomicrobiae (OR = 1.452, CI = 1.180–1.787, p = 0.004). Similarly, at the order level, Bacteroidales (OR = 1.419, CI = 1.119–1.799, p = 0.004), and Verrucomicrobiales (OR = 1.452, CI = 1.180–1.787, p = 0.0004) were found to have a positive causal effect on the development of DN. At the family level, Victivallaceae (OR = 0.873, CI = 0.780–0.977, p = 0.018) may decrease the risk of DN, while Peptostreptococcaceae (OR = 1.224, CI = 1.019–1.471, p = 0.031), Veillonellaceae, (OR = 1.198, CI = 1.014–1.416, p = 0.034) Verrucomicrobiaceae (OR = 1.452, CI = 1.180–1.787, p = 0.0004) may increase the risk of DN. In addition, at the genus level, Akkermansia (OR = 1.452, CI = 1.180–1.786, p = 0.0004), Catenibacterium (OR = 1.312, CI = 1.079–1.594, p = 0.006), Lachnoclostridium (OR = 1.381, CI = 1.114–1.713, p = 0.003), Parasutterella (OR = 1.257, CI = 1.068–1.480, p = 0.006) may be associated with a higher risk of DN. While Eubacteriumcoprostanoligenesgroup (OR = 0.765, CI = 0.591–0.990, p = 0.042), Clostridiumsensustricto1 (OR = 0.760, CI = 0.595–0.972, p = 0.029) may be associated with a lower risk of DN. At the phylum level, a higher abundance of Bacteroidetes (OR = 1.395, CI = 1.086–1.792, p = 0.009) similarly indicated a significantly higher risk of DN. In general, Bacteroidia, Verrucomicrobiaceae, Peptostreptococcaceae, Veillonellaceae, Verrucomicrobiaceae, Akkermansia, Catenibacterium, Lachnoclostridium, Parasutterell, Bacteroidales, Verrucomicrobiaceae, Bacteroidetes are considered as risk factors for DN, while Victivallaceae, Eubacteriumcoprostanoligenesgroup, Clostridiumsensustricto1 are protective factors for DN. All scatter plots are shown in Fig. 1 and Fig. 2. The forest plot based on IVW analysis results is displayed in Fig. 3. While Fig. 4 shows the causal analysis of gut microbiome taxa and diabetic nephropathy based on MR analyses.

Sensitivity analysis. To verify the reliability of these results, we conducted a sensitivity analysis. The sensitivity analysis included tests for pleiotropy and heterogeneity. In the pleiotropy test, no potential horizontal pleiotropy was detected by using the MR-Egger method and MR-PRESSO analysis (P > 0.05). In the heterogeneity test, the observation of a Cochran's Q p-value greater than 0.05 and leave-one-out analysis indicated the absence of heterogeneity. The results of the sensitivity analysis are shown in Table 1, and more information is presented in Supplementary Table S2.

Table 1. The results of Sensitivity analysis.
<table>
<thead>
<tr>
<th>Classification</th>
<th>Horizontal pleiotropy</th>
<th>Heterogeneity</th>
<th>MR-PRESSO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Egger Intercept</td>
<td>SE</td>
<td>P-value</td>
</tr>
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<td>class.Bacteroidia</td>
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<td>0.021</td>
<td>0.903</td>
</tr>
<tr>
<td>class.Verrucomicrobiae</td>
<td>0.04</td>
<td>0.028</td>
<td>0.184</td>
</tr>
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</tr>
<tr>
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<td>genus.Clostridiumsensustricto1</td>
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<td>genus.Lachnocolstridium</td>
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<td>genus.Parasutterella</td>
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<td>0.021</td>
<td>0.903</td>
</tr>
<tr>
<td>order.Verrucomicrobiales</td>
<td>0.04</td>
<td>0.028</td>
<td>0.184</td>
</tr>
</tbody>
</table>
**Discussion**

We performed a two-sample MR analysis of the potential causal relationship between gut microbiota and diabetic nephropathy by using public data from GWAS. Previous studies only investigated the relationship between gut microbiota and diabetic nephropathy through clinical trials and animal models\textsuperscript{16,17}. These studies have confirmed that there is a certain relationship between gut microbiota and diabetic nephropathy. However, these studies are susceptible to confounding factors, making it difficult to determine whether there is a causal association between gut microbiota and DN. Therefore, through MR analysis, it shows that certain gut microbiota have a causal association with DN risk. These findings showed no influence by heterogeneity or horizontal pleiotropy. This may facilitate the discovery of new biomarkers in future DN studies.

The gut microbiota is a dynamic community of microorganisms made up of 100 trillion microbes living within the gastrointestinal system of the host organism\textsuperscript{18}. Some studies have indicated that dysregulation in the microbiota resulting in a deficiency of short chain fatty acids (SCFAs) such as propionate, acetate, and butyrate, by-products of healthy gut microbiota metabolism, have been demonstrated in obesity, type 1 and type 2 diabetes\textsuperscript{19}. Diabetic nephropathy is the main cause of end-stage renal failure, which is closely related to obesity, T1DM, and T2DM. Besides, it seems that SCFAs can reduce inflammation\textsuperscript{20}. SCFAs is a metabolite of intestinal flora, and its metabolites can also affect the kidney’s blood flow by activating the renin-angiotensin-system (RAAS) system, which is associated with chronic kidney disease\textsuperscript{21}.

In our study, we found that Family Victivallaceae, Genus Eubacteriumcoprostanoligenesgroup, and Genus Clostridiumsensustricto1 were negatively associated with DN, suggesting a protective effect for DN. However, existing observational studies have found an association between an increase in the abundance of Clostridiaceae bacteria and systemic inflammation, which may increase the risk of developing chronic kidney disease\textsuperscript{22}. This conflicts with the results of our MR analysis, which may be due to the presence of confounding factors in the observational studies that could affect the experimental results. On the other hand, the p-values we set during the experiment may be related. Therefore, we used Bonferroni correction and established significance thresholds for MR results at five levels of classification. The Bonferroni correction threshold for each feature level is 0.05/n (where n is the number of independent bacterial taxa on the corresponding classification level). When the p-value is less than the Bonferroni correction threshold, the MR results can be considered significant. However, these MR results did not pass the Bonferroni multiple testing correction. Among the remaining 12 positive results (e.g., Bacteroidia), all suggest a causal relationship with diabetic nephropathy and an increased risk of its occurrence. Some related studies have indicated that an increase in the abundance of Bacteroidia is associated with the severity of chronic kidney disease, possibly due to the production and accumulation of uremic toxins\textsuperscript{23}. In addition, this bacterial group can also activate the RAAS system by releasing inflammatory factors\textsuperscript{24}, consistent with the mechanisms mentioned earlier. Some literature also mentions that bacterial groups such as Allobaculum and Anaerosporobacter increase the risk of developing diabetic kidney disease by increasing the release of TMAO\textsuperscript{25}. On the other hand, an increase in the
abundance of Firmicutes reduces the risk of DN, but no causal relationship between these bacterial groups and DN was found in this MR analysis.

Our research has certain limitations. Firstly, the MR analysis was conducted on a European population, and it remains to be verified whether the results represent the global population. Secondly, there are five stages of diabetic nephropathy, and the relationship between different gut microbiota and these five stages of diabetic nephropathy still needs to be validated. Thirdly, although we have established a causal relationship between gut microbiota and diabetic nephropathy, the mechanisms through which gut microbiota influences diabetic nephropathy are not fully understood. Fourthly, by using a p-value of $<1 \times 10^{-5}$ as a threshold and selecting a limited number of SNPs as IVs, we may only be able to explain a small portion of the variation in exposure, thereby affecting the statistical power of causal estimation.

Materials and methods

Study design. We aim to investigate the association between gut microbiota and diabetic nephropathy by two-sample Mendelian randomization studies. All data were obtained from GWAS databases, with gut microbiota data sourced from MiBioGen and diabetic nephropathy data sourced from FinnGen. To minimize the impact of confounding factors on the results, MR analysis needs to satisfy the following three major assumptions. (1) Select SNPs significantly associated with gut microbiota as instrumental variables (IVs), and these IVs should be strongly correlated with the exposure. (2) There should be no relationship between IVs and the outcome, which is diabetic nephropathy, except through their effect on the exposure. (3) IVs should be unrelated to any confounding factors. (Fig. 5)

Ethics statement. The data used in this study are sourced from public GWAS databases, thus ethical committee approval is not required. Each study included in this article has been reviewed by its respective ethical institution.

Data sources. Summary-level data for the human gut microbiota were obtained from the MiBioGen databases. This encompassed 211 bacterial taxa units, which consisted of 131 genera, 35 families, 20 orders, 16 classes, and 9 phyla. Additionally, summary statistics for DN were extracted from a dataset stored in the FinnGen biobank analysis round 9. This dataset comprised 4,111 DN cases and 308,539 controls.

Instrumental variables selection. Firstly, we need to select instrumental variables that are strongly correlated with the gut microbiota. To avoid a limited number of SNPs, we will employ a statistical threshold of $P < 1 \times 10^{-5}$ for the selection process. Then, we set the threshold for $r^2$ as 0.001 and $KB = 10000$ to eliminate linkage disequilibrium. Second, we will align the exposure data and outcome data based on the principle that the selected SNPs have the same alleles, thereby excluding palindromic and incompatible SNPs.

To assess whether weak instrument bias could potentially impact estimates of causality, we will evaluate the strength of the instrumental variables using the F statistic. If the corresponding F-statistic > 10, it is considered that no significant weak instrumental bias exists.

MR analysis. MR analysis includes five basic methods, namely MR Egger, Weighted median (WM), Inverse variance weighted (IVW), Simple mode, and Weighted mode. IVW is the most fundamental method, and the
other methods can be used as supplements to IVW. After harmonizing the data, if the P-value in the IVW method is less than 0.05, it can be preliminarily considered that there is a causal relationship between the microbial community and DN. If P > 0.05, there is no statistical significance. Further verification can be done using MR Egger or other methods. Then, a heterogeneity test is conducted, primarily observing the P-value of Cochran's Q test. If P > 0.05, there is no heterogeneity. Conversely, if heterogeneity exists, biased SNPs can be removed using MR-PRESSO. The next step is to perform a pleiotropy test, observing the P-value. If P > 0.05, no statistical significance indicates no pleiotropy. Finally, a sensitivity analysis is conducted. (Fig. 6)

Conclusion

Through Mendelian randomization analysis, our study has confirmed a causal relationship between gut microbiota and diabetic nephropathy. The results of our MR analysis offer new insights for diagnosis and the gut microecological-based therapy of diabetic nephropathy.

Declarations

Data availability

Publicly available datasets are analyzed in this study. GWAS summary data for DN are publicly available at the Finngen website (https://www.finngen.fi/en). The summary statistics of microbial features from the MiBioGen cohort are available at www.mibiogen.org.

Author Contributions

The main tasks of the article involved conceptualizing and selecting the topic, conducting literature review and screening, extracting and analyzing data, creating tables, and MR analyzing, all of which were carried out by R.L.. The initial draft of the paper was completed by R.L.. R.C. has made modifications and improvements to the initial draft. R.C. supervised and supported the entire writing process. Finally, R.L. completed the submission work. All authors have made significant contributions to this article.

References


Figures
Figure 1

Summary of scatter plots of potential positive associations between gut microbiome and AD risk (A–L). Each dot in the graph represents an SNP locus. The vertical axis of the graph is the effect of the instrumental variable on the outcome, the horizontal axis is the effect of the instrumental variable on the exposure, and the ratio of the two effects is the effect of exposure on the outcome, that is, the slope of the regression line corresponds to the causal effect of exposure on the outcome in the graph. Horizontal and vertical crosses show a 95% confidence interval for each association. The estimates for the MR Analysis were slightly different, but the overall upward trend, suggests that exposure (this gut microbiome) may have a positive causal effect on the outcome (DN). DN, diabetic nephropathy; SNPs, single nucleotide polymorphisms; MR, Mendelian randomization; IVW, inverse variance weighted.
Summary of scatter plots of potential negative associations between gut microbiome and DN risk (A–C). IVW estimates show that Victivallaceae, Eubacteriumcoprostanoligenesgroup and Clostridiumsensustricto1, and DN show a downward-sloping trend, suggesting a potential negative correlation between them. DN, diabetic nephropathy; SNPs, single nucleotide polymorphisms; MR, Mendelian randomization; IVW, inverse variance weighted.

<table>
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<td>1.395(1.086,1.792)</td>
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</table>

Figure 3
Figure 4

Causal analysis of gut microbiome taxa and diabetic nephropathy based on MR analyses (locus-wide significance, $P<1 \times 10^{-5}$). From outside to inside, the $P$ values of IVW, MR Egger, WM, Simple mode, and Weighted mode are represented, respectively. The GM taxa name represented by each ID can be found in Supplementary Table S3.
Figure 5

Three assumptions of MR.

Figure 6

Flowchart of MR design.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTableS1.docx
- SupplementaryTableS2.docx
- SupplementaryTableS3.xlsx