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Associations Between Gut Microbiota and Diabetic Nephropathy: A Mendelian Randomization Study

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ABSTRACT

Objectives: Diabetic nephropathy (DN) is a severe complication of diabetes mellitus, and its pathogenesis remains incompletely understood. Emerging evidence suggests a potential link between gut microbiota and DN. This study aimed to explore the causal relationship between gut microbiota and DN using a two-sample Mendelian randomization (MR) approach.

Methods: Gut microbiota data were obtained from the MiBioGen consortium, which provides the most comprehensive genome-wide association studies (GWAS) on gut microbiota. Summary-level genetic data for DN were sourced from publicly available GWAS data provided by the FinnGen consortium. The primary analysis was conducted using the inverse variance–weighted (IVW) method, complemented by sensitivity analyses to evaluate pleiotropy and heterogeneity.

Results: Fourteen gut microbiota species demonstrated significant genetic associations with DN in the MR analysis, including five negatively and nine positively associated species, as determined by the IVW method. No evidence of pleiotropy or heterogeneity was observed, ensuring the robustness of the findings.

Conclusions: This study provides novel insight into the causal role of gut microbiota in DN pathogenesis, uncovering specific microbial species that may contribute to disease progression. These findings offer a promising avenue for future research and therapeutic development targeting gut microbiota.

1 | Introduction

Diabetic nephropathy (DN), a debilitating complication of diabetes mellitus, afflicting around 40% of individuals with diabetes [1], is characterized by progressive renal damage due to sustained hyperglycemia. It is a major cause of end-stage renal disease (ESRD) worldwide, accounting for more than half of the individuals undergoing dialysis and kidney transplant therapy [2]. DN is a multifactorial disease, involving complex interactions between genetic, metabolic, and environmental factors [3]. Despite extensive research, the precise mechanisms underlying its pathogenesis remain incompletely understood, necessitating innovative approaches to unravel the intricate web of causality.

Yujun Xiong and Xingyun Zhu are contributed equally to this work.

The emerging scientific literature has previously suggested a potential association between gut microbiota and DN. Several studies have shed light on alterations in gut microbiota composition among individuals with DN compared to those without renal complications [4–6]. These observed changes encompass variations in microbial diversity and shifts in the relative abundance of specific bacterial taxa, particularly those involved in short-chain fatty acid production and dietary component metabolism [7, 8]. Alterations in microbiota composition, leading to dysbiosis, result in an overproduction of uremic toxins, such as indoxyl sulfate and p-cresyl sulfate. Concurrently, there is a reduction in renoprotective metabolite levels. These changes are associated with increased oxidative stress, uremia, inflammation, and the progressive deterioration of kidney function, contributing to the advancement of kidney diseases [9].

One promising approach to investigate the potential causal relationship between gut microbiota and DN is Mendelian randomization (MR). MR is a robust methodology that leverages genetic variants as instrumental variables to assess causality. By utilizing genetic variants strongly associated with a specific exposure as proxies, MR can provide more robust evidence for causal inference compared to traditional observational studies [10]. MR mitigates the impact of confounding and reverse causation, offering a unique opportunity to examine causal relationships systematically [11]. In the context of DN, we aim to employ MR to explore the causal relationship between gut microbiota and DN and identify specific harmful or protective bacterial taxa in DN.

2 | Materials and Methods

2.1 | Study Design

In general, we conducted a two-sample MR study to assess the causal relationship between gut microbiota and DN, as shown in Figure 1.

The selection criteria for identifying instrumental variables (IVs) were as follows: (a) single-nucleotide polymorphisms (SNPs) linked to each genus with locus-wide significance ($p < 1 \times 10^{-5}$) were considered potential IVs; (b) data from the European samples within the 1000 Genomes Project served as the reference panel for calculating linkage disequilibrium (LD) among the SNPs. SNPs with an R^2 value of less than 0.001 (using a clumping window size of 10,000 kb) were further analyzed, and only those SNPs exhibiting the most significant p-values were retained for subsequent analysis; (c) SNPs with an F statistic < 10 should be excluded; (d) in cases where palindromic SNPs were present, the alleles on the forward strand were determined using allele frequency information.

2.2 | Data Sources

In this study, we employed the genome-wide association study (GWAS) dataset sourced from the MiBioGen consortium, encompassing a cohort of 18,340 participants, to scrutinize the exposure variable, gut microbiota [12]. This specific GWAS endeavor meticulously scrutinized a total of 211 gastrointestinal

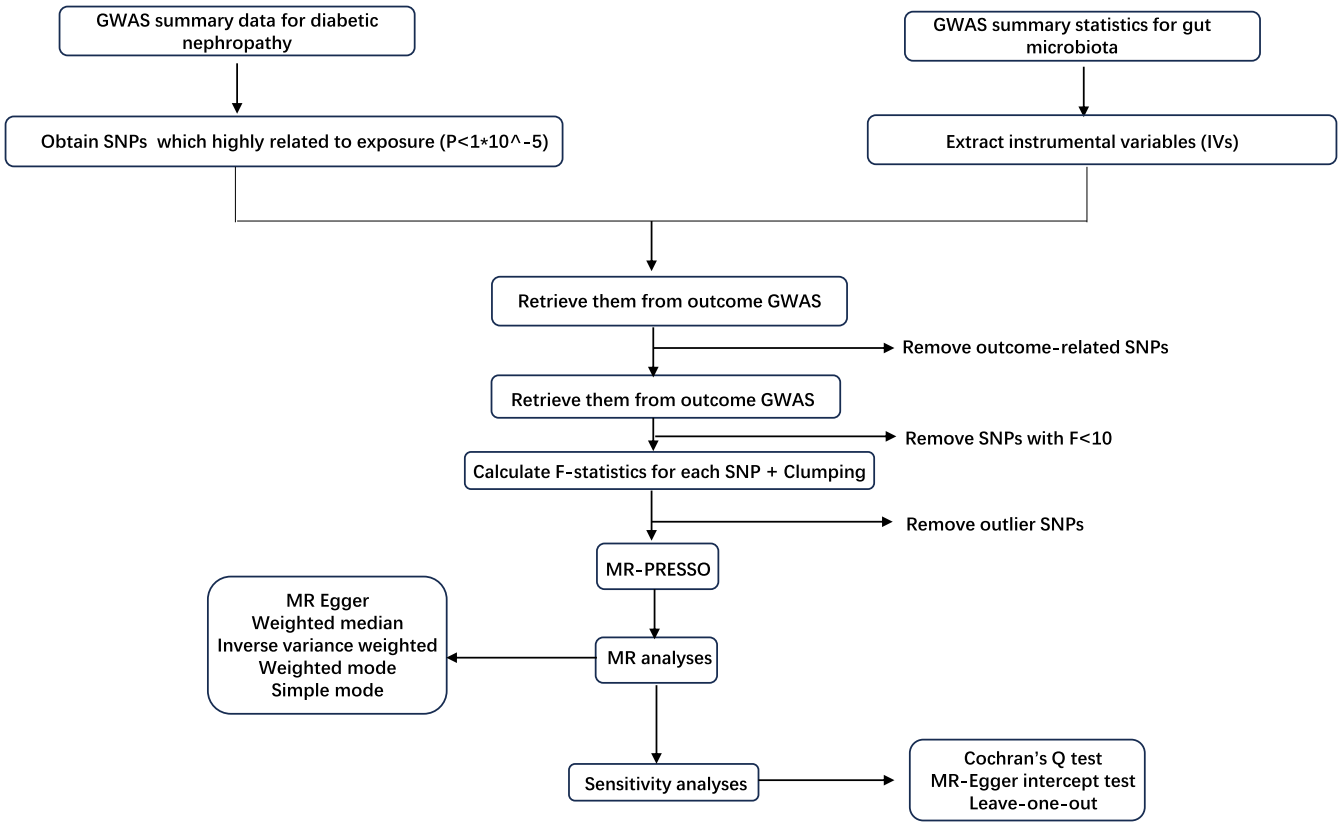

FIGURE 1 | The flowchart of the Mendelian randomization study revealing the causal relationship between gut microbiota and diabetic nephropathy.

microbiota taxa through the utilization of the 16S ribosomal RNA sequencing technique [13].

For the outcome, DN, the GWAS summary statistics consisting of 213,746 individuals, specifically 3283 patients with DN and 210,463 controls, were from the FinnGen database R8. Detailed information is listed in Table 1.

2.3 | Statistical Analysis

In our study, we initiated by harmonizing SNPs with identical alleles from the data source, followed by conducting a two-sample MR analysis. The primary analysis method employed for assessing the causal relationship between gut microbiota and DN was the inverse variance weighting (IVW) method. Acknowledging the assumption that all instrumental variables are valid, it is important to note that the IVW method is susceptible to the impact of instrumental variable pleiotropy and heterogeneity. Nevertheless, in the absence of these influences, IVW is deemed the most accurate method, particularly when other methods fail to produce conclusive results [14]. We utilized odds ratios (ORs) of the exponential β for categorical outcomes along with corresponding confidence intervals (CIs) to estimate effect sizes of causality. A significance threshold of $p < 0.05$ was applied.

To ensure the robustness and sensitivity of our findings, we also performed additional analyses, including MR-Egger, weighted median, simple mode, and MR-PRESSO (Mendelian Randomization Pleiotropy Residual Sum and Outlier). Heterogeneity was assessed using Cochrane's Q test calculated in the IVW methods, while potential pleiotropy was evaluated and corrected using the MR-Egger intercept test. The "leave-one-out" method was employed to evaluate the causal genetic impact of potential outlier SNPs and to ascertain whether the exclusion of these SNPs influenced the MR estimates.

3 | Results

Based on the criteria for selecting IVs, a total of 2308 SNPs were employed as IVs to investigate 210 bacterial genera ($p < 1 \times 10^{-5}$). The *F*-statistic for all IVs exceeded 10, signifying that the chosen SNPs exhibited robust IV effects, thus minimizing the potential for weak instrument bias.

TABLE 1 | Details of the exposure and outcome.

Trait	Consortium	Samples	Link	Year
Exposure				
211 GM taxa	MiBioGen	/	https://mibiogen.gcc.rug.nl/	2021
Outcome				
Diabetic nephropathy	FinnGen	/	https://www.finngen.fi/en	2021

As shown in Table 2, fourteen bacterial genera were found to be significantly associated with DN in at least the IVW method. IVW estimates suggest *Eubacterium ventriosum*, Ruminococcus gauvreauii, ErysipelotrichaceaeUCG003, Lactobacillales, and Proteobacteria might be protective factors for DN, while Akkermansia, Verrucomicrobiaceae, Verrucomicrobiae, Coprococcus, Catenibacterium, Bacteroidia, Marvinbryantia, Bacteroidales, and Verrucomicrobiales may increase the risks of DN (Figure 2). The MR results were presented via scatter plots in Figure 3A,B to reveal the potential positive and negative associations between gut microbiota and DN.

The summary of sensitivity analysis is presented in Table 3. In the MR-Egger regression analysis, no evidence of directional pleiotropic effects was observed for any of the 14 bacterial taxa, as indicated by *p*-values greater than 0.05 (Figure 3A,B). There was no significant heterogeneity identified by the IVW method of Cochrane's Q test, revealing that all *p*-values were more than 0.05. The outcomes from the leave-one-out method indicated that certain individual SNPs might introduce bias in genetic prediction (Figure 4A,B). Horizontal pleiotropy, which refers to the possibility of the IVs affecting outcomes through pathways other than the intended one, was evaluated using the MR-Egger intercept method. The results revealed no indications of horizontal pleiotropy, suggesting that the chosen IVs were not significantly influencing outcomes through alternative pathways (Table 4).

4 | Discussion

In our current investigation, we utilized comprehensive GWAS summary-level data to conduct MR analysis, aiming to evaluate the potential causal relationship between gut microbiota and DN. Our research findings suggest that various components of the gut microbiota may influence the risk of DN, either positively or negatively. Specifically, our study revealed that *Eubacterium ventriosum*, *Ruminococcus gauvreauii*, Erysipelotrichaceae UCG003, Lactobacillales, and Proteobacteria exhibited an inverse association with the risk of DN, while nine other bacterial taxa demonstrated a positive association with the risk of DN.

It is noteworthy that dietary constituents have the potential to impact DN by influencing the gut microbiota [15]. Specifically, the gut microbiota is known to contribute to the production of various metabolites, including short-chain fatty acids (SCFAs), which serve to mitigate bacterial translocation, uphold intestinal integrity, and curtail intestinal inflammation [16]. These SCFAs, such as acetate, propionate, and butyrate, have been shown to possess anti-inflammatory and antioxidant properties [15, 17]. Therefore, their negative association with DN risk observed in this study suggests that SCFAs produced by certain gut bacteria may play a protective role in DN pathogenesis [18].

Previous studies have reported that *Eubacterium ventriosum*, Lactobacillus, and Proteobacteria species, as well as the Ruminococcaceae family, were proven to be butyrate-producing

TABLE 2 | Significant Mendelian randomization estimates of the associations from gut microbiota to diabetic nephropathy.

Taxa	Exposure	MR method	No. of SNP	OR	*p*
Genus	*Eubacterium ventriosum*	MR-Egger	15	0.802	0.692
		Weighted median	15	0.757	0.083
		Inverse variance weighted	15	0.767	0.030
		Simple mode	15	0.766	0.291
		Weighted mode	15	0.759	0.288
Genus	Akkermansia	MR-Egger	11	1.372	0.462
		Weighted median	11	1.359	0.077
		Inverse variance weighted	11	1.443	0.003
		Simple mode	11	1.468	0.171
		Weighted mode	11	1.412	0.177
Genus	Ruminococcus gauvreauii	MR-Egger	12	0.319	0.061
		Weighted median	12	0.781	0.149
		Inverse variance weighted	12	0.742	0.026
		Simple mode	12	0.772	0.469
		Weighted mode	12	0.743	0.343
Genus	ErysipelotrichaceaeUCG003	MR-Egger	15	0.765	0.393
		Weighted median	15	0.758	0.076
		Inverse variance weighted	15	0.778	0.029
		Simple mode	15	0.753	0.276
		Weighted mode	15	0.747	0.260
Family (id 4036)	Verrucomicrobiaceae	MR-Egger	11	1.373	0.461
		Weighted median	11	1.358	0.072
		Inverse variance weighted	11	1.444	0.003
		Simple mode	11	1.461	0.176
		Weighted mode	11	1.411	0.139
Class (id 4029)	Verrucomicrobiaceae	MR-Egger	11	1.375	0.459
		Weighted median	11	1.358	0.071
		Inverse variance weighted	11	1.444	0.003
		Simple mode	11	1.466	0.187
		Weighted mode	11	1.410	0.185
Genus	Coprococcus	MR-Egger	11	1.796	0.116
		Weighted median	11	1.509	0.025
		Inverse variance weighted	11	1.368	0.022
		Simple mode	11	1.675	0.082
		Weighted mode	11	1.603	0.115
Genus	Catenibacterium	MR-Egger	4	0.838	0.912
		Weighted median	4	1.271	0.062
		Inverse variance weighted	4	1.278	0.031
		Simple mode	4	1.222	0.317
		Weighted mode	4	1.222	0.329

(Continues)

TABLE 2 | (Continued)

Taxa	Exposure	MR method	No. of SNP	OR	*p*
Class	Bacteroidia	MR-Egger	13	1.254	0.589
		Weighted median	13	1.636	0.016
		Inverse variance weighted	13	1.403	0.036
		Simple mode	13	1.767	0.098
		Weighted mode	13	1.767	0.069
Order	Lactobacillales	MR-Egger	15	0.939	0.871
		Weighted median	15	0.782	0.169
		Inverse variance weighted	15	0.748	0.045
		Simple mode	15	0.788	0.438
		Weighted mode	15	0.742	0.290
Genus	Marvinbryantia	MR-Egger	10	1.636	0.389
		Weighted median	10	1.278	0.177
		Inverse variance weighted	10	1.369	0.023
		Simple mode	10	1.260	0.419
		Weighted mode	10	1.236	0.398
Order	Bacteroidales	MR-Egger	13	1.254	0.589
		Weighted median	13	1.636	0.014
		Inverse variance weighted	13	1.403	0.036
		Simple mode	13	1.767	0.087
		Weighted mode	13	1.767	0.071
Phylum	Proteobacteria	MR-Egger	12	1.005	0.991
		Weighted median	12	0.710	0.069
		Inverse variance weighted	12	0.714	0.017
		Simple mode	12	0.662	0.196
		Weighted mode	12	0.732	0.254
Order	Verrucomicrobiales	MR-Egger	11	1.375	0.459
		Weighted median	11	1.358	0.063
		Inverse variance weighted	11	1.444	0.003
		Simple mode	11	1.466	0.181
		Weighted mode	11	1.410	0.173

Abbreviations: MR, mendelian randomization; OR, odds ratio; SNP, single-nucleotide polymorphism.

bacteria [19], while Coprococcus may result in less production of butyrate [20]. Butyrate exhibits multifaceted properties within the gastrointestinal milieu. It activates G-protein coupled receptors, namely GPR41 and GPR43, thereby modulating immune responses and dampening inflammation [21]. Furthermore, butyrate promotes the synthesis of antimicrobial peptides by intestinal epithelial cells, bolstering the innate immune system's ability to combat pathogenic microorganisms [22].

In contrast, the phylum Bacteroidota (synonym Bacteroidetes) was found to reduce sulfate and may lead to a decrease in butyrate production, which may increase oxidative stress and further exacerbate the development of diabetes and DN [23].

As previously stated, the gut microbiota serves as a notable source of uremic toxins, such as para-cresol sulfate and indoxyl sulfate [24]. Elevated levels of indoxyl sulfate in the bloodstream have the potential to harm renal cells, specifically tubular cells and podocytes [25, 26]. Podocytes are a critical component of the glomerular filtration barrier and play a pivotal role in controlling the passage of proteins from the capillary lumen to

Bowman's space [27]. Consequently, any injuries or abnormalities in podocytes lead to substantial proteinuria and the onset of nephrotic syndrome. Hence, the changes in the composition of bacteria may accelerate the development of DN through derived uremic toxins.

Our study offers several notable advantages. Firstly, it represents the inaugural MR analysis aimed at establishing a causal link between gut microbiota and DN. This approach effectively mitigates the influence of confounding variables and offers potential candidate bacteria for subsequent functional investigations [28].

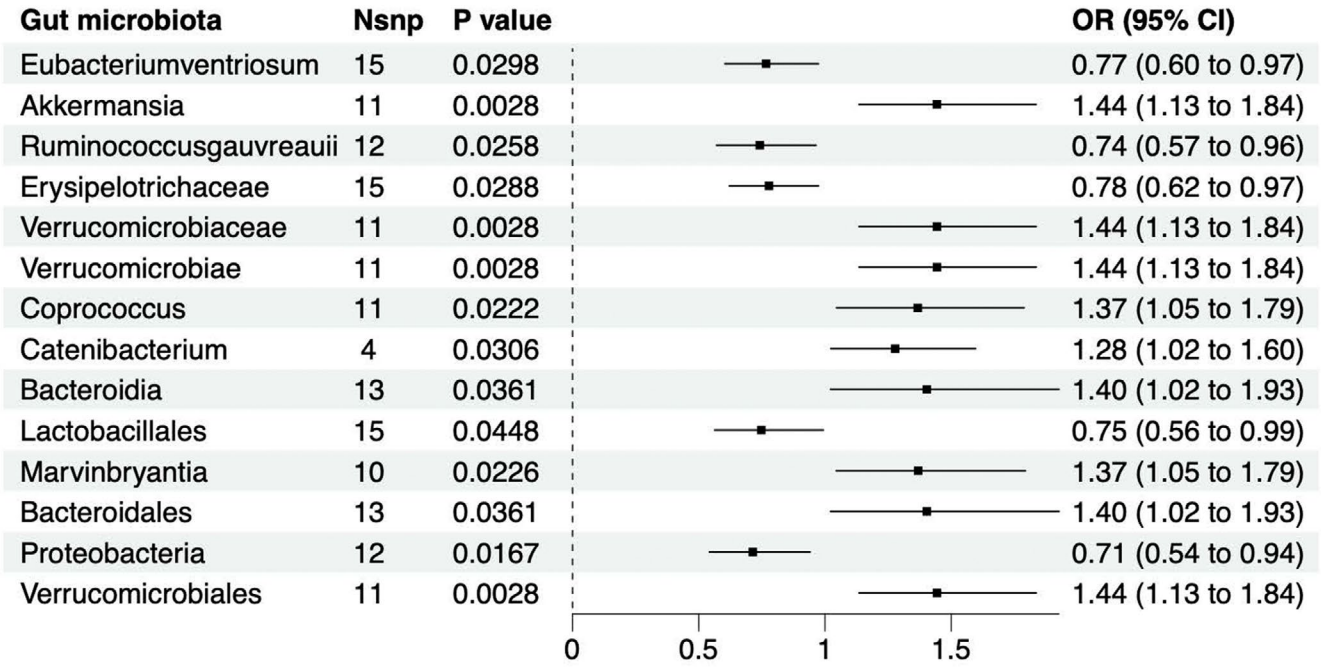

FIGURE 2 | Forrest plot for summary causal effects of gut microbiota on diabetic nephropathy risk based on inverse variance–weighted MR method.

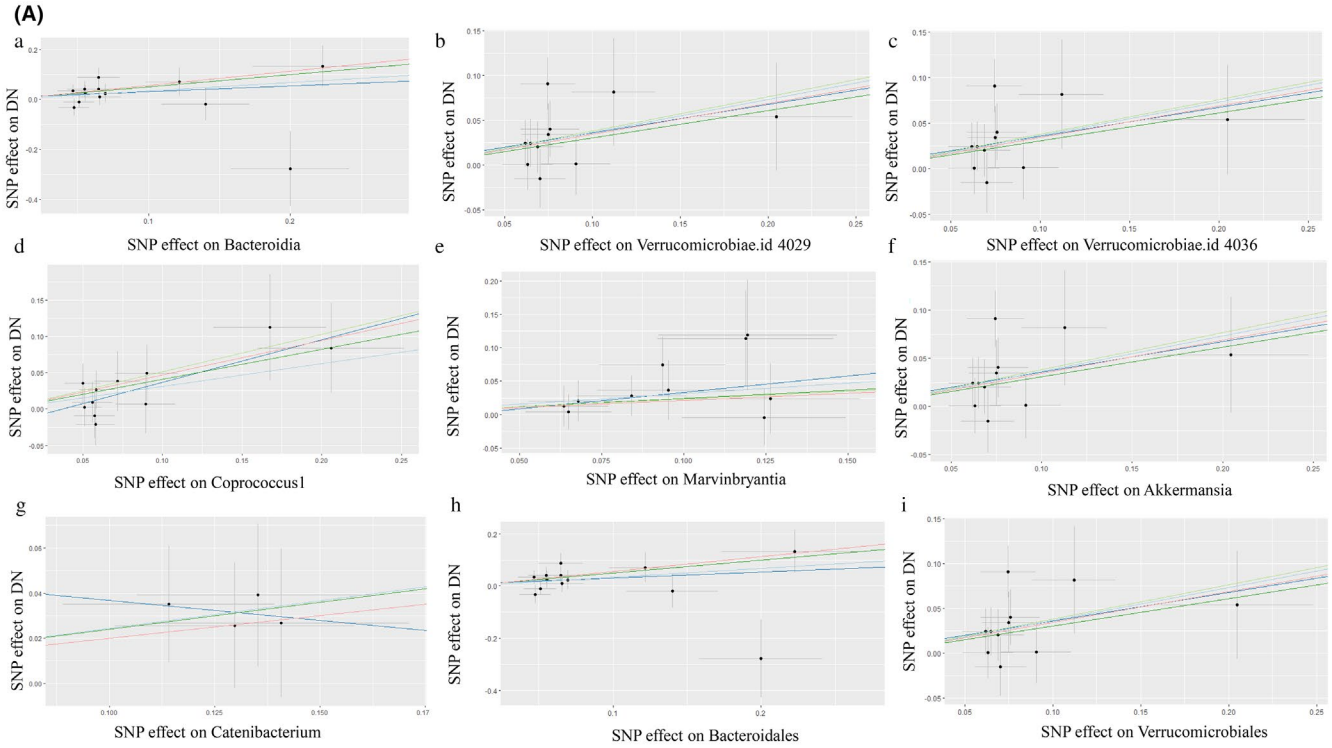

FIGURE 3 | (A) Summary of scatter plots of potential positive associations between gut microbiota and diabetic nephropathy risk. (B) Summary of scatter plots of potential negative associations between gut microbiota and diabetic nephropathy risk.

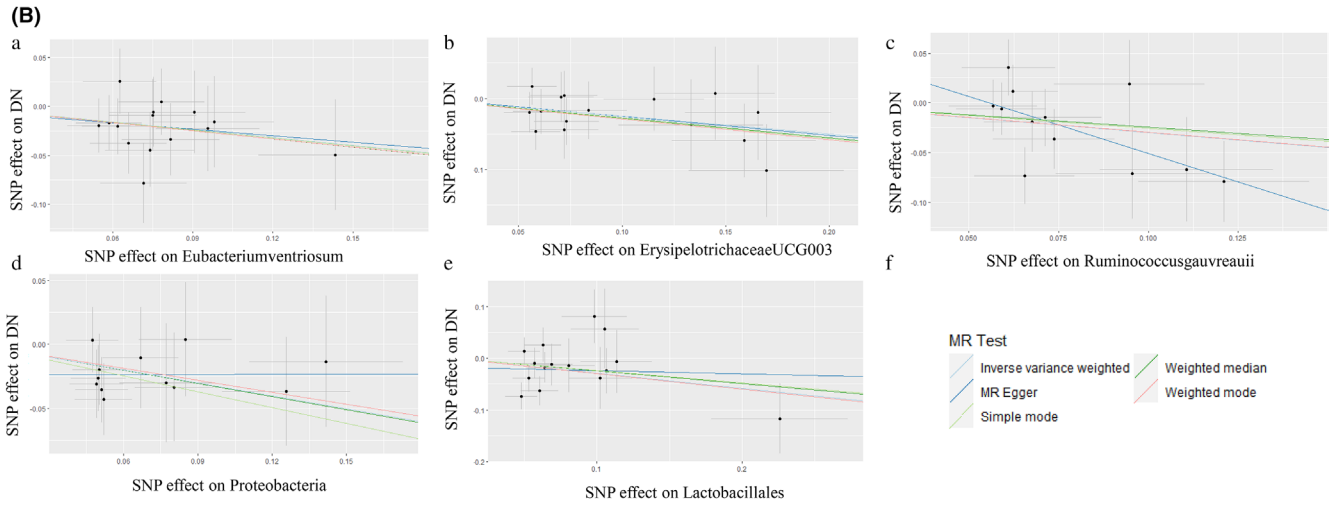

FIGURE 3 | (Continued)

TABLE 3 | The heterogeneity results from the Cochran's Q test in the inverse variance–weighted method.

Taxa	Exposure	Q	*p*
Genus	*Eubacterium ventriosum*	5.821	0.971
Genus	Akkermansia	8.618	0.569
Genus	Ruminococcus gauvreauii	12.792	0.307
Genus	ErysipelotrichaceaeUCG003	6.195	0.961
Family (id 4036)	Verrucomicrobiaceae	8.616	0.569
Class (id 4029)	Verrucomicrobiaceae	8.609	0.570
Genus	Coprococcus	5.081	0.886
Genus	Catenibacterium	0.215	0.975
Class	Bacteroidia	14.878	0.248
Order	Lactobacillales	18.215	0.197
Genus	Marvinbryantia	4.939	0.840
Order	Bacteroidales	14.878	0.248
Phylum	Proteobacteria	3.484	0.983
Order	Verrucomicrobiales	8.608	0.570

Additionally, our MR findings hold substantial implications for public health, as they complement prior research on the interplay between gut microbiota and DN, offering a novel genetic perspective on their connection. From a disease prevention standpoint, the regulation of gut microbiota could potentially guide preventive strategies for DN. Furthermore, on a diagnostic note, it underscores the importance of screening for DN in individuals displaying gut microbiota irregularities.

However, there are notable limitations in our research that warrant attention. It is essential to consider these constraints when interpreting the data. First, while the majority of participants in the GWAS meta-analysis of gut microbiota data were of European descent, potential confounding from demographic stratification remains, limiting the generalizability of our findings to non-European populations. Moreover, MR methods utilize genetic variations to address causal inference questions in epidemiology rather than genetic inquiries per se. In this two-sample MR analysis, we discussed the overall association between gut microbiota and DN but did not investigate the direct cause-and-effect relationship. Hence, further research is essential to uncover the precise mechanisms, targets, and pathways linking gut microbiota and DN. Consequently, cautious interpretation of these findings is warranted.

5 | Conclusion

This study provides robust evidence for a causal relationship between gut microbiota and cirrhosis in European populations.

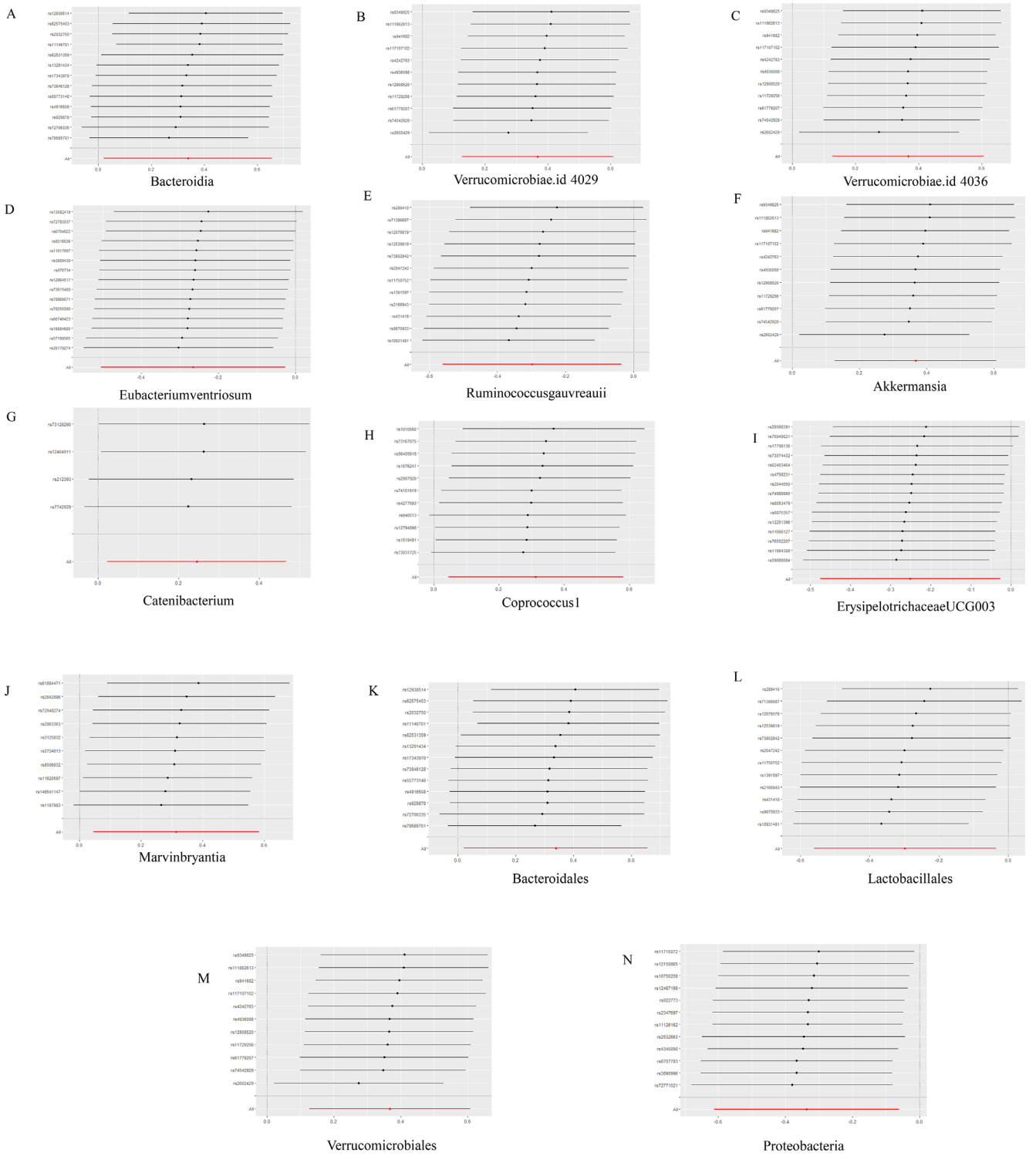

FIGURE 4 | Leave-one-out analysis of gut microbiota on diabetic nephropathy.

Moreover, specific bacterial taxa with potential regulatory roles in the initiation and progression of DN were identified. These findings enhance our understanding of the complex interactions between gut microbiota and health outcomes, highlighting the gut microbiota as a potential therapeutic target. Future randomized controlled trials are warranted to elucidate the protective effects of probiotics on DN and to uncover the underlying mechanisms.

TABLE 4 | Pleiotropy results from MR-Egger intercept analysis.

Taxa	Exposure	Egger intercept	SE	*p*
Genus	*Eubacterium ventriosum*	−0.003	0.041	0.935
Genus	Akkermansia	0.004	0.032	0.900
Genus	Ruminococcus gauvreauii	0.063	0.039	0.139
Genus	ErysipelotrichaceaeUCG003	0.002	0.025	0.954
Family (id 4036)	Verrucomicrobiaceae	0.004	0.032	0.901
Class (id 4029)	Verrucomicrobiaceae	0.004	0.032	0.904
Genus	Coprococcus	−0.022	0.024	0.399
Genus	Catenibacterium	0.054	0.183	0.794
Class	Bacteroidia	0.009	0.028	0.768
Order	Lactobacillales	−0.018	0.028	0.529
Genus	Marvinbryantia	−0.016	0.046	0.743
Order	Bacteroidales	0.009	0.028	0.768
Phylum	Proteobacteria	−0.024	0.026	0.373
Order	Verrucomicrobiales	0.004	0.032	0.904

Abbreviation: SE, standard error.

Author Contributions

Y.X. and Q.L. conceived and designed the study, acquired the data, and drafted the manuscript; H.X. and Z.Z. analyzed the data; X.Z. contributed to the interpretation of the results and critical revision of the manuscript for important intellectual content; Y.X. developed the software and provided technical support. Q.L. had the primary responsibility for final content. All authors have read and approved the final manuscript.

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Ethics Statement

The study was conducted utilizing publicly accessible data derived from the MiBioGen and FinnGen studies, adhering to the principles outlined in the Declaration of Helsinki 1964 and its subsequent revisions. Ethical approval was obtained for both the MiBioGen and FinnGen studies, and all participants provided informed consent before their participation. The databases contain patient data that have been de-identified, ensuring the confidentiality of personal information.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The raw data supporting the conclusions of this article can be found here: https://mibiogen.gcc.rug.nl/, https://r8.finngen.fi/.

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