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Association between gut microbiota and diabetic nephropathy: a two-sample mendelian randomization study

Shisheng Han[1†], Yinqing Chen[1†], Yan Lu[1], Meng Jia[1], Yanqiu Xu[1*] and Yi Wang[1*]

Abstract

Background Observational studies have demonstrated the alterations of gut microbiota composition in diabetic nephropathy (DN), however, the correlation between gut microbiota and DN remains unclear.

Methods A two-sample Mendelian randomization (MR) analysis was designed to estimate the association between gut microbiota and DN. The summary statistics of gut microbiota from phylum level to genus level were obtained from a large-scale, genome-wide association study involving 18,340 individuals, and the data at the species level was derived from the study of TwinsUK Registry, including 1126 twin pairs. The summary statistics of DN were originated from the latest release data of FinnGen (R7, 299623 participants). The MR estimation was calculated using inverse variance weighted, weighted median, MR-Egger regression, and MR-PRESSO. Heterogeneity was assessed using Cochrane's Q test.

Results Inverse variance weighted results indicated that the order *Bacteroidetes* and its corresponding class and phylum [odds ratio (OR), 1.58; 95% confidence interval (CI), 1.15–2.17], the family *Verrucomicrobiaceae* and its corresponding class and order (OR, 1.46; 95% CI, 1.14–1.87), the genera *Akkermansia* (OR, 1.46; 95% CI, 1.14–1.87) and *Catenibacterium* (OR, 1.33; 95% CI, 1.07–1.66) might be associated with a higher risk of DN; whereas the genera *Coprococcus2* (OR, 0.68; 95% CI, 0.51–0.91) and *Eubacterium_coprostanoligenes_group* (OR, 0.69; 95% CI, 0.52–0.92) might play protective roles in DN.

Conclusions This MR study suggested that several gut bacteria were potentially associated with DN, further studies are required to validate these findings.

Keywords Gut microbiota, Diabetic nephropathy, Mendelian randomization

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Background

Diabetic nephropathy (DN) is a common cause of end-stage kidney disease worldwide, and is associated with an increased risk of cardiovascular events and all-cause mortality among patients suffering diabetes mellitus (DM) [1, 2]. Although inflammation, oxidative stress, and dysregulation of renin-angiotensin-aldosterone system have been demonstrated to be involved in the pathogenesis of DN, the underlying mechanisms are still not fully understood, resulting in poor therapeutic effects [3]. Recently, gut microbiota has become of interest due to its prognostic and therapeutic implications [4]. Growing evidence suggested the correlation between gut microbiota and DN, and the alterations of gut bacterial composition in patients with DN were analyzed by several observational studies [5, 6]. However, most previous studies were cross-sectional designs, and it was difficult to confirm the potential relationship between exposure and outcome. Additionally, the correlation between gut bacterial composition and DN might be affected by age, sex, ethnicity, diet, lifestyle and other confounding factors [7], which limits the strength of any inference regarding the association between gut microbiota and DN. Determining the correlation between the gut microbiota and DN might be beneficial in formulating therapeutic strategies for DN. Mendelian randomization (MR) is a novel approach to explore the causal relationship between exposure and disease outcome. Using genetic variants as instrumental variables (IVs), MR analysis can prevent the potential effects of confounding factors [8], since the allocation of genotypes from parent to offspring is random [9]. Recently, MR has been applied to explore the potential association between gut microbiota and DM and its complications, such as diabetic retinopathy [10, 11]. In this study, we conducted a two-sample MR analysis to assess the potential association between gut microbiota and DN, and to identify the taxa of microorganisms potentially involved in the pathogenesis of DN.

Methods

Aim and design

This two-sample MR was designed to investigate the potential relationships between gut microbiota and DN, and was conducted in adherence to the statement for strengthening the reporting of observational studies in epidemiology using mendelian randomization (STROBE-MR) [12].

Data sources

The summary data of single nucleotide polymorphisms (SNPs) correlated with human gut microbiota were derived from the genome-wide association study (GWAS) from the MiBioGen consortium [13, 14]. Briefly, a large-scale, multi-ethnic, genome-wide meta-analysis for the effects of host genetic variants on gut bacterial composition was conducted through microbiota quantitative trait loci mapping analysis, involving 18,340 individuals, of which, 72.33% were Europeans. 16S rRNA gene sequencing was adopted for profiling microbial taxa by targeting three distinct variable regions, including V1-V2, V3-V4, V4. After adjustment for age, sex, technical covariates and genetic principal components, 122,110 SNPs and 211 taxa were included for analysis, including 9 phyla, 16 classes, 20 orders, 35 families, and 131 genera. The data at the species level were obtained from the GWAS study of the TwinsUK Registry, including 1126 twin pairs and 4 eligible species with strong association with genetic variants [15]. The GWAS summary statistics of DN were originated from the latest release data of FinnGen in June 2022, including 3256 patients with DN and 296,367 controls, which were systematically adjusted for age, sex, genetic relatedness, genotyping batch, and principal components [16].

MR assumptions and IVs selection

To reliably investigate the association between gut microbiota and DN, potential SNPs were selected strictly as IVs, and the following three assumptions should be satisfied in this MR study: (1) SNPs should be closely associated with gut bacterial taxa; (2) SNPs should be independent of potential confounders, which might affect gut microbial composition and DN; (3) SNPs influenced DN only through gut microbiota. The study process and IVs selecting procedure are shown in Fig. 1.

Multiple steps were implemented to identify appropriate SNPs as IVs to meet the three assumptions of this MR study. First, SNPs associated with gut microbiota taxa at genome-wide significance level (P value$<5\times10^{-8}$) were preliminarily selected as IVs to satisfy the assumption 1. Considering that few SNPs were available, we further extracted the SNPs at the locus-wide significance threshold (P value$<1\times10^{-5}$), which was mostly adopted in MR analysis to elucidate a greater variation [17]. Second, independent variants were screened through a clumping procedure by setting a linkage-disequilibrium (LD) threshold of 10,000 kilobases apart and a correlation index $r^2\leq0.001$. Palindromic SNPs and those with a minor allele frequency (MAF) less than 0.01 were removed because of their low confidence level [18]. F statistic was calculated to determine whether the SNP was powerful enough to represent the association with gut bacterial taxa, and a threshold of $F>10$ was further selected [19]. Several confounders were found to be the potential factors affecting both gut microbiome and DN, including: body mass index [20, 21], hypertension [22, 23], glucose [24], dyslipidemia [25, 26], smoking [27, 28], physical activity [29, 30], and salt intake [31, 32]. Therefore, the third step was performed to meet the

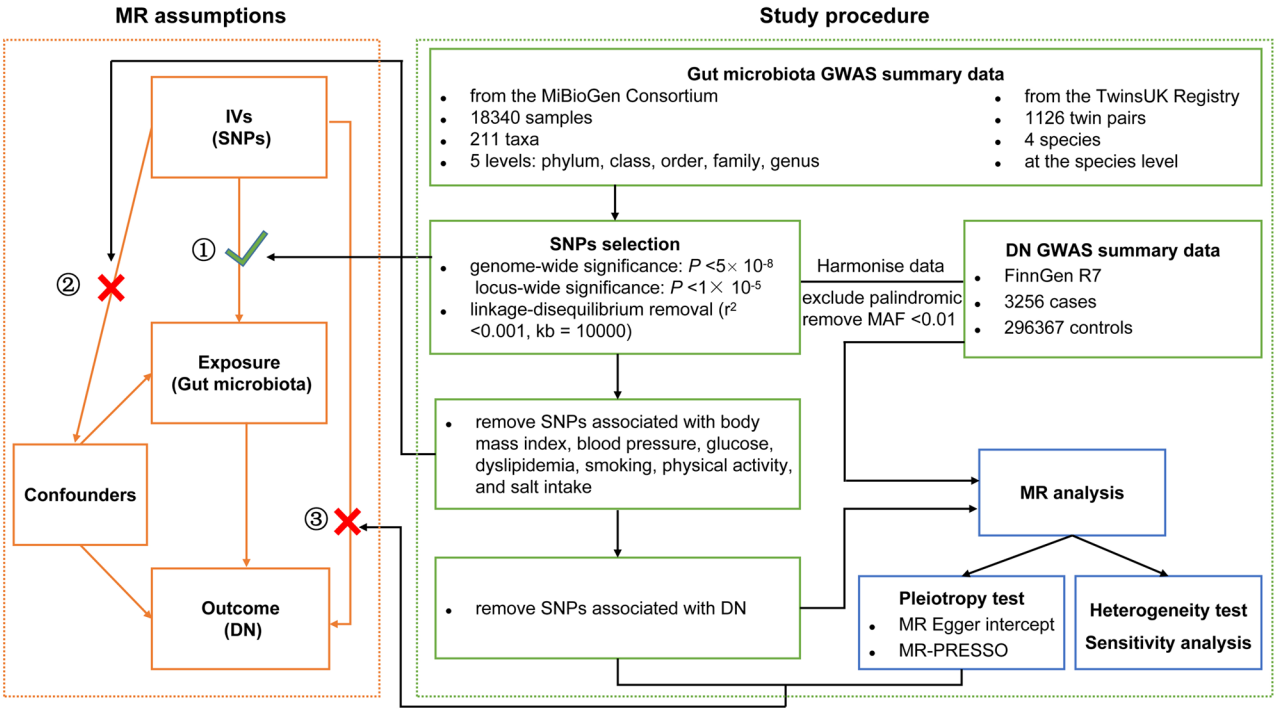

Fig. 1 Flow chart of this Mendelian randomization study. IV, instrumental variable; SNP, single nucleotide polymorphism; GWAS, genome-wide association study; MR, Mendelian randomization

assumption 2 through excluding the SNPs with statistical correlations to these confounders. The final step was to remove SNPs that were directly associated with DN to initially fit assumption 3.

Statistical analysis

Statistical analysis was conducted using the "TwoSampleMR" package of R software (Version 4.1.1). The potential association between gut bacterial taxa and DN was estimated by MR analysis. Odds ratio (OR) and corresponding 95% confidence intervals (CIs) were calculated for the effect size, and the effect was considered significance when P value <0.05. Additionally, we also conducted reverse MR analysis on the bacterial taxa that were found to be associated with DN.

If there was only one SNP available as IV, the Wald ratio (WR) was used for estimating the correlation between gut bacteria and DN. Cochran's Q test was adopted for evaluating the heterogeneity among multiple SNPs. A fixed-effect model or random-effect model inverse-variance weighted (IVW) method was performed to calculate the effect size according to the heterogeneity. A consistent assessment can be provided by the IVW method, when each SNP satisfies all the three assumptions of valid IVs. Therefore, the estimate from the IVW method was considered as the primary result in the absence of heterogeneity and horizontal pleiotropy [33]. Additionally, the weighted median (WM) method and the MR-Egger regression were also conducted for MR analysis. The WM method can provide consistent estimates, if more than 50% of the SNPs were invalid instruments [34]. MR Egger's results remained reliable under the context of significant pleiotropy [35].

Pleiotropy test and sensitivity analysis

The intercept of MR Egger regression was performed to access potential pleiotropy, and horizontal pleiotropy was considered to exist if P value <0.05. MR-PRESSO global test was also employed to evaluate the existence of horizontal pleiotropy. Moreover, MR-PRESSO test could find and rectify horizontal pleiotropic outliers, thereby calculating a accurate estimate by removing possible outliers [36]. The leave-one-out method was conducted as sensitivity analysis to validate the robustness of MR results.

Results

Identification of IVs associated with gut microbiota

After the LD clumping procedure, exposure and outcome data harmonizing, and palindromic and MAF screening, 2250 SNPs were selected preliminary as IVs associated with the 211 bacterial taxa at the locus-wide significance level ($P<1\times10^{-5}$). A total of 193 SNPs were found to be associated with potential confounders, including body mass index, blood pressure, glucose, total cholesterol, triglycerides, high density lipoprotein cholesterol, low density lipoprotein cholesterol cholesterol, smoking,

physical activity, and salt intake (See additional file 1), of which 147 SNPs were further removed from the candidates. The remaining 2103 SNPs were also independent of DN, and were identified as IVs for MR analysis. All the SNPs showed adequate validity with *F* statistic > 10. The detailed characteristics of these IVs are shown in additional file 2. Using the same procedure, 12 SNPs associated with 18 bacterial taxa (2 orders, 5 families, 11 genera) at genome-wide significance level, were identified for MR analysis.

MR analysis using SNPs with genome-wide significance ($P < 5 \times 10^{-8}$)

When gut microbiota was considered as a whole based on the 12 SNPs at the genome-wide significance threshold, MR analysis indicated that gut microbiota alteration might be a risk factor for DN (OR, 1.18; 95% CI, 1.01–1.38; $P=0.03$) (Fig. 2A-B). More specifically, this association might be attributed to the family *Oxalobacteraceae* (OR, 1.70; 95% CI, 1.09–2.67, $P=0.02$) according to the WR method for each taxonomy (Fig. 2A). However, the statistical efficiency of this result was limited, since only one SNP was available.

MR analysis using SNPs with locus-wide significance ($P < 1 \times 10^{-5}$)

The detailed MR results using SNPs with locus-wide significance are shown in Additional file 3. Overall, seventeen taxonomies were identified to be associated with DN (Fig. 3A). At the phylum level, *Bacteroidetes* (OR, 1.58; 95% CI, 1.15–2.17) was found to be the risk factor of DN (Table 1; Fig. 3D).

At the class level, *Bacteroidia* (OR, 1.56; 95% CI, 1.17–2.09) and *Verrucomicrobiae* (OR, 1.46; 95% CI, 1.14–1.87) were associated with increased risks of DN. At the order level, *Bacteroidales*, *Verrucomicrobiales*, and *Rhodospirillales* (OR, 1.20; 95% CI, 1.01–1.43) were identified as risk factors of DN; whereas *Burkholderiales* (OR, 0.73; 95% CI, 0.53–0.99) was associated with a decreased risk of DN. The family *Verrucomicrobiaceae* shared the same statistical estimate with its corresponding order and class (Fig. 3C).

At the genus level, *Akkermansia* (OR, 1.46; 95% CI, 1.14–1.87), *Catenibacterium* (OR, 1.33; 95% CI, 1.07–1.66), *Lachnoclostridium* (OR, 1.37; 95% CI, 1.02–1.84), *Lachnospiraceae_UCG001* (OR, 1.27; 95% CI, 1.02–1.57), *Parasutterella* (OR, 1.23; 95% CI, 1.01–1.50), and *Streptococcus* (OR, 1.39; 95% CI, 1.03–1.88) might be associated with an increased risk of DN; whereas *Coprococcus2* (OR, 0.68; 95% CI, 0.51–0.91), *Ruminococcaceae_UCG014* (OR, 0.75; 95% CI, 0.58–0.96), and *Eubacterium_coprostanoligenes_group* (OR, 0.69; 95% CI, 0.52–0.92) might play a protective role in DN (Fig. 3E - H).

MR analysis at the species level

Four species, involving *Eggerthella_Lenta*, *Faecalibacterium_Prausnitzii*, *Akkermansia_Muciniphila*, and *Veillonella_Dispar*, were found to be associated with 15 SNPs, and were analyzed for their potential relationships with DN. No effects of these species on the risk for DN were found according to the MR results (Table 2).

Horizontal pleiotropy analysis

To minimize the pleiotropy, we have excluded SNPs associated with potential confounders and those directly correlated with DN. At the statistical level, MR Egger regression and MR-PRESSO global test were adopted for detecting potential pleiotropy. We did not find any pleiotropy in the MR analysis of the seventeen taxa with potential association with DN, as well as the bacteria

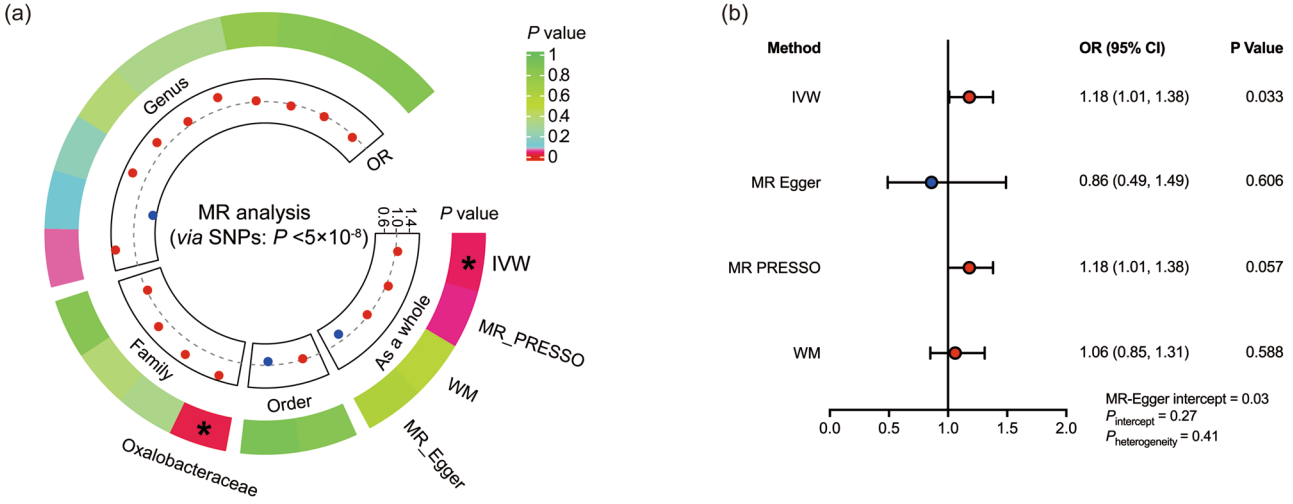

Fig. 2 MR analysis using SNPs with genome-wide significance. (**a**) The results MR analysis based on inverse variance weighted method; (**b**) MR results of gut microbiota as a whole for DN risk

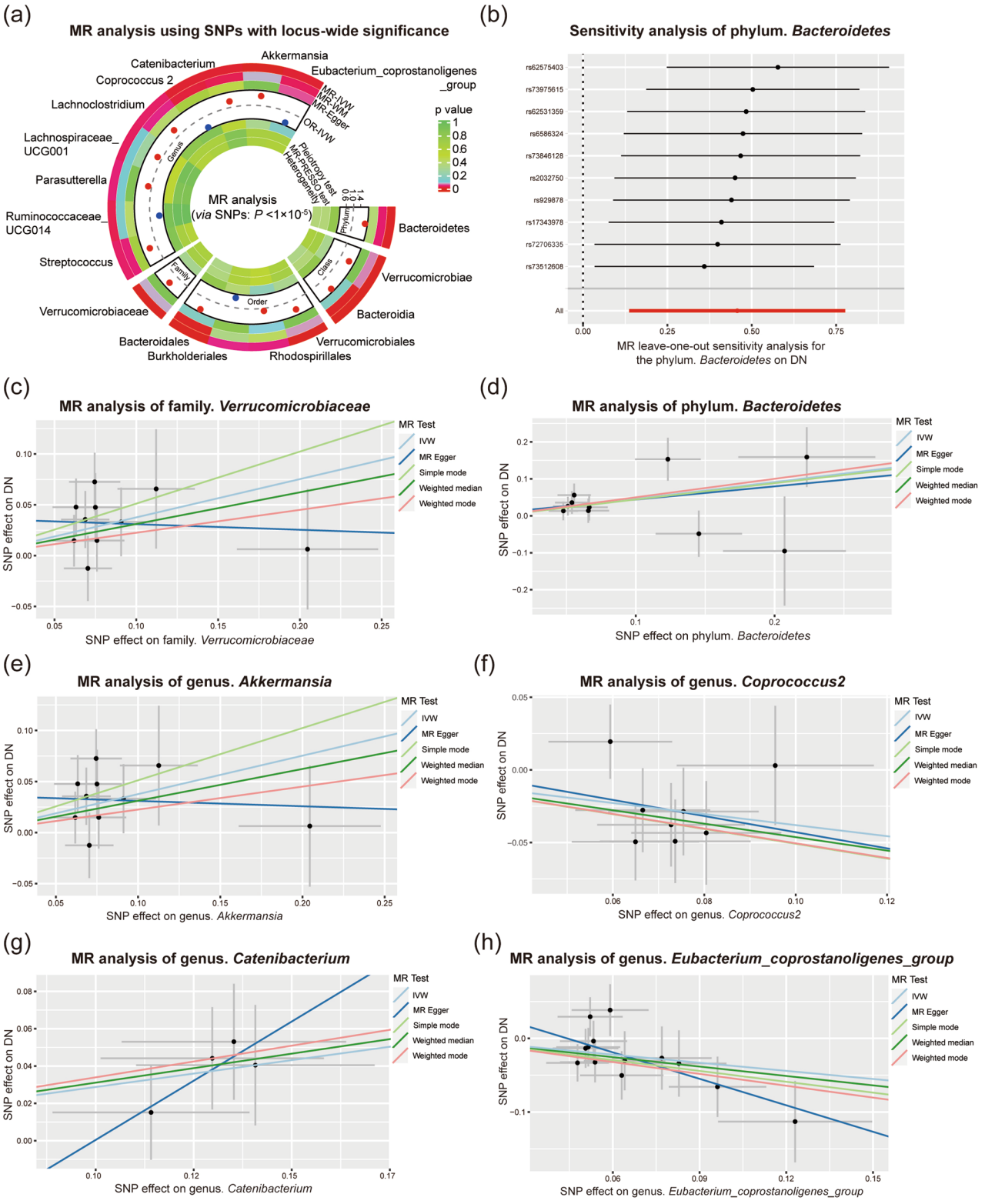

Fig. 3 MR analysis using SNPs with locus-wide significance. (**a**) The results of MR analysis, heterogeneity test, pleiotropy test; (**b**) Sensitivity analysis of the MR analysis between phylum *Bacteroidetes* and DN; (**c**) Scatter plot of MR analysis for the family *Verrucomicrobiaceae* and DN; (**d**) Scatter plot of MR analysis for the phylum *Bacteroidetes* and DN; (**e**) Scatter plot of MR analysis for the genus *Akkermansia* and DN; (**f**) Scatter plot of MR analysis for the genus *Coprococcus2* and DN; (**g**) Scatter plot of MR analysis for the genus *Catenibacterium* and DN; (**h**) Scatter plot of MR analysis for the genus *Eubacterium_coprostanoligenes_group* and DN

Table 1 MR analysis using SNPs at the locus-wide significance level

Taxa	Gut bacteria	nSNP	MR results		Horizontal pleiotropy			Heterogeneity		Sensitivity
			OR (95% CI)	*P* value	Intercept	*P* value	MR_PRESSO[a]	Q statistic	*P* value	
Phylum	*Bacteroidetes*	10	1.58 (1.15, 2.17)	5.17E-03	0.01	0.78	0.37	9.81	0.37	stable
Class	*Bacteroidia*	13	1.56 (1.17, 2.09)	2.57E-03	-0.01	0.77	0.37	13.53	0.33	stable
Class	*Verrucomicrobiae*	10	1.46 (1.14, 1.87)	2.91E-03	0.04	0.31	0.66	6.95	0.64	stable
Order	*Bacteroidales*	13	1.56 (1.17, 2.09)	2.57E-03	-0.01	0.77	0.35	13.53	0.33	stable
Order	*Verrucomicrobiales*	10	1.46 (1.14, 1.87)	2.91E-03	0.04	0.31	0.68	6.95	0.64	stable
Order	*Rhodospirillales*	14	1.20 (1.01, 1.43)	4.44E-02	-0.05	0.20	0.53	12.03	0.53	unstable
Order	*Burkholderiales*	9	0.73 (0.53, 0.99)	4.89E-02	-0.03	0.38	0.62	6.42	0.60	unstable
Family	*Verrucomicrobiaceae*	10	1.46 (1.14, 1.87)	2.92E-03	0.04	0.30	0.68	6.95	0.64	stable
Genus	*Akkermansia*	10	1.46 (1.14, 1.87)	2.91E-03	0.04	0.31	0.65	6.95	0.64	stable
Genus	*Coprococcus2*	8	0.68 (0.51, 0.91)	9.63E-03	0.01	0.88	0.64	5.29	0.63	stable
Genus	*Catenibacterium*	4	1.33 (1.07, 1.66)	9.81E-03	-0.13	0.55	0.88	0.76	0.86	stable
Genus	*Eubacterium_coprostanoligenes_group*	13	0.69 (0.52, 0.92)	1.03E-02	0.05	0.15	0.65	10.07	0.61	stable
Genus	*Lachnospiraceae_UCG001*	11	1.27 (1.02, 1.57)	2.94E-02	-0.03	0.51	0.94	4.44	0.93	unstable
Genus	*Lachnoclostridium*	11	1.37 (1.02, 1.84)	3.37E-02	-0.01	0.80	0.64	8.35	0.59	unstable
Genus	*Parasutterella*	13	1.23 (1.01, 1.50)	4.01E-02	0.01	0.52	0.89	6.35	0.90	unstable
Genus	*Ruminococcaceae_UCG014*	10	0.75 (0.58, 0.96)	2.22E-02	0.00	0.91	0.99	2.32	0.99	unstable
Genus	*Streptococcus*	10	1.39 (1.03, 1.88)	3.39E-02	0.01	0.87	0.65	7.52	0.58	unstable

[a] *P* value of MR_PRESSO global test; OR, odds ratio; CI, Confidence interval; nSNP, number of single nucleotide polymorphism

Table 2 MR analysis at the species level

Species	MR method	nSNP	MR results		Heterogeneity		Sensitivity
			OR (95% CI)	*P* value	Q statistic	*P* value	
MR analysis using SNPs at the locus-wide significance level							
Eggerthella_Lenta[a]	IVW	3	1.00 (0.99, 1.00)	0.58	1.88	0.39	stable
Eggerthella_Lenta	WM	3	1.00 (0.99, 1.00)	0.97			
Eggerthella_Lenta	Egger	3	1.00 (0.99, 1.01)	0.38			
Faecalibacterium_Prausnitzii[b]	IVW	9	1.01 (0.97, 1.06)	0.44	8.72	0.37	stable
Faecalibacterium_Prausnitzii	WM	9	0.99 (0.94, 1.05)	0.96			
Faecalibacterium_Prausnitzii	Egger	9	1.05 (0.98, 1.12)	0.16			
Akkermansia_Muciniphila	IVW	2	1.06 (0.99, 1.13)	0.08	0.59	0.44	stable
Veillonella_Dispar	WR	1	0.99 (0.98, 1.01)	0.91			
MR analysis using SNPs at the genome-wide significance level							
Eggerthella_Lenta	WR	1	1.00 (0.99, 1.00)	0.16	-	-	-
Faecalibacterium_Prausnitzii	IVW	2	1.02 (0.95, 1.09)	0.53	0.14	0.71	stable

Horizontal pleiotropy test: [a]$P = 0.40$, [b]$P = 0.20$; IVW, inverse-variance weighted; WR, wald ratio; SNP, single nucleotide polymorphism; MR, Mendelian randomization; OR, odds ratio; CI, confidence interval

as a whole. For positive pleiotropic test, outliers were excluded for correction, however, the final results were not changed after MR-PRESSO analysis (See additional file 3).

Sensitivity analysis

The phylum *Bacteroidetes*, the orders *Bacteroidales* and *Verrucomicrobiales*, the class *Bacteroidia* and *Verrucomicrobiae*, the family *Verrucomicrobiaceae*, and the genera *Coprococcus2*, *Catenibacterium*, *Akkermansia*, and *Eubacterium_coprostanoligenes_group* showed robust association with DN in sensitivity analyses (Fig. 3B; Table 1). The statistical associations between the other seven taxa and DN disappeared when some SNPs were omitted in sensitivity analyses, including the orders of *Rhodospirillales* and *Burkholderiales*, the genera of *LachnospiraceaeUCG001*, *Lachnoclostridium*, *Parasutterella*, *Ruminococcaceae_UCG014*, and *Streptococcus*, suggesting the uncertainty of causality (Table 1).

Reverse MR analysis

According the pre-designed procedure for IV selection, nine SNPs associated with DN at the genome-wide significance level were identified for the reverse MR analysis (See additional file 4). Our reverse MR analysis revealed a potential positive association between DN and the genus *Streptococcus* (OR, 1.05; 95% CI, 1.00-1.09), however, this correlation was not robust in sensitivity analysis (Table 3). No significant association between DN and other observed gut microbiota was found in the reverse MR analyses (See additional file 5). There was no available data for reverse MR analysis of DN and gut microbiota at the species level.

Discussion

Overwhelming evidence has suggested the association between gut dysbiosis and DN, such as the alterations of gut bacterial composition and the potential mechanisms through which gut microbiota affecting the occurrence and development of DN [37], however, the potential relationship between specific taxa and DN is still unclear. Here, we attempted to explore the correlation between gut microbiota and susceptibility to DN from a host genetic perspective using a MR analysis approach. The phylum *Bacteroidetes*, the classes *Bacteroidia* and *Verrucomicrobiae*, the orders *Bacteroidales* and *Verrucomicrobiales*, the family *Verrucomicrobiaceae*, and the genera *Akkermansia* and *Catenibacterium*, were found to be associated with a increased risk of DN, whereas the genera *Eubacterium_coprostanoligenes_group* and *Coprococcus2* were linked to a decreased risk of DN.

The phylum *Bacteroidetes* is the predominantly autochthonous microbiome in the gut, while *Verrucomicrobiota* represents fewer taxa [38]. *Bacteroidetes* have been shown to be the main contributors of lipopolysaccharide (LPS) biosynthesis [39]. Interestingly, damaged gut barrier and high intestinal permeability were also found in DN, which might induce the translocation of bacteria and leak of harmful substances into circulation, such as LPS, and further aggravate the inflammatory response [40]. The LPS mediated inflammation is often initiated by activating the Toll-like receptor 4 (TLR4) signaling pathway [41]. TLR4 is a sensor that trigger immune responses against bacterial components, resulting in the production of downstream inflammatory cytokines and leukocyte adhesion molecules [42]. The abundance of *Bacteroidetes* were significantly higher in patients with DM than in healthy individuals, accompaning with a positive association with the expression of TLR4 [43]. Meanwhile, the expression of TLR4 was significantly enhanced in the glomerular capillary endothelial cells and glomerular

Table 3 Reverse MR analysis using SNPs associated with diabetic nephropathy at the genome-wide significance level

Taxa	Gut bacteria	nSNP	MR results		Horizontal pleiotropy			Heterogeneity		Sensitivity
			OR (95% CI)	*P* value	Intercept	*P* value	MR_PRESSO[a]	Q statistic	*P* value	
Phylum	*Bacteroidetes*	7	1.00 (0.97, 1.03)	0.93	0.02	0.13	0.33	8.00	0.24	stable
Class	*Bacteroidia*	7	1.00 (0.97, 1.04)	0.86	0.02	0.16	0.32	8.08	0.23	stable
Class	*Verrucomicrobiae*	7	1.02 (0.97, 1.07)	0.46	0.01	0.53	0.21	11.57	0.07	stable
Order	*Bacteroidales*	7	1.00 (0.97, 1.03)	0.86	0.02	0.16	0.33	8.08	0.23	stable
Order	*Burkholderiales*	7	0.99 (0.96, 1.02)	0.54	0.01	0.34	0.87	2.97	0.81	stable
Order	*Rhodospirillales*	7	1.04 (0.99, 1.08)	0.09	0.01	0.95	0.51	5.79	0.45	unstable
Order	*Verrucomicrobiales*	7	1.02 (0.97, 1.07)	0.46	0.01	0.53	0.21	11.57	0.07	stable
Family	*Oxalobacteraceae*	6	1.00 (0.95, 1.06)	0.90	-0.01	0.58	0.73	2.05	0.84	unstable
Family	*Verrucomicrobiaceae*	7	1.02 (0.97, 1.07)	0.46	0.01	0.53	0.20	11.56	0.07	stable
Genus	*Akkermansia*	7	1.02 (0.97, 1.07)	0.47	0.01	0.53	0.24	11.48	0.07	stable
Genus	*Catenibacterium*	5	1.04 (0.97, 1.12)	0.29	0.01	0.92	0.99	0.22	0.99	stable
Genus	*Coprococcus2*	7	1.00 (0.96, 1.04)	0.87	-0.01	0.43	0.73	3.46	0.75	stable
Genus	*Eubacterium_coprostanoligenes_group*	7	1.01 (0.98, 1.04)	0.35	-0.01	0.44	0.62	4.46	0.61	stable
Genus	*Lachnoclostridium*	7	0.99 (0.97, 1.03)	0.88	-0.01	0.53	0.82	1.98	0.92	stable
Genus	*Lachnospiraceae_UCG001*	7	1.00 (0.96, 1.04)	0.60	0.01	0.42	0.53	5.95	0.43	stable
Genus	*Parasutterella*	7	1.01 (0.98, 1.05)	0.41	0.01	0.98	0.89	2.82	0.83	stable
Genus	*Ruminococcaceae_UCG014*	7	0.98 (0.95, 1.01)	0.26	0.01	0.58	0.68	4.64	0.59	stable
Genus	*Streptococcus*	7	1.05 (1.00, 1.09)	0.02	-0.02	0.15	0.26	10.40	0.11	unstable

[a] *P* value of MR_PRESSO global test; OR, odds ratio; CI, Confidence interval; nSNP, number of single nucleotide polymorphism

mesangial cells under diabetic condition [44]. Cytokine productions of the TLR signaling pathway, such as interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and transforming growth factor-β (TGF-β), were also found to be increased in the renal cortex of the glomeruli of diabetic mice, which promoted the accumulation of type I collagen in the glomeruli and the occurrence of microalbuminuria [45]. Additionally, several studies have demonstrated the parallel correlation between *Bacteroides* and IL6 [46] and TNF-α [47]. The family *Verrucomicrobiaceae*, belongs to the phylum *Verrucomicrobia*, was found to be enriched in type 2 diabetic patients with chronic kidney disease [48], and be significantly increased in the fecal sample of non-obese diabetic Goto-Kakizaki rats, as well as their associated fecal metabolites, which were associated with inflammation of diabetes [49]. These evidences indicated that *Bacteroidetes* and *Verrucomicrobiaceae* might cause DN by promoting inflammation.

At the genus level, *Eubacterium_coprostanoligenes_group* and *Coprococcus2* were found to protect against DN according to our results. These genera were butyrate-producing bacteria, and were found to be negatively correlated with serum HbA1c [50, 51]. Butyrate has proven to be effective in the prevention and treatment of DN through inhibiting histone deacetylase, inducing autophagy processes, and ameliorating inflammation [52]. Additionally, the abundance of *Eubacterium_coprostanoligenes_group* was negatively correlated with the liver weight, serum triglyceride, serum glucose levels in high-fat diet-fed mice [53]. Using a microbial-metabolite network, *Eubacterium_coprostanoligenes_group* was found to be the major hub genus involving dyslipidemia, and was positively related to sphingosine and its downstream pathway glycosphingolipid biosynthesis [54]. Decreased abundance of *Eubacterium_coprostanoligenes_group* resulting in the reduction of fecal and serum sphingosine, which was associated with the increase of urinary albumin in patients with DN [55]. These evidences suggested that *Eubacterium_coprostanoligenes_group* might decrease the risk of DN through producing short chain fatty acids and improving glucose and lipid metabolism.

This study suggested that the enrichment of *Akkermansia* (belongs to *Verrucomicrobiaceae*) was a risk factor for DN, which was consistent with the systematic review focusing on the alteration of gut microbiota in diabetic kidney disease [6]. However, most studies supported the beneficial role of *Akkermansia* for both DM and DN. Supplementation with *Akkermansia muciniphila* improved metabolic parameters in obese insulin-resistant volunteers, such as insulin resistance [56], however, our results did not find the association between *Akkermansia muciniphila* and DN at the species level. Additionally, diabetic patients taking metformin had higher relative abundance of *Akkermansia* in the gut microbiota [57]. Therefore, the specific role of *Akkermansia* in the pathogenesis of DN still needs further validation. Although the enrichment of *Catenibacterium* was reported to be associated with insulin-resistant and high levels of A1c in in subjects with pre-diabetes [58, 59], the effect of *Catenibacterium* for DN is currently lacking.

The advantage of this work was that the potential association between gut microbiota and DN were analyzed comprehensively from the phylum level to the species level, using bidirectional MR analysis. The summary GWAS data were obtained from the latest large sample populations, which enhanced reliability of our results. Several limitations should be taken into consideration. Although the genus *Streptococcus* and DN may have a bidirectional relationship, the mutual association is still questionable, due to the unstable sensitivity analysis. The analysis primarily relied on GWAS studies involving participants of European descent, potentially restricting the generalizability of the findings to other ethnic groups. Additionally, the MR analysis at the species level was conducted using a small sample size, possibly yielding less reliable results and limited generalizability.

Conclusions

This MR study found that several gut bacterial taxa were potentially associated with DN, such as *Bacteroidetes*, *Verrucomicrobiaceae*, *Akkermansia*, *Catenibacterium*, *Eubacterium_coprostanoligenes_group*, and *Coprococcus2*. Further studies are required to elucidate the protective effect of medication targeting specific bacteria on DN.

Abbreviations

CI	Confidence interval
DM	Diabetes mellitus
DN	Diabetic nephropathy
GWAS	Genome-wide association study
IL-6	Interleukin-6
IV	Instrumental variable
IVW	Inverse-variance weighted
LD	Linkage-disequilibrium
LPS	Lipopolysaccharide
MAF	Minor allele frequency
MR	Mendelian randomization
OR	Odds ratio
SNP	Single nucleotide polymorphism
STROBE-MR	Strengthening the reporting of observational studies in epidemiology using mendelian randomization
TLR4	Toll-like receptor 4
TGF-β	Transforming growth factor-β
TNF-α	Tumor necrosis factor-α
WR	Wald ratio

Supplementary Information

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Supplementary Material 1: GWAS sources of potential confounders

Supplementary Material 2: Characteristics of selected SNPs for MR analysis

Supplementary Material 3: MR results, heterogeneity test, pleiotropy test of the association between gut microbiota and DN using SNPs with locus-wide significance

Supplementary Material 4: Characteristics of selected SNPs for reverse MR analysis

Supplementary Material 5: Reverse MR results, heterogeneity test, pleiotropy test, and sensitivity analyses between DN and gut microbiota using SNPs with genome-wide significance

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Author contributions
YX and YW designed this work. YC, YL, and MJ collected and analyzed the data. SH interpreted the results and drafted the manuscript. All authors read and approved the final manuscript.

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Data availability
The datasets analysed during the current study are available in the MiBioGen repository (https://mibiogen.gcc.rug.nl, Dataset name: MBG.allHits.p1e4.txt; MiBioGen_QmbQTL_summary_phylum.zip; MiBioGen_QmbQTL_summary_class.zip; MiBioGen_QmbQTL_summary_order.zip; MiBioGen_QmbQTL_summary_family.zip; MiBioGen_QmbQTL_summary_genus.zip) [13, 14], the originated publication of TwinsUK registry (doi: https://doi.org/10.1016/j.chom.2016.04.017) [15], and the FinnGen repository (https://r7.finngen.fi, Dataset name: summary_stats_finngen_R7_DM_NEPHROPATHY) [16].

Declarations

Ethics approval and consent to participate
Because this is a reanalysis of the summary-level data from previously collected data, and each data from GWAS study was ethically approved by their respective institutions, further ethics approval and informed consent for participants are not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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