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# Genetic evidence supporting the causal role of gut microbiota in chronic kidney disease and chronic systemic inflammation in CKD: a bilateral two-sample Mendelian randomization study

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**Background:** The association of gut microbiota (GM) and chronic kidney disease (CKD), and the relevancy of GM and chronic systemic inflammation in CKD, were revealed on the basis of researches on gut–kidney axis in previous studies. However, their causal relationships are still unclear.

**Objective:** To uncover the causal relationships between GM and CKD, as well as all known GM from eligible statistics and chronic systemic inflammation in CKD, we performed two-sample Mendelian randomization (MR) analysis.

**Materials and methods:** We acquired the latest and most comprehensive summary statistics of genome-wide association study (GWAS) from the published materials of GWAS involving GM, CKD, estimated glomerular filtration rate (eGFR), c-reactive protein (CRP) and urine albumin creatine ratio (UACR). Subsequently, two-sample MR analysis using the inverse-variance weighted (IVW) method was used to determine the causality of exposure and outcome. Based on it, additional analysis and sensitivity analysis verified the significant results, and the possibility of reverse causality was also assessed by reverse MR analysis during this study.

**Results:** At the locus-wide significance threshold, IVW method and additional analysis suggested that the protective factors for CKD included family *Lachnospiraceae* ($P$=0.049), genus *Eubacterium eligens* group ($P$=0.002), genus *Intestinimonas* ($P$=0.009), genus *Streptococcu* ($P$=0.003) and order *Desulfovibrionales* ($P$=0.001). Simultaneously, results showed that genus *LachnospiraceaeUCG010* ($P$=0.029) was a risk factor for CKD. Higher abundance of genus *Desulfovibrio* ($P$=0.048) was correlated with higher eGFR; higher abundance of genus *Parasutterella* ($P$=0.018) was correlated with higher UACR; higher abundance of class *Negativicutes* ($P$=0.003), genus *Eisenbergiella* ($P$=0.021), order *Selenomonadales* ($P$=0.003) were correlated with higher CRP levels; higher abundance of class *Mollicutes* (0.024), family *Prevotellaceae*

(*P*=0.030), phylum *Tenericutes* (*P*=0.024) were correlated with lower levels of CRP. No significant pleiotropy or heterogeneity was found in the results of sensitivity analysis, and no significant causality was found in reverse MR analysis.

**Conclusion:** This study highlighted associations within gut-kidney axis, and the causal relationships between GM and CKD, as well as GM and chronic systemic inflammation in CKD were also revealed. Meanwhile, we expanded specific causal gut microbiota through comprehensive searches. With further studies for causal gut microbiota, they may have the potential to be new biomarkers for targeted prevention of CKD and chronic systemic inflammation in CKD.

KEYWORDS

two-sample Mendelian randomization, gut microbiota, chronic kidney disease, inflammation, causality two-sample mendelian randomization, causality

# 1 Introduction

Recently, a worldwide trend of increasing prevalence has appeared in chronic kidney disease (CKD) (1). The most important and commonly used CKD-classifying quantitative traits include estimated glomerular filtration rate (eGFR) and urinary albumin-to-creatinine ratio (UACR) (2, 3). Based on these traits, an epidemiological investigation revealed (4) that the prevalence of CKD is estimated to be as high as 13.4% (11.7-15.1%) worldwide. What's more, CKD significantly increases the mortality of patients, and heavily burdens the financial and healthcare system (5). In addition, Patients with CKD are usually in a chronic inflammatory status, as manifested by the elevated levels of inflammatory markers in CKD patients, which are caused by multiple factors (6). To some extent, renal functions and severity of inflammation in CKD patients can be reflected by levels of c-reactive protein (CRP) or other inflammatory markers (7). Interestingly, levels of the same inflammatory markers are inconsistent within the same period of CKD patients. Besides explicit risk factors including diabetes mellitus (DM) (8), hypertension (HTN) (9), and oxidative st (10) that can influence the inflammation of CKD patients, more risk factors remain to be explored.

Targeting explicit risk factors, the only interventions available for improving renal function and chronic systemic inflammation in patients with CKD are lifestyle changes, pharmacotherapy, and optimization of dialysis conditions (11). However, these interventions are either costly or lack sufficient clinical evidence to support their therapeutic effects. Hence, it is essential to identify potential risk factors for CKD and chronic systemic inflammation in CKD, and develop new preventive and therapeutic measures for them.

Gut microbiota (GM) is a crucial regulator of human health (12). As a critical regulator of human health, it has gradually received attention recently for being considered one of the new potential risk factors of CKD and chronic systemic inflammation in CKD (13, 14). Meijers et al. (15), in 2011, proposed the concept known as gut-kidney axis, revealing that GM might interact with CKD and chronic systemic inflammation in CKD through it. Lau Wei Ling et al. (16) further indicated that GM of CKD patients appeared to be ecologically imbalanced, and the degree of GM imbalance directly influenced the severity of inflammation in the dialysis population. Some studies asserted that interventions on GM could reduce the levels of inflammatory factors and delay disease progression in CKD patients (17). Conversely, some studies suggested that altering types and abundance of GM could not improve renal function and chronic systemic inflammation in CKD patients (18). Nevertheless, their findings were limited by confounding bias, small sample size and reverse causality. Therefore, the association effects they showed were not equivalent to causality. The causal relationships between GM and CKD, as well as GM and chronic systemic inflammation in CKD are still unclear.

Mendelian randomization (MR), as a novel genetic statistical method, can be an effective alternative to traditional epidemiological study approaches. It uses genetic variants strongly associated with exposure factors as instrumental variables (IVs) to statistically evaluate the causality of exposure and outcome (19). The advantage of MR over traditional epidemiological study approaches is that its random assignment method is determined by the DNA genotype. Thus, the influence of external factors on the robustness of causality can be limited to the greatest extent possible (20). In this research, the latest genome-wide association study (GWAS) statistics were used in two-sample MR analysis, and four sets of causal relationships were analyzed at the genetic level: GM and CKD, GM and eGFR, GM and UACR, GM and CRP. Also, we determined the specific causal GM in these four sets. The results supplemented gaps in existing research and might provide new ideas for improving and enriching treatment measures.

# 2 Materials and methods

## 2.1 Study design

In this study, four sets of causal relationships were assessed by two-sample MR method: GM and CKD, GM and eGFR, GM and

UACR, GM and CRP. The overall study flow is displayed in Figure 1. To ensure the reliability of MR results, three basic assumptions (21) must be conformed: (I) IVs significantly correlated with exposure are used in the analysis. (II) The IVs are independent of confounding factors affecting exposure and outcome. (III) The IVs are not horizontally pleiotropic, that is, the IVs have effects on outcome only through exposure.

## 2.2 Data sources of instruments variables

The largest GWAS meta-analysis of GM to date is from the MiBioGen consortium, containing 25 cohorts totaling 18,340 participants of European ancestry from 11 countries (22). From 211 bacterial taxa in total, this study ultimately identified 122,110 variant sites at 5 levels: phylum, class, order, family, and genus. To ensure the accuracy of the data, our study excluded 15 bacterial taxa of unknown family or genus, leaving 196.

Accurate measurement of renal function is difficult to achieve, so the use of biomarkers is necessary (23). Thus, we selected four sets of IVs to represent the renal function and chronic systemic inflammation. Among them, CKD is considered a chronic disease associated with impairment of renal function. eGFR is an important indicator of renal filtration function (24). UACR, a reflection of the degree of urinary protein, is the main clinical diagnostic criteria for CKD (25). CRP is one of the inflammatory markers measuring the severity of the inflammation status (26). The CKD, eGFR and UACR related summarized level GWAS data were collected from the CKDGen consortium's meta-analysis of GWAS from participants of European ancestry. Wuttke et al. have reported CKDGen consortium in detail (27), so we did not elaborate here. With 480698 samples from 23 European ancestry cohorts, the GWAS meta-analysis of CKD comprised 41395 samples in the trial group and 439303 samples in the control group. GWAS meta-analysis of eGFR comprised 567460 European ancestry samples from 54 cohorts. The data of UCAR included 54 GWAS summary statistics of 564,257 participants. GWAS summary statistics related to CRP were obtained from The MRC IEU OpenGWAS data infrastructure (28). The dataset name is c-reactive protein (ID: ieu-b-4764) and it contains 8036590 single nucleotide polymorphism (SNP) in a sample size of 61308.

## 2.3 Selection of instruments variables

Quality checks of SNPs were performed to obtain eligible IVs: (I) GM related SNPs must reach a threshold ($P < 5\times10^{-8}$) with genome-wide significance. To obtain more comprehensive results, another set of SNPs reaching locus-wide significance level ($P < 1\times10^{-5}$) as IV was selected. (II) No linkage disequilibrium (LD) existed among GM-associated IVs, and a clumping process ($r^2 < 0.001$, clumping distance = 10000kb) was performed on the screened SNPs to retain independent ones. (III) Moreover, for SNPs that were not available in GWASs of the outcome, we used the LD proxy search on the online platform (https://snipa.helmholtz-muenchen.de/snipa3/index.php/) to replace them

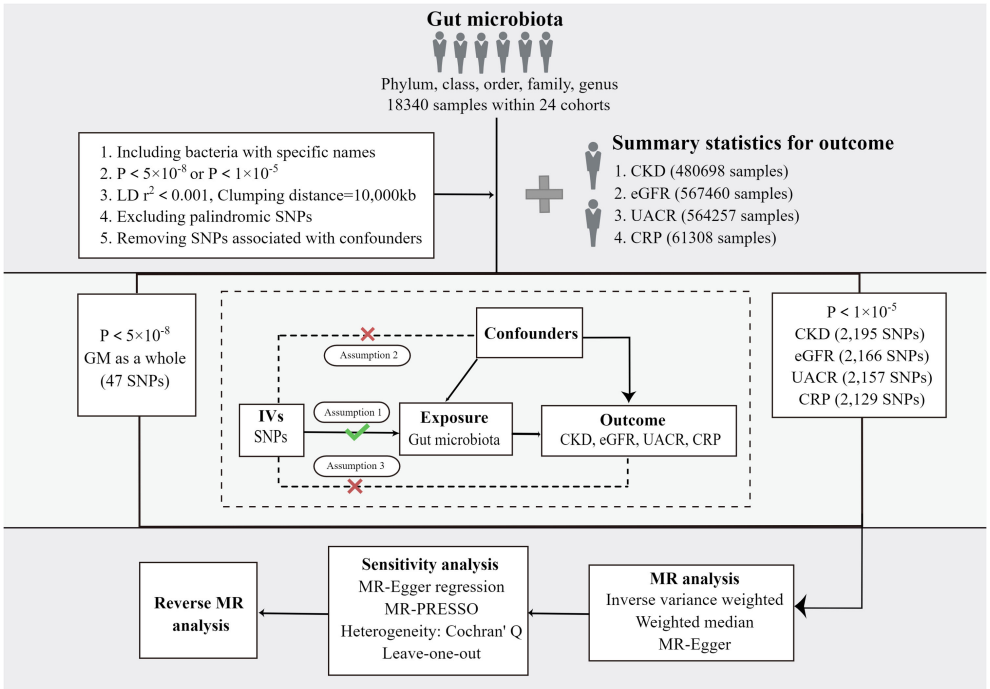


FIGURE 1
The study design and major assumptions of the Two-sample MR study. GM, gut microbiota; LD, linkage disequilibrium; SNP, single nucleotide polymorphism; IV: instruments variable; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; UACR, urine albumin creatine ratio; CRP, c-reactive protein; MR-PRESSO, Mendelian Randomization Pleiotropy RESidual Sum and Outlier.

with the proxy SNPs identified in high-LD (r2 > 0.8) or discard them if the proxies were not available (IV) The effects of GM-associated IVs on both exposure and outcome corresponded to the same alleles, so the palindromic SNPs were removed. (V) To avoid SNPs associated with potential risk factors for outcome, the PhenoScanner V2 website was used to retrieve these SNPs and exclude those associated with potential confounders or risk factors. (VI) To avoid bias caused by weak IVs, we calculated the intensity of IVs using $F=R^2 (n-k-1)/k (1-R^2)$ (29, 30). $R^2$ represents the exposure variance explained by the selected SNPs, n is the sample size, and k represent the number of included instrumental variables. We excluded weak IVs with F < 10 (31).

## 2.4 Ethics statement

In this study, all the summary-level data were published available de-identified ones, which were authorized by the Ethical Standards Committee. No independent ethical approval was necessary during the research.

## 2.5 Mendelian randomization analysis

When no horizontal pleiotropy was available, the IVW method was used in this study as the primary method for inferring 4 sets of causal relationships: GM and CKD, GM and eGFR, GM and UACR, GM and CRP (32). To detect the presence of heterogeneity, we performed Cochran' Q test. If there was significant heterogeneity ($P < 0.05$), a random-effects IVW model was adopted, otherwise a fixed-effects IVW model was applied (33). Moreover, to obtain more robust results under broader conditions, the weighted median (WM) approach and MR-Egger method were adopted to complement the IVW method. The additional methods need to satisfy the respective model assumptions: the WM approach assumes that at least half of the SNPs are free of pleiotropy (34). If the number of SNPs possessing pleiotropy exceeds 50%, the MR-Egger inference is still robust (35). In this study, the causality of exposure and outcome was considered to exist if the results of the main MR analysis reached a nominal significance ($P < 0.05$). The result would be regarded as significant and stable if it was supported by one or more additional methods simultaneously (36), and we would provide a focused discussion on such results.

To avoid the interference of pleiotropy to MR hypothesis, sensitivity analysis on the study results was performed: MR-Egger regression was used to estimate the potential horizontal pleiotropy of the included SNPs, and the results were considered to have horizontal pleiotropy if $P < 0.05$ (35). Considering the lower precision and statistical efficacy of MR-Egger regression, we used Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) to examine any deviations to reflect pleiotropy bias and to give the causal effect of excluding outliers (37). The results of the sensitivity analysis for all IVs are presented in the Supplementary Tables 1, 2. In addition, we conducted leave-one-out sensitivity analysis on significant results, in order to determine whether the significant causal association of the MR analysis was caused by a single IV (38).

## 2.6 Reverse-direction Mendelian randomization analysis

In MR analysis, additional reverse-direction MR analysis on stable and significant results was performed to test whether gene-predicted CKD, eGFR, UACR, and CRP would be causal to GM. The steps of reverse-direction MR analysis were the same as those of MR analysis.

# 3 Results

## 3.1 Selection of IVs related GM

Through a series of quality control measures, at the genome-wide significance level ($P < 5\times10^{-8}$), 12 SNPs, 11 SNPs, 12 SNPs and 12 SNPs were used for genetic prediction of CKD, eGFR, UACR and CRP, respectively. At the locus-wide significance level ($P < 1\times10^{-5}$), 2195 SNPs, 2166 SNPs, 2157 SNPs and 2129 SNPs were used for gene prediction of CKD, eGFR, UACR and CRP, respectively (Supplementary Tables 3, 4). And the F-statistic values of the SNPs were all met (Supplementary Tables 3, 4) (39). Based on the locus-wide significance level, we identified 16, 17, 4, and 10 bacterial taxa causally associated with CKD, eGFR, UACR, and CRP in the primary MR analysis, respectively, as detailed in Supplementary Table 5 and Figures 2, 3. After additional MR analysis and sensitivity analysis, only 6, 1, 1 and 6 bacterial taxa remained robust to the results of CKD, eGFR, UACR, and CRP, respectively (Table 1).

## 3.2 Locus-wide significance threshold $P<1\times10^{-5}$

### 3.2.1 CKD

Primary MR analysis showed that 16 bacterial taxa were associated with CKD risk (Supplementary Table 5; Figure 2). However, only 6 bacterial taxa remained robust in subsequent additional and sensitivity analysis (Table 1). Precisely, higher genetically predicted *Lachnospiraceae*, *Eubacteriumeligens* group, *Intestinimonas*, *Streptococcus*, and *Desulfovibrionales* were related to a lower risk of CKD [odds ratio (OR): 0.927, 95% confidence interval (CI): 0.79-0.98, *P*=0.049 for *Lachnospiraceae*; and OR: 0.830, 95% CI: 0.74-0.93, *P*=0.002 for *Eubacteriumeligens* group, and OR: 0.924, 95% CI: 0.87-0.98, *P*=0.009 for *Intestinimonas*, and OR: 0.892, 95% CI: 0.82-0.96, *P*=0.003 for *Streptococcus*, and OR: 0.873, 95% CI: 0.81-0.94, *P*=0.001 for *Desulfovibrionales*]. Conversely, higher genetic prediction of *LachnospiraceaeUCG010* was related to a higher risk of CKD [OR: 1.096, 95% CI: 1.01-1.19, *P*=0.029].

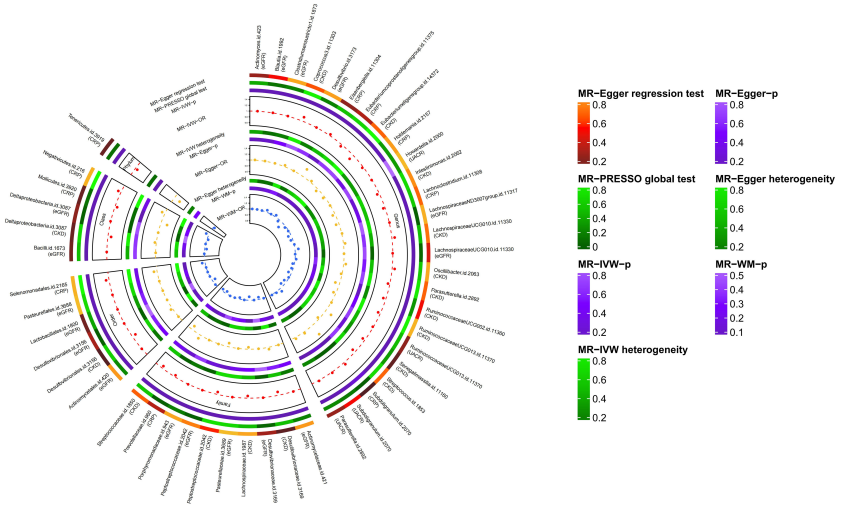


FIGURE 2
Results of MR study and sensitivity analysis between GM and CKD, GM and eGFR, GM and UACR, GM and CRP (locus-wide significance, $P$<1x10$^{-5}$).

| Bacterial Taxa and Methods | Outcome | Lower risk | Higher risk | OR (95%CI) | P-value |
|---|---|---|---|---|---|
| *Significant results without pleiotropy and heterogeneity* | | | | | |
| ***Family Lachnospiraceae*** | | | | | |
| Inverse variance weighted | CKD | | | 0.93 (0.86-0.99) | 0.049 |
| Weighted median | CKD | | | 0.88 (0.86-0.98) | 0.019 |
| ***Genus Eubacteriumeligens group*** | | | | | |
| Inverse variance weighted | CKD | | | 0.83 (0.74-0.93) | 0.002 |
| Weighted median | CKD | | | 0.80 (0.70-0.93) | 0.003 |
| ***Genus Intestinimonas*** | | | | | |
| Inverse variance weighted | CKD | | | 0.92 (0.87-0.98) | 0.009 |
| Weighted median | CKD | | | 0.89 (0.87-0.99) | 0.015 |
| ***Genus LachnospiraceaeUCG010*** | | | | | |
| Inverse variance weighted | CKD | | | 1.10 (1.01-1.19) | 0.029 |
| Weighted median | CKD | | | 1.12 (1.00-1.24) | 0.042 |
| ***Genus Streptococcus*** | | | | | |
| Inverse variance weighted | CKD | | | 0.89 (0.83-0.96) | 0.003 |
| Weighted median | CKD | | | 0.90 (0.80-0.99) | 0.042 |
| ***Order Desulfovibrionales*** | | | | | |
| Inverse variance weighted | CKD | | | 0.87 (0.81-0.94) | 0.001 |
| Weighted median | CKD | | | 0.89 (0.79-0.99) | 0.047 |

0.6 0.8 1 1.2 1.4

| Bacterial Taxa and Methods | Outcome | Lower risk | Higher risk | Beta (95%CI) | P-value |
|---|---|---|---|---|---|
| *Significant results without pleiotropy and heterogeneity* | | | | | |
| ***Genus Desulfovibrio*** | | | | | |
| Inverse variance weighted | eGFR | | | 0.003 (0.00002-0.006) | 0.048 |
| Weighted median | eGFR | | | 0.005 (0.001-0.009) | 0.025 |
| ***Genus Parasutterella*** | | | | | |
| Inverse variance weighted | UACR | | | 0.021 (-0.0004-0.100) | 0.018 |
| MR-Egger | UACR | | | 0.050 (-0.0004-0.100) | 0.044 |
| ***Class Mollicutes*** | | | | | |
| Inverse variance weighted | CRP | | | -0.079 (-0.148--0.010) | 0.024 |
| MR-Egger | CRP | | | -0.248 (-0.451--0.045) | 0.040 |
| ***Class Negativicutes*** | | | | | |
| Inverse variance weighted | CRP | | | 0.098 (0.033-0.163) | 0.003 |
| Weighted median | CRP | | | 0.096 (0.014-0.178) | 0.021 |
| ***Family Prevotellaceae*** | | | | | |
| Inverse variance weighted | CRP | | | -0.059 (-0.113--0.006) | 0.030 |
| Weighted median | CRP | | | -0.082 (-0.155--0.009) | 0.027 |
| ***Genus Eisenbergiella*** | | | | | |
| Inverse variance weighted | CRP | | | 0.047 (0.007-0.087) | 0.021 |
| Weighted median | CRP | | | 0.061 (0.008-0.113) | 0.023 |
| ***Order Selenomonadales*** | | | | | |
| Inverse variance weighted | CRP | | | 0.098 (0.014-0.177) | 0.003 |
| Weighted median | CRP | | | 0.096 (0.033-0.163) | 0.021 |
| ***Phylum Tenericutes*** | | | | | |
| Inverse variance weighted | CRP | | | -0.079 (-0.148--0.010) | 0.024 |
| MR-Egger | CRP | | | -0.248 (-0.451--0.045) | 0.040 |

-0.5 -0.4 -0.3 -0.2 -0.1 0 0.1 0.2 0.3 0.4 0.5

FIGURE 3
MR results of GM taxa with a significant causal relationships to CKD, EGFR, UACR and CRP (locus-wide significance, $P$<1x10$^{-5}$).

TABLE 1 Significant MR analysis results of causal links between GM and CKD, eGFR, UACR, CRP ($P<1\times10^{-5}$).

| Human gut microbiota | Nsnps | Traits | Method | OR | OR (95% CI) | Beta | *P*-value | MR-Egger Regression | | Heterogeneity (IVW) | | Mean F |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Egger Intercept | *P*-value | Cochran's Q | *P*-value | |
| Family *Lachnospiraceae* | 17 | CKD | WM | 0.882 | 0.794-0.980 | -0.126 | 0.019 | -0.00063 | 0.923 | 15.430 | 0.493 | 21.040 |
| | | | IVW | 0.927 | 0.860-1.000 | -0.075 | 0.049 | | | | | |
| Genus *Eubacteriumeligens group* | 7 | CKD | WM | 0.803 | 0.695-0.927 | -0.22 | 0.003 | -0.00802 | 0.702 | 1.434 | 0.964 | 20.734 |
| | | | IVW | 0.83 | 0.739-0.931 | -0.187 | 0.002 | | | | | |
| Genus *Intestinimonas* | 17 | CKD | WM | 0.899 | 0.825-0.980 | -0.107 | 0.015 | 0.00153 | 0.830 | 13.151 | 0.662 | 21.407 |
| | | | IVW | 0.924 | 0.871-0.980 | -0.079 | 0.009 | | | | | |
| Genus *LachnospiraceaeUCG010* | 10 | CKD | WM | 1.117 | 1.004-1.244 | 0.111 | 0.042 | 0.00312 | 0.746 | 9.017 | 0.436 | 21.578 |
| | | | IVW | 1.096 | 1.010-1.190 | 0.092 | 0.029 | | | | | |
| Genus *Streptococcus* | 15 | CKD | WM | 0.895 | 0.805-0.996 | -0.111 | 0.042 | -0.00420 | 0.710 | 10.831 | 0.699 | 22.512 |
| | | | IVW | 0.892 | 0.827-0.963 | -0.114 | 0.003 | | | | | |
| Order *Desulfovibrionales* | 12 | CKD | WM | 0.894 | 0.800-0.999 | -0.113 | 0.047 | -0.01546 | 0.066 | 8.528 | 0.665 | 21.488 |
| | | | IVW | 0.873 | 0.808-0.944 | -0.136 | 0.001 | | | | | |
| Genus *Desulfovibrio* | 10 | eGFR | WM | 1.005 | 1.001-1.009 | 0.005 | 0.025 | 0.00007 | 0.894 | 12.589 | 0.182 | 21.674 |
| | | | IVW | 1.003 | 1.000-1.007 | 0.003 | 0.048 | | | | | |
| Class *Mollicutes* | 11 | CRP | MR-egger | 0.78 | 0.637-0.956 | -0.248 | 0.04 | 0.01580 | 0.120 | 16.648 | 0.083 | 21.298 |
| | | | IVW | 0.924 | 0.862-0.990 | -0.079 | 0.024 | | | | | |
| Class *Negativicutes* | 11 | CRP | WM | 1.101 | 1.014-1.194 | 0.096 | 0.021 | -0.00032 | 0.962 | 2.713 | 0.987 | 21.709 |
| | | | IVW | 1.103 | 1.034-1.177 | 0.098 | 0.003 | | | | | |
| Family *Prevotellaceae* | 15 | CRP | WM | 0.921 | 0.856-0.991 | -0.082 | 0.027 | -0.01305 | 0.063 | 15.349 | 0.355 | 22.150 |
| | | | IVW | 0.943 | 0.894-0.994 | -0.059 | 0.03 | | | | | |
| Genus *Eisenbergiella* | 10 | CRP | WM | 1.063 | 1.008-1.120 | 0.061 | 0.023 | -0.02104 | 0.229 | 8.352 | 0.499 | 21.278 |
| | | | IVW | 1.048 | 1.007-1.091 | 0.047 | 0.021 | | | | | |
| Order *Selenomonadales* | 11 | CRP | WM | 1.101 | 1.015-1.194 | 0.096 | 0.021 | -0.00032 | 0.962 | 2.713 | 0.987 | 21.709 |
| | | | IVW | 1.103 | 1.034-1.177 | 0.098 | 0.003 | | | | | |
| Phylum *Tenericutes* | 11 | CRP | MR-egger | 0.78 | 0.637-0.956 | -0.248 | 0.04 | 0.01580 | 0.120 | 16.648 | 0.083 | 21.298 |
| | | | IVW | 0.924 | 0.862-0.990 | -0.079 | 0.024 | | | | | |

*(Continued)*





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TABLE 1 Continued

| Human gut microbiota | Nsnps | Traits | Method | OR | OR (95% CI) | Beta | *P*-value | MR-Egger Regression | | Heterogeneity (IVW) | | Mean F |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Egger Intercept | *P*-value | Cochran's Q | *P*-value | |
| Genus *Parasutterella* | 15 | UACR | MR-egger | 1.051 | 1.000-1.105 | 0.05 | 0.044 | -0.00287 | 0.007 | 9.032 | 0.770 | 22.369 |
| | | | IVW | 1.021 | 1.004-1.039 | 0.021 | 0.018 | | | | | |

Nsnps, number of single nucleotide polymorphisms; OR, odds ratio; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; CRP, c-reactive protein; UACR, urine albumin creatine ratio; IVW, inverse variance weighted method; WM, weighted median method.

### 3.2.2 eGFR

Seventeen bacterial taxa associated with eGFR were identified from primary MR analysis (Supplementary Table 5; Figure 2). After additional and sensitivity analysis, only the results for *Desulfovibrio* remained robust, as detailed in Table 1. Results from the IVW method showed that higher abundance of *Desulfovibrio* was related to higher eGFR [beta: 0.003, 95% CI: $2.37\times10^{-5}$-$6.48\times10^{-3}$, *P*=0.048], and this result was also supported by the WM approach [beta: 0.005, 95% CI: $5.79\times10^{-4}$-$8.51\times10^{-3}$, *P*=0.025], suggesting a protective effect of *Desulfovibrio* on eGFR decline.

### 3.2.3 UACR

Primary MR analysis confirmed that 4 bacterial taxa were associated with UACR (Supplementary Table 5; Figure 2). Sensitivity analysis and additional analysis only supported the results for *Parasutterella* (Table 1). IVW results showed that higher abundance of *Parasutterella* was a risk factor for proteinuria and was related to higher levels of UACR [beta: 0.021, 95% CI: 0.01-0.04, *P*=0.018].

### 3.2.4 CRP

Ten bacterial taxa were proved to be causally related to CRP in the primary MR analysis (Supplementary Table 5; Figure 2). However, sensitivity and additional analysis supported the results for only 6 bacterial taxa, as shown in Table 1. MR analysis of IVW showed that genetically predicted *Mollicutes* [beta: -0.079, 95% CI: -0.15–0.01, *P*=0.024], *Prevotellaceae* [beta: -0.059, 95% CI: -0.11–0.01, *P*=0.030] and *Tenericutes* [beta: -0.079, 95% CI: -0.15–0.01, *P*=0.024] were associated with lower CRP levels and were protective factors against inflammation. Conversely, genetically predicted *Negativicutes* [beta: 0.098, 95% CI: 0.03–0.16, P=0.003], *Eisenbergiella* [beta: 0.047, 95% CI: 0.01–0.09, *P*=0.021], and *Selenomonadales* [beta: 0.098, 95%CI: 0.03–0.16, *P*=0.003] were associated with higher CRP levels (Table 1).

As shown in Supplementary Table 6, the intercepts of the MR-Egger regressions all did not deviate from the null, suggesting no evidence for horizontal pleiotropy ($P > 0.05$ for all intercepts). We further validated the MR-Egger regression results using MR-PRESSO, and no evidence of outliers was found (Supplementary Table 5). Leave-one-out analysis found no outliers as in MR-PRESSO (Supplementary Figures 1A–N). Without heterogeneity and pleiotropy, outcomes of the above MR analysis were reliable and robust.

## 3.3 Genome-wide significance threshold $P<5\times10^{-8}$

At this stage, we analyzed gut microbiota at five levels (phylum, class, order, family, and genus) based on SNPs available in the gut microbiome GWAS summary data. The results showed no significant causal relationship with CKD, eGFR, UACR or CRP when MR analysis was conducted on each taxonomic level (Table 2). In sensitivity analysis, no intercepts of MR-Egger regression were diverged from the null value, suggesting the

TABLE 2 MR analysis results of causal links between GM and CKD, eGFR, UACR, CRP ($P<5\times10^{-8}$).

| Human gut microbiota | Nsnps | Traits | Method | OR | OR (95%) | Beta | *P*-value | MR-Egger Regression | | Heterogeneity (IVW) | | F |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Egger Intercept | *P*-value | Cochran's Q | *P*-value | |
| Level of class | 1 | CKD | Wald ratio | 0.973 | 0.795-1.190 | -2.781E-02 | 0.787 | NA | NA | NA | NA | 85.376 |
| Level of family | 6 | CKD | MR-Egger | 1.507 | 0.915-2.482 | 4.101E-01 | 0.183 | -0.054 | 0.159 | 3.277 | 0.657 | 40.243 |
| Level of family | 6 | CKD | WM | 0.987 | 0.895-1.088 | -1.342E-02 | 0.789 | | | | | |
| Level of family | 6 | CKD | IVW | 0.976 | 0.903-1.055 | -2.419E-02 | 0.542 | | | | | |
| Level of genus | 12 | CKD | MR-Egger | 0.881 | 0.709-1.093 | -1.271E-01 | 0.276 | 0.012 | 0.318 | 15.089 | 0.178 | 35.942 |
| Level of genus | 12 | CKD | WM | 0.967 | 0.902-1.038 | -3.313E-02 | 0.354 | | | | | |
| Level of genus | 12 | CKD | IVW | 0.984 | 0.923-1.048 | -1.640E-02 | 0.613 | | | | | |
| Level of order | 3 | CKD | MR-Egger | 1.344 | 0.683-2.645 | 2.955E-01 | 0.550 | -0.039 | 0.539 | 0.807 | 0.668 | 48.587 |
| Level of order | 3 | CKD | WM | 0.990 | 0.869-1.128 | -1.036E-02 | 0.876 | | | | | |
| Level of order | 3 | CKD | IVW | 0.994 | 0.891-1.109 | -5.723E-03 | 0.918 | | | | | |
| Level of phylum | 1 | CKD | Wald ratio | 0.983 | 0.765-1.263 | -1.731E-02 | 0.893 | NA | NA | NA | NA | 58.164 |
| Level of class | 1 | eGFR | Wald ratio | 1.004 | 0.997-0.011 | 3.588E-03 | 0.317 | NA | NA | NA | NA | 85.376 |
| Level of family | 6 | eGFR | MR-Egger | 0.997 | 0.978-0.017 | -2.835E-03 | 0.793 | 0.000 | 0.800 | 4.365 | 0.498 | 40.243 |
| Level of family | 6 | eGFR | WM | 1.000 | 0.997-1.003 | 2.133E-04 | 0.909 | | | | | |
| Level of family | 6 | eGFR | IVW | 1.000 | 0.998-1.003 | -1.305E-04 | 0.928 | | | | | |
| Level of genus | 12 | eGFR | MR-Egger | 1.055 | 0.984-1.131 | 5.373E-02 | 0.123 | -0.001 | 0.101 | 17.306 | 0.099 | 35.942 |
| Level of genus | 12 | eGFR | WM | 1.015 | 0.995-1.035 | 1.442E-02 | 0.532 | | | | | |
| Level of genus | 12 | eGFR | IVW | 1.013 | 0.997-1.028 | 1.248E-02 | 0.887 | | | | | |
| Level of order | 3 | eGFR | MR-Egger | 1.002 | 0.978-1.028 | 2.212E-03 | 0.890 | 0.000 | 0.991 | 0.369 | 0.831 | 48.587 |
| Level of order | 3 | eGFR | WM | 1.002 | 0.998-1.007 | 2.165E-03 | 0.346 | | | | | |
| Level of order | 3 | eGFR | IVW | 1.002 | 0.998-1.006 | 2.041E-03 | 0.320 | | | | | |
| Level of phylum | 1 | eGFR | Wald ratio | 1.005 | 0.996-1.014 | 4.616E-03 | 0.317 | NA | NA | NA | NA | 58.164 |
| Level of class | 1 | UACR | Wald ratio | 1.036 | 0.995-1.078 | 3.498E-02 | 0.090 | NA | NA | NA | NA | 85.376 |

*(Continued)*

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TABLE 2 Continued

| Human gut microbiota | Nsnps | Traits | Method | OR | OR (95%) | Beta | *P*-value | MR-Egger Regression | | Heterogeneity (IVW) | | F |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Egger Intercept | *P*-value | Cochran's Q | *P*-value | |
| Level of family | 6 | UACR | MR-Egger | 0.995 | 0.887-0.118 | -4.601E-03 | 0.942 | 0.002 | 0.752 | 2.325 | 0.803 | 40.243 |
| Level of family | 6 | UACR | WM | 1.013 | 0.989-1.037 | 1.245E-02 | 0.308 | | | | | |
| Level of family | 6 | UACR | IVW | 1.015 | 0.996-1.034 | 1.510E-02 | 0.114 | | | | | |
| Level of genus | 12 | UACR | MR-Egger | 0.935 | 0.860-1.016 | -6.714E-02 | 0.145 | 0.009 | 0.070 | 52.240 | 0.000 | 35.942 |
| Level of genus | 12 | UACR | WM | 1.010 | 0.991-1.028 | 9.548E-03 | 0.306 | | | | | |
| Level of genus | 12 | UACR | IVW (random) | 1.015 | 0.987-1.044 | 1.491E-02 | 0.302 | | | | | |
| Level of order | 3 | UACR | MR-Egger | 0.960 | 0.814-1.131 | -4.129E-02 | 0.709 | 0.008 | 0.588 | 0.735 | 0.692 | 48.587 |
| Level of order | 3 | UACR | WM | 1.020 | 0.990-1.051 | 1.991E-02 | 0.195 | | | | | |
| Level of order | 3 | UACR | IVW | 1.022 | 0.996-1.048 | 2.132E-02 | 0.105 | | | | | |
| Level of phylum | 1 | UACR | Wald ratio | 1.044 | 0.993-1.097 | 4.270E-02 | 0.093 | NA | NA | NA | NA | 58.164 |
| Level of class | 1 | CRP | Wald ratio | 0.883 | 0.782-0.997 | -1.247E-01 | 0.044 | NA | NA | NA | NA | 85.376 |
| Level of family | 6 | CRP | MR-Egger | 1.166 | 0.839-1.620 | 1.537E-01 | 0.412 | -0.026 | 0.276 | 3.645 | 0.602 | 40.243 |
| Level of family | 6 | CRP | WM | 0.939 | 0.875-1.007 | -6.334E-02 | 0.079 | | | | | |
| Level of family | 6 | CRP | IVW | 0.946 | 0.896-0.999 | -5.505E-02 | 0.046 | | | | | |
| Level of genus | 12 | CRP | MR-Egger | 1.051 | 0.913-1.210 | 5.020E-02 | 0.499 | -0.010 | 0.218 | 15.913 | 0.144 | 36.263 |
| Level of genus | 12 | CRP | WM | 0.972 | 0.926-1.022 | -2.799E-02 | 0.266 | | | | | |
| Level of genus | 12 | CRP | IVW | 0.961 | 0.920-1.004 | -3.969E-02 | 0.075 | | | | | |
| Level of order | 3 | CRP | MR-Egger | 1.455 | 0.918-2.307 | 3.753E-01 | 0.356 | -0.056 | 0.318 | 3.592 | 0.166 | 48.587 |
| Level of order | 3 | CRP | WM | 0.920 | 0.837-1.011 | -8.324E-02 | 0.083 | | | | | |
| Level of order | 3 | CRP | IVW | 0.952 | 0.863-1.050 | -4.958E-02 | 0.323 | | | | | |
| Level of phylum | 1 | CRP | Wald ratio | 0.907 | 0.779-1.055 | -9.809E-02 | 0.205 | NA | NA | NA | NA | 58.164 |

Nsnps, number of single nucleotide polymorphism; OR, odds ratio; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; CRP, c-reactive protein; UACR, urine albumin creatine ratio; IVW, inverse variance weighted method; WM, weighted median method; NA, Not Applicable.

absence of evidence for horizontal pleiotropy (P > 0.05). MR-PRESSO results showed outliers in sensitivity analysis of UACR at the genus level, then we removed rs35866622, rs602075 and re-analyzed the results, the results did not change significantly (p=0.1095194, beta=0.0125, OR=1.0126). The results of MR analysis could not prove a significant causal relationship between GM and CKD, eGFR, UACR or CRP, because there were too few eligible IVs (Supplementary Table 2).

### 3.4 Reverse-direction Mendelian randomization analysis

Finally, reverse MR analysis was performed for the 14 bacterial taxa presenting significant results with CKD, eGFR, UACR and CRP. After additional analysis and sensitivity analysis, no significant and stable results were found. The specific information is presented in Supplementary Table 7.

## 4 Discussion

This research was not the first one exploring the cause-and-effect relationship between GM and CKD. However, the innovative points of this article remedied the shortcomings of the past studies: (I) GM data used here was the latest large GWAS dataset, which contains 196 bacterial taxa at 5 levels, consisting of 9 phyla, 16 classes, 20 orders, 32 families, 119 genera, in contrast, previous studies only estimated the effect of 8 microbiota genera on CKD (40). The bacterial taxa we studied in this research included but were not limited to those analyzed in previous studies. (II) The selection of IVs was more rigorous, and two thresholds were used to select independent SNPs. In contrast, previous studies did not select SNPs strongly associated with exposure and did not perform the aggregation process, which might seriously affect the reliability and robustness of MR analysis results. (III) Chronic systemic inflammation in CKD is a strong predictor of CKD prognosis, and UACR is sensitive and specific to the overall renal injury profile. Hence, this study revealed not only the causal relationship between GM and CKD, eGFR, but also GM and UACR, CRP. In conclusion, our findings expanded the bacterial taxa associated with CKD and revealed that GM played a regulatory role in CKD and its inflammation state.

The human intestine contains more than 1,000 bacterial taxa, $10^{14}$ in total, which are involved in the metabolic processes of substances in the body and regulate immune function. Several studies (41, 42) found that the composition and abundance of GM were different between healthy individuals and CKD patients. The diversity and different abundance of bacterial taxa may contribute to the development of CKD and CKD co-morbidities such as chronic systemic inflammation. Therefore, the causal relationships between GM and CKD, as well as GM and chronic systemic inflammation in CKD, were explored in this study. After additional analysis and sensitivity analysis, 14 bacterial taxa had a significant causal relationship with our outcome at the phylum, class, order, family, and genus levels (Figure 4).

Family *Lachnospiraceae* isa critical member of GM. Previous studies demonstrated that *Lachnospiraceae* took an essential part in the pathogenesis of CKD (43, 44), however, there were conflicting findings among these studies. Lai et al. (45) found that the abundance of *Lachnospiraceae* was increased in mice with kidney damage and in patients with CKD, which might predict that it was a risk bacterial taxon for CKD. Conversely, other observational studies (44) showed that the abundance of *Lachnospiraceae* in stool samples from patients with CKD was reduced. Moreover, the abundance of *Lachnospiraceae* was negatively correlated with serum creatinine, urea nitrogen and other indicators of renal function, and our study supported these findings. The reason may be that *Lachnospiraceae* is the primary producer of short-chain fatty acids (SCFAs) (43). SCFAs act as a link between GM and within-host environments. They play essential roles in correcting pH value and ensuring the integrity of the intestinal epithelium (46). SCFAs also induce renin secretion and the development of renal cell subtypes to control blood pressure, thereby delaying the progression of CKD (47).

Furthermore, a study by Hu et al. (48) found an increased abundance of genus *Intestinimonas* in CKD patients, suggesting that it might be a risk factor for CKD. The mechanism may be the involvement of genus *Intestinimonas* in the production of tryptophan-derived uremic toxins, which damage the renal vasculature and the circulatory system (49). However, these findings are inconsistent with our conclusions. It is possible that gene-gene interactions and gene-environment interactions result in the same changes in GM but cause different results. In addition, these observational studies may draw biased conclusions because of their smaller sample size (12 rats) and shorter trial duration (2 weeks). In addition to the bacteria mentioned above that are causally related to CKD, in the present study, genus *Eubacterium eligens* group, genus *Streptococcus*, and order *Desulfovibrionales*, all showed a positive effect on CKD, whereas genus *LachnospiraceaeUCG010* indicated a negative effect on CKD.

Two-sample MR analysis also found that higher abundance of genus *Desulfovibrio* was positively associated with higher levels of eGFR. An observational study (50) supporting our view confirmed that the abundance of order *Desulfovibrionales* was low in stool samples from patients presenting with eGFR <15 mL/min/1.73 $m^2$, implying that order *Desulfovibrionales* was a beneficial bacterial taxon for eGFR. Genus *Desulfovibrio* is a bacterial genera pattern of order *Desulfovibrionales*, which may be relevant to the findings of this study. However, Zhao et al. (51) suggested that the abundance of genus *Desulfovibrio* was higher in patients with CKD whose eGFR was significantly reduced. Although the direction of association between genus *Desulfovibrio* and eGFR varied considerably in different studies, the current consensus concluded that genus *Desulfovibrio* could metabolize renoprotective SCFAs (14). It also produces more hydrogen sulfide, causing metabolic impairment and toxicity in the kidney (52, 53), which consequently affects eGFR.

In the MR analysis of GM and proteinuria, our results showed that genus *Parasutterella* was positively associated with higher UACR. This finding is also supported by previous studies: Feng et al. (54) found that GM disorders such as genus *Parasutterella*

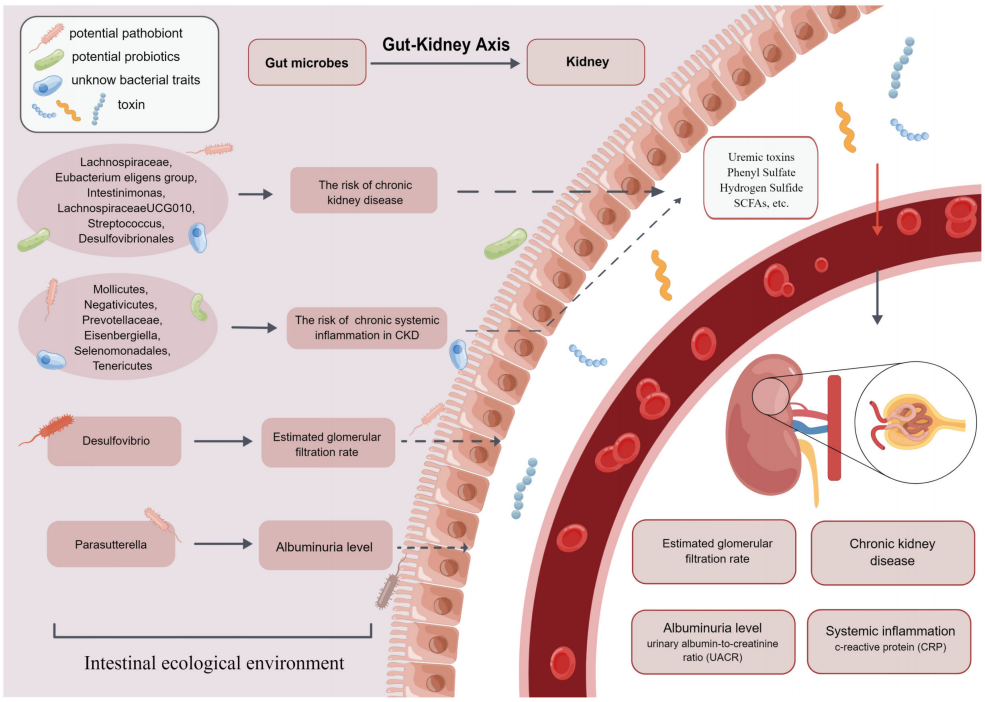


FIGURE 4
The present MR study reveals that GM causally influences CKD, eGFR, UACR and CRP, which supports the existence of gut-kidney axis.

increased in abundance was positively correlated with increased proteinuria in CKD model rats. The mechanism may involve phenyl sulfate (PS) (17), an enteric-derived uremic toxin (55) that can increase proteinuria by damaging podocytes (56). The level of PS is found to be significantly associated with UACR in microalbuminuric patients. It is the only element that predicts the progression of UACR in microalbuminuric patients over the next two years (57). A few bacterial taxa such as *Parasutterella* and *Citrobacter* contain TPL, which synthesizes tyrosine into phenol, which is further metabolized to PS (58). Therefore, GM intervention will constitute a new approach towards the prevention and treatment of proteinuria.

Researchers have long worked on the relationship between GM and chronic systemic inflammation in CKD. One of the common comorbidities of CKD is chronic systemic inflammation. To some extent, chronic inflammation can reflect the renal function status of CKD patients, and it is predictive of CKD prognosis and related comorbidities (7, 59). Previous study (60) suggested that the amount and abundance of GM producing SCFAs were increased, whereas GM producing uremic toxins were increased in CKD patients. Toxins disrupted intestinal tight junctions, penetrated from the damaged intestinal mucosa into the blood, and produced systemic chronic inflammation (60). The regulation of imbalanced GM has become an increasingly popular topic in correcting chronic inflammation. It has been considered (61) that regulating GM abundance by supplementation with probiotics and prebiotics is a proven prevention and treatment method to modify the inflammatory status of CKD patients effectively. Our findings indirectly confirmed that differences in GM abundance do have an effect on inflammatory status.

In the present study, high abundance of family *Prevotellaceae*, class *Mollicutes*, and phylum *Tenericutes* were negatively correlated with CRP. Vaziri et al. (62) found that in stool samples from ESRD patients, family *Prevotellaceae* abundance decreased, leading to increased levels of inflammatory factors (such as CRP) in patients. This was consistent with some of our findings. Family *Prevotellaceae* is the main bacterial taxon producing SCFAs. SCFAs play an important role in maintaining intestinal epithelial integrity and activating FFAR2 to suppress inflammation (63). Li et al. (64) found that patients with CKD4-5 had improved inflammatory status after appropriate intake of SCFAs. Therefore, further study of GM such as family *Prevotellaceae*, class *Mollicutes* and phylum *Tenericutes* may provide theoretical support for the therapeutic strategy to improve chronic systemic inflammation in CKD by intervening GM. In addition to the mentioned GM above are associated with chronic systemic inflammation in CKD, our results also showed that high abundance of class *Negativicutes*, genus *Eisenbergiella*, and order *Selenomonadales* were positively associated with CRP levels.

The mechanisms of GM effects on chronic systemic inflammation in CKD have been proposed so far. The main mechanisms focus on the impact of intestinal endotoxins and GM metabolites (e.g., IS, PCS) (65) on inflammatory responses. This includes the induction of renal tubular oxidative stress and inflammatory responses by IS and PCS (65), and the induction of cytokine secretion by renal interstitial cells to promote inflammatory responses (66). However, no human or rodent model studies have yet explored class *Negativicutes*, genus *Eisenbergiella*, and order *Selenomonadales*, so researches for these

bacteria taxa may become an approach to discovering novel markers for predicting chronic inflammation in CKD.

This study has certain advantages and limitations. First, the advantage is that MR analysis was used, which is a novel genetic statistical method to detect causality. It can avoid confounders and reverse causality. Limitations include (I) GM data were only classified above the genus level. Thus, the causality of CKD and chronic systemic inflammation in CKD could not be concluded at species, strain, or more specialized levels. (II) Since the GWAS data only included European ancestry, the conclusions of this research could not be extended to cover other races. (III) Considering the multi-stage statistics process and biological plausibility, stringent multiple testing calibration may overlook the potential GM with CKD and chronic systemic inflammation in CKD. Hence, we did not strictly follow the multi-corrected p-values to screen GM. (IV) Classes are subcategories of phyla. So the SNP data contained in phylum, class, order, family, and genus may have significant overlap, which may lead to the reproducibility of MR analysis results.

## 5 Conclusion

Four sets of causal relationships were confirmed in this work: GM and CKD, GM and eGFR, GM and UACR, GM and CRP; however, the reverse causality was not identified. In addition, the present study identified specific bacterial taxa associated with CKD, eGFR, UACR, and CRP, respectively, which might be novel biomarkers for further research of CKD and chronic systemic inflammation in CKD. The study of these bacterial taxa may contribute to the prevention and treatment of CKD and chronic systemic inflammation in CKD, and provide a theoretical basis for studying the mechanism governing gut-kidney axis.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary Files, further inquiries can be directed to the corresponding author.

## Author contributions

FR: Formal Analysis, Writing – original draft. QiuJ: Formal Analysis, Writing – review and editing. QiJ: Formal Analysis, Writing – review and editing. YQ: Data curation, Formal Analysis, Writing – review and editing. XR: Data curation, Writing – review and editing. TL: Writing – review and editing. YZ: Conceptualization, Funding acquisition, Investigation, Writing – review and editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2023.1287698/full#supplementary-material

SUPPLEMENTARY FIGURE 1
**(A)** The result of sensitivity analysis of the causal effect of family *Lachnospiraceae* on CKD risk. **(B)** The result of sensitivity analysis of the causal effect of genus *Eubacteriumeligens* group on CKD risk. **(C)** The result of sensitivity analysis of the causal effect of genus *Intestinimonas* on CKD risk. **(D)** The result of sensitivity analysis of the causal effect of genus *LachnospiraceaeUCG010* on CKD risk. **(E)** The result of sensitivity analysis of the causal effect of genus *Streptococcus* on CKD risk. **(F)** The result of sensitivity analysis of the causal effect of genus *Desulfovibrionales* on CKD risk. **(G)** The result of sensitivity analysis of the causal effect of order *Desulfovibrio* on eGFR. **(H)** The result of sensitivity analysis of the causal effect of class *Mollicutes* on CRP. **(I)** The result of sensitivity analysis of the causal effect of class *Negativicutes* on CRP. **(J)** The result of sensitivity analysis of the causal effect of family *Prevotellaceae* on CRP. **(K)** The result of sensitivity analysis of the causal effect of genus *Eisenbergiella* on CRP. **(L)** The result of sensitivity analysis of the causal effect of order *Selenomonadales* on CRP. **(M)** The result of sensitivity analysis of the causal effect of phylum *Tenericutes* onCRP. **(N)** The result of sensitivity analysis of the causal effect of genus *Parasutterella* on UACR.

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